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Minnesota State University, Mankato

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The effects of surface albedo and initial lignin concentration on
photodegradation of *Sorghum bicolor* litter

Joshua A. Niere

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science
in
Environmental Science

Minnesota State University
Department of Biological Sciences
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Mankato MN 56001

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The Effects of Surface Albedo on Rate of Photodegradation of *Sorghum bicolor* (Wild Type and Double Mutant)

Joshua A. Niere

This thesis has been examined and approved by the following member of the student's committee.

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Abstract

Photodegradation has been recognized as a contributor to litter decomposition in a wide variety of ecosystems, however many of the mechanisms that drive it remain unknown. The primary focus of this study was to investigate the effect of surface albedo on the rate at which plant litter photodegrades. The first hypothesis that was tested was that surfaces with higher albedo will increase the rate of mass loss. The second hypothesis was that a wild type *Sorghum bicolor* with higher lignin concentration will degrade more rapidly than a double mutant variety. Three different artificial surface covers (aluminum foil, black paint, and white paint) were used to mimic the surface albedo of natural surfaces. Two varieties of *Sorghum bicolor* (wild type (WT) & double mutant (DM)) that differed in initial litter chemistry were placed on the surfaces and exposed to varying levels of solar radiation for 200-d. Mass loss, cell wall constituent (hemicellulose, cellulose and lignin) concentrations and bulk-soluble phenolic concentrations were examined every 50-d, for the duration of the experiment. In support of our first hypothesis, decomposition of the WT and DM litter was generally faster on the aluminum surfaces than on the black and white surfaces. Litter collected from the aluminum surfaces lost an average of 1.71% more mass than the black surfaces and an average of 3.08% more mass than the white surfaces. In contrast to our second hypothesis, the higher lignin, WT litter, photodegraded at a slower rate than did the lower lignin, DM litter. Following the 200-d collection, DM litter lost approximately 5% more mass, with WT losing an average of 47.5% of initial mass, and DM losing an average of 52.6% across all surface types

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Introduction

A fundamental understanding of the carbon cycle is of increasing importance as atmospheric carbon levels continue to rise rapidly across the globe. However, there are currently several important components of the carbon cycle that are not yet fully understood. Carbon sinks play a major role in storing excess carbon found in the atmosphere. One of the Earth's major sinks, the terrestrial biosphere, is responsible for holding approximately 2,000 Gt of carbon. This pool is held in both living biomass (600-1,000 Gt) and dead biomass (1,200 Gt) (Falkowski 2000). Decomposition is responsible for releasing more carbon annually than fossil fuel combustion, supporting the need for further research (Gholz *et al.* 2000). To date, the majority of research focused on the decomposition of dead biomass. However, photodegradation, or the mineralization of carbon as carbon dioxide through photochemical interactions with ultraviolet (UV; 280-400 nm) and photosynthetically active radiation (PAR; 400-700 nm) is believed to be a primary contributor to decomposition in arid and semiarid ecosystems (Austin & Vivanco 2006).

The focus of this study was to examine how varying levels of surface albedo impact the rate of photodegradation of two different strains of sorghum with varying lignin levels. The study tested the following hypotheses: (1) surfaces with higher albedo will increase the rate of mass loss; (2) *Sorghum bicolor* with higher lignin concentration will see more rapid mass loss.

Literature Review

Decomposition-

Cotrufo *et al.* (2010) defines litter decomposition as “the process through which dead organic material is broken down into particles of progressively smaller size, until the structure can no longer be recognized, and organic molecules are mineralized to their prime constituents.” The terrestrial biosphere is a major sink of carbon holding approximately 2,000 Gt of carbon, with around 1,200 Gt of this carbon being held within dead biomass (Falkowski 2000). Decomposition is a major contributor of carbon to the atmosphere as it is responsible for the release of the carbon that is held within dead biomass. Overall, decomposition is responsible for the release of more carbon annually than through the burning of fossil fuels (Gholz *et al.* 2000). Decomposition of plant litter can occur via both biotic and abiotic processes. Previous studies have focused primarily on the role that biotic processes play on decomposition. Studies dealing with biotic decomposition, focus primarily on decomposition by microorganisms, and how these microorganisms are impacted by variables such as moisture, temperature, and other environmental factors (Mellio *et al.* 1982; Nagy *et al.* 1982; Aerts *et al.* 1997). Not until recently have studies begun to focus more on abiotic factors, such as degradation by chemical or physical processes as contributors to decomposition (Vossbrinck *et al.* 1979).

Swift *et al.* (1979) established the P-O-Q triangle which illustrates the individual factors that impact litter decomposition, along with how they interact. “P” represents the physical-chemical environment, “O” represents the organisms responsible for decomposition and the “Q” represents overall resource quality. This figure illustrates the complex nature of

decomposition, and all of the factors that can modify the rate at which it occurs. Research on decomposition has been extensive covering various species and biomes, however the complexities of the process leave the need for further study.

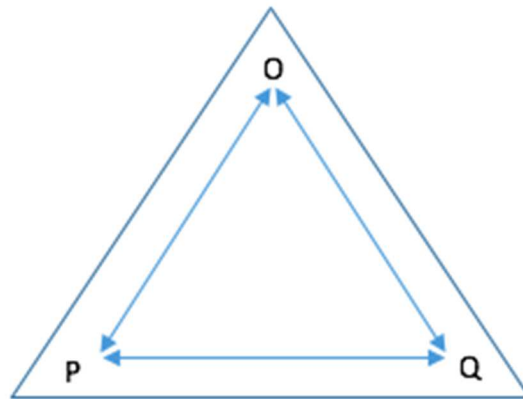


Figure 1. The POQ triangle established in Swift et al (1979). “P” represents physical-chemical environment, “O” represents organisms responsible for decomposition, and “Q” represents resource quality.

Substrate quality, which is the overall chemical makeup of the litter that is being acted upon, is one of the primary components that alter the rate at which litter decays (Waksman and Tenney 1927). Studies have shown contradictory results when it comes to what component of plant litter is responsible for determining the rate at which litter decomposes. Some studies have found that nitrogen content determines that rate at which litter decomposes (Findlay 1934; Merrill and Cowling 1966), while other studies have found that lignin actually plays a more significant role in determining the rate of decomposition than nitrogen (Fogel and Cromack 1977). The quality of substrate has the ability to make litter more or less susceptible to microorganisms and environmental variables depending on concentrations. For example, lignin provides a rigidity to litter that limits microbial breakdown, however it is vulnerable to

photodecomposition (Austin *et al.* 2009). This shows the overall complexities of decomposition and supports the need for further research.

Along with substrate quality, litter decay is also controlled by climate. Although, climate plays a role in decomposition, it is still unknown which climate variable has the most significant impact on decomposition. Studies have shown that temperature and precipitation play a role in decomposition, however results have shown that temperature is dependent on precipitation to achieve the maximum rate of decomposition. Murphy *et al.* (1998) studied the effects of climate on decomposition along an environmental gradient. Their results demonstrated that decomposition rates were higher at sites that were cold but had high levels of moisture. It appears that temperature alone does not increase rates of decomposition, as available moisture must be high enough in order for temperature to have a role in decomposition. These results show why it is difficult to establish the role that climate plays in decomposition.

Previous studies have focused primarily on biotic decomposition, as a result of organisms found within soil, and how these organisms are impacted by environmental variables including temperature, water availability and litter chemical quality (Melilo *et al.* 1982; Nagy *et al.* 1982; Aerts 1997; Lin *et al.* 2014). These studies helped in developing an understanding of decomposition in mesic ecosystems, however, they failed to account for decomposition in arid and semiarid ecosystems, in which environmental conditions differ (Austin and Vivanco 2006). Research done in arid ecosystems has shown that litter typically does not immobilize nitrogen, and initial nitrogen concentration does not impact the rate of decay (Parton *et al.* 2007; Vanderbilt *et al.* 2008; Gallo *et al.* 2009). This suggests that abiotic decomposition is more prolific in arid ecosystems than is microbial breakdown. Photodegradation, or the

mineralization of carbon as carbon dioxide through photochemical interactions with ultraviolet (UV; 280-400 nm) and photosynthetically active radiation (PAR; 400-700 nm) is believed to be a primary contributor to decomposition in arid and semiarid ecosystems. (Austin and Vivanco 2006).

Photodegradation-

Prior research in arid ecosystems has found that decomposition rates are faster than what is expected as a result of microbial breakdown alone (Whitford *et al.* 1981). Pauli (1964) first hypothesized that solar radiation may be causing the faster than expected rates of decomposition in arid ecosystems, now coined as photodegradation. Photodegradation is defined as the breakdown of organic matter through photochemical interactions with ultraviolet (UV; 280-400 nm) and photosynthetically active radiation (PAR; 400-700 nm; King *et al.*, 2012). Originally, it was believed that the UV-B range (280-320 nm) was responsible for photodegradation, however, further research has found that UV-A (320-400 nm) and short-wave visible range (400-500 nm) radiation both play an equal or greater role in photodegradation (Brandt *et al.*, 2009, Day *et al.* 2015).

Initially, the bulk of research pertaining to photodegradation looked at how plant litter decomposes in water-limited ecosystems. Austin and Vivanco (2006) evaluated the role that solar radiation, soil biotic activity and soil resource availability plays on litter decomposition in the semi-arid Patagonian steppe. Manipulative experiments were used in order to examine the role that photodegradation plays in the decomposition process. Three different radiation treatments were used in order to better evaluate how radiation modifies the rate in which litter

decomposes. The three treatments included: (1) Aclar filters, which allow the transmission of >95% of solar radiation; (2) Mylar filters, which block all radiation below 310 nm; and (3) Mylar filters covered with reflective aerosol paint that blocks >90% of solar radiation. Following experimentation, the results supported that photodegradation is a control on above-ground decomposition in semi-arid ecosystems. Similar results have been realized in several other experiments, therefore supporting that photodegradation plays a role in plant litter decomposition (Gallo *et al.*, 2006; Brandt *et al.*, 2007; Day *et al.*, 2007, 2015).

Water-limited ecosystems were assumed to see more rapid photodegradation due to there being limited microbial activity. However, research has shown that photodegradation plays a role in other ecosystems as well. Brandt *et al.* (2010) analyzed the role that photodegradation plays in litter decomposition across an ecosystem precipitation gradient. Three different grassland sites were chosen for experimentation in Minnesota, Colorado and New Mexico that represented mesic, semiarid and arid grasslands respectively. The exposure of *B. gracilis* to UV radiation resulted in an increase in mass loss and a higher rate of decay at each of the three sites. These results provide evidence that photodegradation plays a role in more than just semi-arid and arid ecosystems.

Direct/Indirect Photolysis-

Photolysis, is the breakdown of organic material by solar radiation, and an overall an important actor of decomposition (Nagy *et al.* 1982). Photolysis can be both direct or indirect, and the mechanisms behind both are not yet completely understood. With direct photolysis, the solar radiation acts directly on the substrates (lignin, cellulose, hemicellulose) of the plant

litter with no intervention from any other chemical components. In comparison, with indirect photolysis, photo-synthesizers within the plant absorb the solar radiation and transfer it to other molecules ($\bullet\text{OH}$, $^1\text{O}_2$, H_2O_2 , Organic Reactive Intermediates) These molecules then go on to break down the substrates of the plant litter.

Studies have supported both direct and indirect photodegradation of plant material, resulting in the overall mechanisms of photolysis remaining unknown. Several studies have seen litter lignin levels decrease, along with other constituents, when exposed to solar radiation (Rozema *et al.* 1997; Day *et al.* 2007; Henry *et al.* 2008; Austin and Ballaré 2010). These studies appear to show that direct photolysis is the primary mechanism involved in photodegradation. However, there have been other studies that saw decreases in cellulose but not lignin when exposed to solar radiation (Brandt *et al.* 2007, 2010). This would likely be attributed to indirect photolysis.

Cell-Wall Chemistry-

The secondary cell wall of plants is composed of three primary constituents; hemicellulose, cellulose and lignin. Of the three, cellulose makes up the majority. Cellulose is a β -1,4 –linked glucose polysaccharide. Cellulose microfibrils are hydrophobic and also help to protect litter biomass from being acted upon by decomposition, due to its recalcitrance (Somerville *et al.*, 2006). Hemicellulose, is the least common of the three cell wall constituents and is more easily acted upon by decomposition. Hemicellulose chains are thought to combine with cellulose fibrils to form cross-links that provide extra rigidity to the cell wall. The final of the three primary cell wall constituents is lignin, and it is the second most common cell wall

constituent following cellulose. Lignin encases the other cell wall constituents and becomes a major source of recalcitrance. Along with the structural integrity that lignin provides, it also provides mechanical and elastic support and creates a chemical barrier which limits the influence of microbial pathogens (Davison, 2013). Besides the three primary polymers, there are other components of the cell wall including protein, ash, etc. All of these cell-wall components along with the primary constituents can add to the overall recalcitrance of the cell wall via cross-linking and the forming of a matrix that is resistant to both chemical and biological degradation. Overall, concentrations of these cell wall constituents vary greatly depending on species. These variations can result in varying structural makeup of plant material and how the plant litter reacts to decomposition processes.

Lignin-

Lignin is an aromatic compound within the cell wall of plants. Lignin provides extra rigidity along with making the cell walls impervious to water (Whetten *et al.* 1995). Behind carbon, lignin is the most abundant terrestrial biopolymer and accounts for approximately 30% or organic carbon within the biosphere (Boerjan *et al.* 2003). The quantity and structure of lignin varies between taxa, species, and cells. This is a result of being influenced through development or as the result of environmental cues (Campbell *et al* 1996).

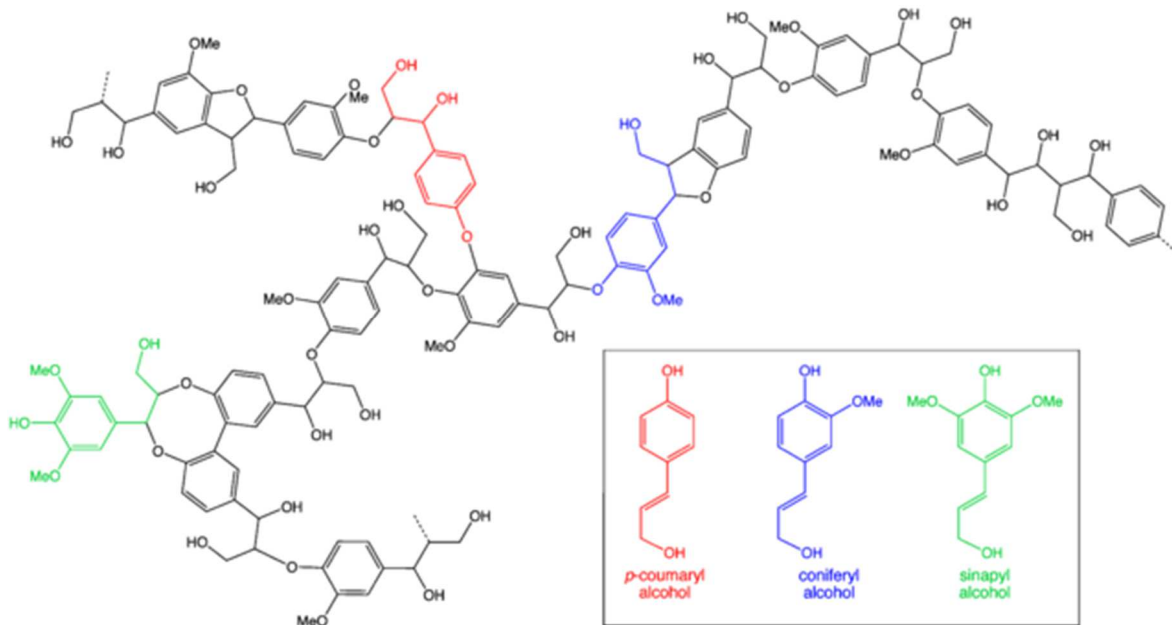


Figure 2: Structure of lignin. Credit: *Lignin*: from Wikimedia Commons

The main lignin biosynthetic pathway, produces three different hydroxycinnamyls, also known as monolignols. The three monolignols that are produced are coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol. These three different alcohols are derived from phenylalanine, which goes through a multistep process (Whetten *et al.* 1995). When these monolignols are incorporated into the lignin polymer, they are referred to as guaiacyl (G-), syringyl (S-) and p-hydroxyphenyl (H-) lignin units (Boerjan 2003). The levels of these three lignin units, and the overall amount of lignin within plant litter, can be modified through mutations that limit the production of lignin subunits. For example, *Sorghum bicolor*, has two different *bmr* mutations that cause reduction in lignin levels. Pillonel *et al.* (1991) discovered that sorghum *bmr-6* has a mutation that impacts cinnamyl alcohol dehydrogenase (CAD) activity. The mutation itself has

yet to be identified, however, plants that contain the mutation see decreased levels of lignin along with decreased fusion of cinnamylaldehydes to lignin polymers. Bout and Vermerris (2003) identified the caffeic acid *O*-methyl transferase (COMT) nonsense mutation within *bmr-12*, which results in the COMT protein being absent. This mutation results in lowered levels of syringyl (S-) lignin.

In Moorhead and Callaghan (1994) it was hypothesized that lignin is the primary cell wall constituent that is susceptible to photodegradation. Up to this point there has been little evidence that supports this hypothesis. However, Austin and Ballaré (2010) looked at lignin-free, pure cellulose substrates, and how they reacted to solar radiation. Over the duration of the experiment, the cellulose substrates were not degraded by solar radiation. However, with the addition of a lignin solution to the cellulose substrates, photodegradation increased. Overall, these results show the need for continued research in order to understand the mechanisms of photolysis.

Surface Albedo-

Surface albedo, also known as surface reflectance, is the amount of energy that is reflected by a surface. Natural surfaces have a large range when it comes to the percentage of solar radiation that they reflect. For example, organically rich (dark) soils reflect approximately 2% of ultraviolet radiation, while snow can reflect up to 94% (Correa and Ceballos 2008; Chadysiene and Girgzdys 2010). This large range when it comes to the albedo of natural surfaces supports the assumption that surface reflectance likely plays a role in photodegradation. In Rozema *et al.* (1999) soil reflectivity was mentioned as a possible driver

for photodegradation. It was hypothesized that sandy soils would increase albedo of a natural surface and therefore would result in increased photodegradation in adjacent litter. In King *et al.* (2012) a similar hypothesis was made pertaining to snow. It was hypothesized that since snow is highly reflective, that it would increase the rate at which photodegradation occurs in adjacent litter. Although these hypotheses have been established, they have yet to be tested, therefore leaving a void in the understanding of the role that surface albedo plays in photodegradation.

Methods

Surface Selection-

The surface albedo of soil (dark, organically rich) is approximately 2%, coarse sand (0.2-2.0 mm) is approximately 9% and snow is between 74-94% depending on age and moisture (Correa and Ceballos, 2008). Using a UV/visible spectrometer (Lambda 35, Perkin Elmer Incorporated, Waltham, MA, USA), equipped with a 50-mm machined integrating sphere (Spectralon, Perkin Elmer Incorporated, Waltham, MA, USA), reflectance of several artificial surfaces was measured in order to determine surface covers that best mimicked these natural surfaces. Measurements were taken between 280-760-nm with 10-nm scanning intervals and were compared against a NSIT-traceable standard (Labsphere USRS-99-010, Labsphere, Incorporated, North Sutton, NH, USA). It was determined that the artificial covers that best mimicked the natural surfaces, when applied to plywood, were 0.024-mm thick aluminum foil (Reynold's Wrap, Lake Forest IL, USA), flat black paint (exterior flat black, Glidden, Strongsville OH, USA) and flat white paint (exterior flat white, Glidden, Strongsville OH, USA).

Table I. Representation of surface reflectance levels through the ultraviolet (UV; 280-400 nm) and photosynthetically active radiation (PAR; 400-700 nm) spectrums of the aluminum, black and white surfaces via arrows. A upward arrow (↑) represents a percent reflectance of >80%. A downward arrow (↓) represents a percent reflectance <80%.

| | Ultraviolet Radiation | Photosynthetically Active Radiation |
|------------------|-----------------------|-------------------------------------|
| Aluminum Surface | ↑ | ↑ |
| Black Surface | ↓ | ↓ |
| White Surface | ↓ | ↑ |

The aluminum foil surface reflected between 84-85% of PAR, UV-A and UV-B. The black paint surface reflected $\approx 86\%$ of PAR and $\approx 6\%$ of both UV-A and UV-B. The white paint surface reflected between 2-3% of PAR, UV-A and UV-B.

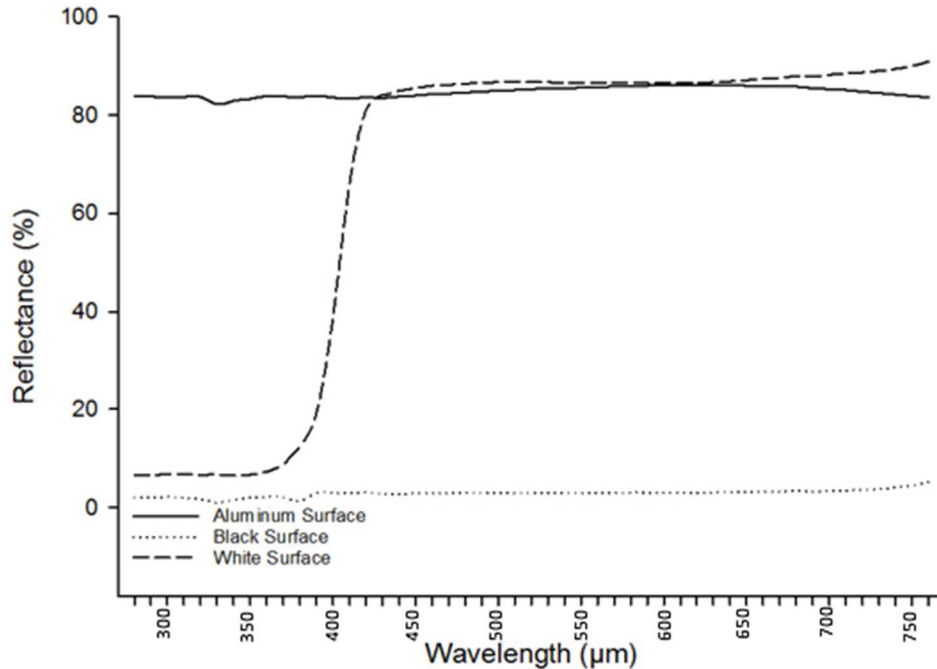


Figure 3. Surface reflectance percentages of study surfaces (aluminum, black and white) at wavelengths between 280 μm and 760 μm .

Surface Construction-

Eighteen surfaces were constructed out of plywood (1.2 m x 1.2 m) with legs that elevated the surfaces 10.2-cm above the ground in order to allow air flow under the surfaces. Each of the three artificial surface types, were applied to six plywood surfaces. Three coats of each paint type were applied and the aluminum foil was attached to the plywood using staples. Sixteen, 0.635 cm holes were drilled into each of the surfaces and 20.32 cm tall wooden dowels (0.635 cm diameter) were placed within the holes to stand the litter bags. Litter bags were placed upright in order to mimic litter stover that

remains in the field following harvest. Eighteen surfaces were placed in a SE direction on the roof of Trafton Science Center.



Figure 4: Photograph of experimental surface design.

During experimentation, surface temperatures were recorded within litterbags, on the surfaces, using a data logger (U23 Pro V2, Onset HOB0, Boume, MA, USA) with a 0.5-cm external temp/rh sensor. Measurements were taken every 5 min and averaged each hour.

Litter Collection-

Two different strains of the plant species *Sorghum bicolor*, a wild type (WT) and double mutant (DM; *bm6/bm12*) variety, were chosen based on initial litter chemistry (Table 1). The three lines, WT, *bm6* and *bm12*, were obtained from the USDA-ARS at University of Nebraska-Lincoln (Pedersen *et al.* 2006a). The *bm6/bm12* stacked hybrid 1. was crossbred at Minnesota State University-Mankato following the method described

in Pedersen *et al.* (2006b). The *Sorghum bicolor* used for this study was grown in the greenhouse at Minnesota State University- Mankato (44°08'N; 93°60'W). Seeds were planted on 1 July 2015 in 25.4-cm pots. Plants were watered regularly until reaching maturity (100 days). Once plants senesced, approximately 40 g of both WT and DM leaf litter was collected and placed into separate paper bags. Following collection, litter was cut into pieces approximately 15.24 cm in length and oven-dried at 60 °C in paper bags for >48 h, prior to being placed into litterbags.

Litterbag Preparation-

Two grams (± 2 g) of litter was placed into Aclar litterbags (Aclar Type 22A film, Proplastics, Linden, NJ, USA). Aclar was chosen due to its ability to transmit 87-89% of UV-B (280-315 nm), 89-92% of UV-A (315-400 nm) and 92-93% of photosynthetically active radiation (PAR, 400-700 nm; Krause *et al.* 1999). Each litter bag measured 45.72 cm x 17.78 cm. One-mm holes (~ 100 per bag) were added above the area containing litter using a sewing machine in order to allow air circulation.



Figure 5: Photograph of experimental bag design.

Litterbags were placed upon each surface, on the roof of Trafton Science Center at

Minnesota State University on 12 June 2016. Eight litterbags (four wild type and four double mutant) were randomly placed on each surface. The litterbags rested directly on each surface, with litter being approximately 1.27-cm above the surface. Litter stood approximately 15.24-cm tall within the litterbags.

Bag Collection-

Thirty-six litterbags (eighteen per variety, one of each variety per surface) were collected at intervals of 50, 100, 150 and 200 days (31 July, 19 September, 8 November and 28 December 2016). Following collection, the litter was removed from the litterbags and oven-dried at 60°C for >48 h prior to being weighed.

Carbon and Nitrogen Analysis-

For C and N analysis, plant material was milled to a fine powder using a Wiley Mill (1-mm mesh screen) and analysis was performed using a flash element analyzer (Leco Truspec CN analyzer, St. Joseph, MO, USA)

ANKOM Analysis

Concentrations of hemicellulose, cellulose and lignin were determined using a sequential extraction technique (Van Soest 1967). Samples were run through a Wiley Mill (1-mm mesh screen) and approximately 0.50 g (± 0.05 g) of ground litter was placed into filter bags (F57; ANKOM Technology, Macedon NY, USA). Chemical analysis was

performed using a fiber analyzer (model A200; ANKOM Technology, Macedon NY, USA) following Warnke and Ruhland (2016).

The first step was to analyze dried samples for Neutral Detergent Fiber (NDF). Samples were submerged in a NDF solution (sodium lauryl sulfate, ethylenediamine-tetraacetic disodium salt dehydrate, sodium tetraborate decahydrate, sodium phosphate dibasic, anhydrous and triethylene glycol). Heat-stable bacterial alpha amylase and sodium sulfite were added to the analyzer along with the NDF solution. The samples were incubated at 100°C for 75 min. Samples were then rinsed twice with an alpha amylase solution, once with hot dH₂O (approximately 80°C) and once with acetone. Samples were then oven-dried at 102°C for 48 h. After 48 h, samples were weighed and % NDF (cellulose, hemicellulose + lignin) was calculated.

Dried samples were then analyzed to determine Acid Detergent Fiber using an ADF solution (20g cetyl trimethylammonium bromide to 1 L 1.00 N H₂SO₄). Samples were incubated in the analyzer at 100°C for 60 minutes. Following incubation, samples were rinsed three times with hot dH₂O (approximately 80°C) and once with acetone. Samples were then oven-dried at 102°C for 48 hours. After 48 hours, samples were cooled and weighed and %ADF (cellulose + lignin) was calculated.

Acid Detergent Lignin (ADL) was determined following ADF analysis. The dried samples were immersed in 72% H₂SO₄ for three hours (agitated every 30 min) and were then rinsed using dH₂O and acetone. Samples were then oven-dried at 60°C for 48 hours prior to being weighed. Samples were then ashed in a muffle furnace at 600°C for 6 h

cooled and weighed. Cellulose concentrations were calculated as %ADF - %ADL, and hemicellulose concentrations were calculated as %NDF - %ADF.

Bulk-Soluble Phenolic Analysis-

Bulk-soluble phenolic concentrations were estimated following Ruhland *et al.* (2013). For 48 h prior to analysis, samples (1-cm²) of plant litter were placed into 15 ml of acidified methanol (MeOH-HCl-H₂O;90:1:1 v/v). Samples were then heated (60°C) for 10 min, cooled and filtered through a 60-µm mesh screen into a quartz cuvette. Bulk-soluble phenolic concentrations were estimated using a spectrometer (HP 8453; Agilent Technologies, Wilmington, DE, USA). Absorbance was measured at 300-nm (UV-B) and 375-nm (UV-A).

Data Analysis-

The Kruskal-Wallis test (IBM SPSS Statistics 25, 2017) was used to examine differences in mass loss, cell solubles, cellulose, hemicellulose, lignin, bulk-soluble phenolics, % carbon, % nitrogen and C:N ratio between treatments (aluminum foil, black paint and white paint) and time of exposure (days). A student t-test (SigmaPlot 13, 2015) was used in order to measure differences between litter types based on treatment. Differences were considered significant at the $P < 0.05$ level.

Results

Initial Litter Chemistry

Initial chemistry differed between litter types (Kruskal Wallis; $P < 0.05$). Cellulose concentrations were 1.3% higher in WT averaging 31.6% while the DM averaged 30.3% (Kruskal Wallis; $P < 0.05$). Initial lignin concentrations were 0.96% higher in the WT averaging 3.33% while the DM averaged 2.37% (Kruskal Wallis; $P < 0.05$). Cell-soluble concentrations were 1.6% lower in WT litter, averaging 43.5% compared to the DM litter, which averaged 45.1% (Kruskal Wallis; $P < 0.05$). There was no difference in initial hemicellulose concentrations between the two varieties (Kruskal Wallis; $P > 0.05$).

Table II. Initial litter chemistry of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant). Values are means of individual plants (n=9 or n=10) including standard errors. P -values were calculated using a two-tailed t-test. A sample size of 9 was used for carbon, nitrogen, C:N and Lignin:N. A sample size of 10 was used for cell solubles, hemicellulose, cellulose and lignin.

| Initial Chemistry | <i>Sorghum bicolor</i> (Wild Type) | <i>Sorghum bicolor</i> (<i>bm6/bm12</i>) | P |
|-------------------|---------------------------------------|---|--------|
| Carbon (%) | 38.48 (0.78) | 38.55 (0.46) | 0.031 |
| Nitrogen (%) | 0.96 (0.05) | 1.23 (0.07) | <0.001 |
| C:N | 40.73 (1.84) | 32.14 (1.60) | <0.001 |
| Cell Solubles (%) | 43.49 (0.35) | 45.14 (0.64) | 0.036 |
| Hemicellulose (%) | 22.81 (0.19) | 23.39 (0.25) | 0.444 |
| Cellulose (%) | 31.63 (0.27) | 30.25 (0.52) | 0.003 |
| Lignin (%) | 3.33 (0.11) | 2.37 (0.20) | 0.003 |
| Lignin:N | 2.91 (0.14) | 1.49 (0.08) | <0.001 |

Internal Litterbag Temperature

Temperatures inside litterbags were recorded for 30-d and averaged 1.8°C warmer than the measured ambient air temperature. Average temperatures, over a 24-hour period, were not different between the three surfaces (Kruskal Wallis; $P > 0.05$). Temperatures within litterbags on all three surfaces were less than 1°C warmer than the ambient temperature between 1800-0600. Daytime (0700-1700) temperatures within

litterbags were between 0.9-5.6°C warmer than ambient temperatures. Minimum daily temperatures were not different between the three surfaces (Kruskal Wallis; $P > 0.05$). Maximum daily temperatures inside litterbags were between 0.8-3.2°C higher on black surfaces than they were on the aluminum and white surfaces (Kruskal Wallis; $P < 0.05$).

Table III. Temperature (°C) above ambient of study surfaces (aluminum, black and white). Values are mean ambient temperatures subtracted from mean surface temperatures (n=84) and standard errors.

| Time | Temperature (°C) | | |
|----------------|------------------|-------------|-------------|
| | Aluminum | Black | White |
| 12 AM | 0.63 (0.03) | 0.56 (0.05) | 0.49 (0.10) |
| 1 AM | 0.60 (0.04) | 0.60 (0.06) | 0.49 (0.10) |
| 2 AM | 0.75 (0.05) | 0.68 (0.08) | 0.70 (0.12) |
| 3 AM | 0.69 (0.05) | 0.55 (0.08) | 0.67 (0.12) |
| 4 AM | 0.70 (0.04) | 0.58 (0.09) | 0.69 (0.14) |
| 5 AM | 0.68 (0.04) | 0.61 (0.09) | 0.67 (0.16) |
| 6 AM | 0.71 (0.04) | 0.77 (0.11) | 0.85 (0.20) |
| 7 AM | 1.07 (0.07) | 1.53 (0.12) | 1.08 (0.21) |
| 8 AM | 2.65 (0.08) | 3.33 (0.12) | 2.30 (0.19) |
| 9 AM | 3.74 (0.10) | 4.84 (0.14) | 3.33 (0.20) |
| 10 AM | 4.18 (0.14) | 5.57 (0.21) | 3.78 (0.19) |
| 11 AM | 4.01 (0.20) | 5.33 (0.25) | 3.66 (0.20) |
| 12 PM | 3.94 (0.22) | 5.47 (0.27) | 3.80 (0.25) |
| 1 PM | 3.54 (0.18) | 5.27 (0.24) | 3.92 (0.22) |
| 2 PM | 2.97 (0.15) | 4.32 (0.20) | 3.55 (0.21) |
| 3 PM | 2.42 (0.15) | 3.01 (0.17) | 2.62 (0.20) |
| 4 PM | 1.90 (0.09) | 2.29 (0.11) | 1.93 (0.14) |
| 5 PM | 0.92 (0.04) | 1.11 (0.07) | 0.97 (0.08) |
| 6 PM | 0.57 (0.02) | 0.59 (0.05) | 0.68 (0.07) |
| 7 PM | 0.44 (0.03) | 0.42 (0.05) | 0.49 (0.07) |
| 8 PM | 0.49 (0.03) | 0.47 (0.05) | 0.57 (0.07) |
| 9 PM | 0.65 (0.05) | 0.64 (0.06) | 0.61 (0.09) |
| 10 PM | 0.49 (0.02) | 0.50 (0.04) | 0.44 (0.08) |
| 11 PM | 0.68 (0.04) | 0.67 (0.06) | 0.49 (0.08) |
| Average | 1.67 (0.29) | 2.11 (0.42) | 1.65 (0.28) |

Mass Loss

Double-mutant litter lost 5% more mass than did the WT after 200-d, with WT losing an average of 47.5% of initial mass, and DM losing an average of 52.6% across all surface types. After 50-d, WT litter on the aluminum and black surfaces lost 4-5% more mass than that on the white surfaces (Kruskal Wallis; $P < 0.05$). The DM litter on the

aluminum surface lost 6.5% more mass than the white surface after 50-d and 3.1% more than the black surface after 200-d (Kruskal Wallis; $P < 0.05$). There were no other effects of surface reflectance on mass loss.

Table IV. Mass remaining of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) after four collections (50, 100, 150, 200 days) studied on three varying surface types (aluminum, black, white). Values are means of individual plants including standard errors, $n \geq 5$ for WT litter and $n \geq 4$ for DM litter.

| Species | Time (Days) | % Mass Remaining | | |
|---|-------------|------------------|--------------|--------------|
| | | Aluminum | Black | White |
| <i>Sorghum bicolor</i> (Wild Type) | 50 | 80.98 (1.43) | 81.62 (1.30) | 85.64 (1.15) |
| | 100 | 66.47 (1.94) | 68.23 (1.97) | 67.65 (2.27) |
| | 150 | 56.53 (1.52) | 58.40 (1.10) | 57.31 (2.63) |
| | 200 | 52.84 (1.24) | 52.25 (1.72) | 52.49 (1.36) |
| <i>Sorghum bicolor</i> (Double Mutant) | 50 | 74.00 (1.98) | 76.85 (1.43) | 80.47 (1.24) |
| | 100 | 57.71 (3.10) | 60.95 (0.97) | 62.93 (2.02) |
| | 150 | 48.83 (1.87) | 50.46 (1.63) | 52.44 (1.71) |
| | 200 | 45.52 (0.39) | 47.83 (0.52) | 48.61 (1.29) |

Carbon and Nitrogen Dynamics

Initial carbon concentrations were not different between the WT and DM litter (Kruskal Wallis; $P > 0.05$; Table 2). There were no differences between initial and final carbon concentrations of the WT after 200-d (Kruskal Wallis; $P > 0.05$). There was a decrease in carbon concentration of 2-3% between the initial and final collections of DM litter (Kruskal Wallis; $P > 0.05$). However, there were no differences in carbon concentrations between the three surface types following the final collection of either litter type and values ranged from 36-38% for WT and 35-37% for DM (Kruskal Wallis; $P > 0.05$).

Table V. Percent carbon of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) initially and after the final collection (200 days), studied on three varying surface types (aluminum, black, white). Values are means of individual plants including standard errors ($n=3$).

| Species | Collection (Days) | % Carbon | | |
|---|-------------------|--------------|--------------|--------------|
| | | Aluminum | Black | White |
| <i>Sorghum bicolor</i> (Wild Type) | Initial (0 Days) | 37.49 (0.44) | 40.09 (1.71) | 37.70 (1.47) |
| | Final (200 Days) | 37.80 (2.10) | 37.46 (0.44) | 36.56 (1.04) |
| <i>Sorghum bicolor</i> (Double Mutant) | Initial (0 Days) | 38.79 (0.47) | 38.91 (0.39) | 37.96 (1.38) |
| | Final (200 Days) | 35.31 (1.27) | 36.08 (1.30) | 36.05 (0.75) |

Initial nitrogen concentrations were 0.27% higher in DM than they were in WT (Kruskal Wallis; $P < 0.05$). The nitrogen concentration of the WT, on the white surface, was 0.35% higher than the initial value after 200-d (Kruskal Wallis; $P < 0.05$).

Table VI. Percent nitrogen of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) initially and after the final collection (200 days), studied on three varying surface types (aluminum, black, white). Values are means of individual plants including standard errors (n=3).

| Species | Collection (Days) | % Nitrogen | | |
|---|-------------------|-------------|-------------|-------------|
| | | Aluminum | Black | White |
| <i>Sorghum bicolor</i> (Wild Type) | Initial (0 Days) | 0.88 (0.04) | 1.02 (0.12) | 0.98 (0.09) |
| | Final (200 Days) | 0.90 (0.05) | 1.03 (0.13) | 1.31 (0.06) |
| <i>Sorghum bicolor</i> (Double Mutant) | Initial (0 Days) | 1.25 (0.12) | 1.32 (0.11) | 1.11 (0.14) |
| | Final (200 Days) | 1.11 (0.11) | 1.01 (0.17) | 1.03 (0.13) |

Double-mutant litter initially had a lower C:N ratio in comparison to WT (Kruskal Wallis; $P < 0.05$). The C:N ratio of the WT litter on the white surface was lower after 200-d, in comparison to the initial value (Kruskal Wallis; $P < 0.05$).

Table VII. Carbon:Nitrogen ratio of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) initially and after the final collection (200 days), studied on three varying surface types (aluminum, black, white). Values are means of individual plants including standard errors (n=3).

| Species | Collection (Days) | Carbon:Nitrogen | | |
|---|-------------------|-----------------|--------------|--------------|
| | | Aluminum | Black | White |
| <i>Sorghum bicolor</i> (Wild Type) | Initial (0 Days) | 42.78 (2.16) | 40.04 (3.24) | 39.36 (4.70) |
| | Final (200 Days) | 42.40 (4.39) | 37.34 (4.13) | 28.00 (0.95) |
| <i>Sorghum bicolor</i> (Double Mutant) | Initial (0 Days) | 33.59 (2.77) | 29.93 (2.92) | 34.91 (2.86) |
| | Final (200 Days) | 32.49 (3.66) | 38.53 (8.26) | 36.34 (5.43) |

Fiber Chemistry

Hemicellulose fractions were 34.5-37.6% and 24.1-27.4% of initial for the WT and DM, respectively, after 200-d. Hemicellulose declined most rapidly during the first 50-d of experimentation, with a consistent reduction in the amount lost over the remainder of the experiment. Following the 50-d collection, the hemicellulose fraction of the WT litter on the aluminum surfaces (59.5%) was between 10-14% lower than both the black (73.6%) and white (69.5%) surfaces (Kruskal Wallis; $P < 0.05$). After the

100-d and 150-d collections, the hemicellulose fraction of the WT litter on the aluminum surface was lower, averaging 41.9% and 37.1%, respectively, in comparison to that collected from the black surface which averaged 51.9% and 43.8% (Kruskal Wallis; $P < 0.05$). Following the 50-d collection, hemicellulose fractions of the DM litter on the aluminum (46.7%) surfaces were between 10-17% lower than both the black (56.5%) and white (63.1%) surfaces (Kruskal Wallis; $P < 0.05$). Similarly, after the 150-d collection, hemicellulose fractions from litter on the aluminum surface (22.6%) were between 5-9% lower in comparison to both the black (31.3) and white (27.9%) surfaces (Kruskal Wallis; $P < 0.05$).

Table VIII. Hemicellulose concentrations of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) remaining after four collections (50, 100, 150, 200 days) studied on three varying surface types (aluminum, black, white). Values are means of individual plants including standard errors, $n \geq 5$ for WT litter and $n \geq 4$ for DM litter.

| Species | Time (Days) | Hemicellulose Concentration % | | |
|---|-------------|-------------------------------|--------------|--------------|
| | | Aluminum | Black | White |
| <i>Sorghum bicolor</i> (Wild Type) | 50 | 59.50 (2.40) | 73.60 (2.22) | 69.50 (2.69) |
| | 100 | 41.88 (1.63) | 51.88 (3.30) | 48.21 (3.07) |
| | 150 | 37.10 (2.07) | 43.84 (1.35) | 39.78 (3.10) |
| | 200 | 35.02 (1.67) | 37.63 (1.14) | 34.46 (1.26) |
| <i>Sorghum bicolor</i> (Double Mutant) | 50 | 46.74 (3.01) | 56.48 (1.12) | 63.06 (6.28) |
| | 100 | 30.18 (2.48) | 34.06 (1.50) | 33.69 (2.82) |
| | 150 | 22.55 (1.78) | 31.27 (1.22) | 27.87 (1.05) |
| | 200 | 24.09 (1.96) | 26.43 (1.92) | 27.42 (1.08) |

Cellulose fractions were 64.0-70.1% and 64.9-70.8% of initial for the WT and DM, respectively, following the 200-d experimentation period. Cellulose fractions declined steadily over the duration of the experiment. After 200-d, cellulose fractions of litter collected from the aluminum surfaces were approximately 7% higher at 70.1% and 69.2% for WT and DM, respectively, in comparison to 64.0% and 64.9% from that on the black surfaces (Kruskal Wallis; $P < 0.05$).

Table IX. Cellulose concentrations of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) remaining after four collections (50, 100, 150, 200 days) studied on three varying surface types (aluminum, black, white). Values are means of individual plants including standard errors, $n \geq 5$ for WT litter and $n \geq 4$ for DM litter.

| Species | Time (Days) | Cellulose Concentration % | | |
|---|-------------|---------------------------|--------------|--------------|
| | | Aluminum | Black | White |
| <i>Sorghum bicolor</i> (Wild Type) | 50 | 83.51 (3.98) | 88.53 (2.76) | 89.64 (3.94) |
| | 100 | 85.63 (3.94) | 83.53 (3.94) | 82.19 (3.55) |
| | 150 | 69.37 (3.42) | 71.85 (3.41) | 74.51 (4.74) |
| | 200 | 70.11 (1.69) | 63.96 (2.18) | 68.45 (5.15) |
| <i>Sorghum bicolor</i> (Double Mutant) | 50 | 85.25 (4.32) | 92.20 (5.52) | 88.73 (3.87) |
| | 100 | 78.14 (5.47) | 79.62 (5.03) | 78.92 (3.57) |
| | 150 | 74.77 (5.18) | 67.60 (3.33) | 74.10 (4.71) |
| | 200 | 69.20 (1.61) | 64.85 (0.96) | 70.84 (2.71) |

Lignin fractions remained at levels above 100% for the duration of the experiment. There were no differences found between litter collected from the three different surface types (Kruskal Wallis; $P > 0.05$). No trends were apparent in lignin fractions.

Table X. Lignin concentrations of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) remaining after four collections (50, 100, 150, 200 days) studied on three varying surface types (aluminum, black, white). Values are means of individual plants including standard errors, $n \geq 5$ for WT litter and $n \geq 4$ for DM litter.

| Species | Time (Days) | Lignin Concentration % | | |
|---|-------------|------------------------|----------------|----------------|
| | | Aluminum | Black | White |
| <i>Sorghum bicolor</i> (Wild Type) | 50 | 198.05 (23.03) | 163.72 (13.24) | 195.01 (34.41) |
| | 100 | 151.36 (30.38) | 147.39 (19.80) | 138.26 (18.56) |
| | 150 | 153.79 (20.15) | 151.87 (31.40) | 113.81 (25.57) |
| | 200 | 128.87 (19.09) | 152.50 (13.96) | 137.88 (18.12) |
| <i>Sorghum bicolor</i> (Double Mutant) | 50 | 164.98 (38.98) | 147.34 (37.94) | 181.68 (25.80) |
| | 100 | 143.98 (25.50) | 168.53 (34.93) | 206.74 (55.76) |
| | 150 | 112.45 (13.22) | 148.39 (24.45) | 133.26 (40.70) |
| | 200 | 117.57 (26.45) | 152.48 (33.72) | 179.88 (38.46) |

Bulk-Soluble Phenolics

There were no differences in bulk-soluble phenolic concentrations for either litter type between the three surfaces at 300 nm or 375 nm (Kruskal Wallis; $P > 0.05$). Although differences were not seen between the surface types, bulk-soluble phenolic concentrations decreased between the 50-d and 200-d collection. Following the 50-d

collection, WT bulk-soluble phenolic concentrations were between 0.061-0.071 $A_{300} \text{ cm}^{-2}$ and 0.026-0.033 $A_{375} \text{ cm}^{-2}$ depending on surface type. After the 200-d collection, concentrations decreased to between 0.041-0.057 $A_{300} \text{ cm}^{-2}$ and 0.014-0.022 $A_{375} \text{ cm}^{-2}$. The DM litter demonstrated similar results, with initial concentrations being between 0.061-0.070 $A_{300} \text{ cm}^{-2}$ and 0.25-0.32 $A_{375} \text{ cm}^{-2}$. After the 200 d collection, concentrations decreased to between 0.036-0.049 $A_{300} \text{ cm}^{-2}$ and 0.007-0.011 $A_{375} \text{ cm}^{-2}$. Bulk-soluble phenolic concentrations were not different between the WT and DM litter (Kruskal Wallis; $P > 0.05$).

Table XI. Bulk-soluble phenolics ($A_{300} \text{ cm}^{-2}$) concentrations of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) remaining after four collections (50, 100, 150, 200 days) studied on three varying surface types (aluminum, black, white). Values are means of individual plants including standard errors, $n \geq 5$ for WT litter and $n \geq 4$ for DM litter.

| Species | Time (Days) | Bulk-Soluble Phenolics ($A_{300} \text{ cm}^{-2}$) | | |
|---|-------------|--|---------------|---------------|
| | | Aluminum | Black | White |
| <i>Sorghum bicolor</i> (Wild Type) | 50 | 0.061 (0.009) | 0.066 (0.009) | 0.071 (0.022) |
| | 100 | 0.043 (0.005) | 0.056 (0.023) | 0.044 (0.011) |
| | 150 | 0.035 (0.006) | 0.037 (0.006) | 0.036 (0.005) |
| | 200 | 0.042 (0.007) | 0.041 (0.007) | 0.057 (0.010) |
| <i>Sorghum bicolor</i> (Double Mutant) | 50 | 0.061 (0.009) | 0.070 (0.015) | 0.061 (0.007) |
| | 100 | 0.052 (0.011) | 0.030 (0.008) | 0.031 (0.008) |
| | 150 | 0.043 (0.004) | 0.025 (0.005) | 0.048 (0.023) |
| | 200 | 0.049 (0.010) | 0.054 (0.013) | 0.036 (0.049) |

Table XII. Bulk-soluble phenolics ($A_{375} \text{ cm}^{-2}$) concentrations of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) remaining after four collections (50, 100, 150, 200 days) studied on three varying surface types (aluminum, black, white). Values are means of individual plants including standard errors, $n \geq 5$ for WT litter and $n \geq 4$ for DM litter.

| Species | Time (Days) | Bulk-Soluble Phenolics ($A_{375} \text{ cm}^{-2}$) | | |
|---|-------------|--|---------------|---------------|
| | | Aluminum | Black | White |
| <i>Sorghum bicolor</i> (Wild Type) | 50 | 0.026 (0.007) | 0.033 (0.005) | 0.033 (0.013) |
| | 100 | 0.013 (0.002) | 0.026 (0.014) | 0.018 (0.007) |
| | 150 | 0.016 (0.004) | 0.015 (0.004) | 0.014 (0.004) |
| | 200 | 0.014 (0.006) | 0.014 (0.004) | 0.022 (0.007) |
| <i>Sorghum bicolor</i> (Double Mutant) | 50 | 0.032 (0.007) | 0.035 (0.007) | 0.025 (0.003) |
| | 100 | 0.021 (0.008) | 0.011 (0.004) | 0.012 (0.003) |
| | 150 | 0.016 (0.003) | 0.011 (0.003) | 0.017 (0.008) |
| | 200 | 0.011 (>0.001) | 0.017 (0.007) | 0.007 (0.001) |

Discussion

In terrestrial ecosystems, temperature has been found to regulate the rate at which litter decomposes (Aerts 1997). The maximum daily temperatures within the litterbags, on the black surfaces, were 0.8-3.2°C warmer than those on the aluminum and white surfaces. The increased temperature is believed to be responsible for the higher mass loss that was seen with the litter collected from the black surfaces. Other studies have had similar results, losing more mass as temperatures increased (Hornsby *et al.* 1995; Hobbie 1996; Salah *et al.* 2010).

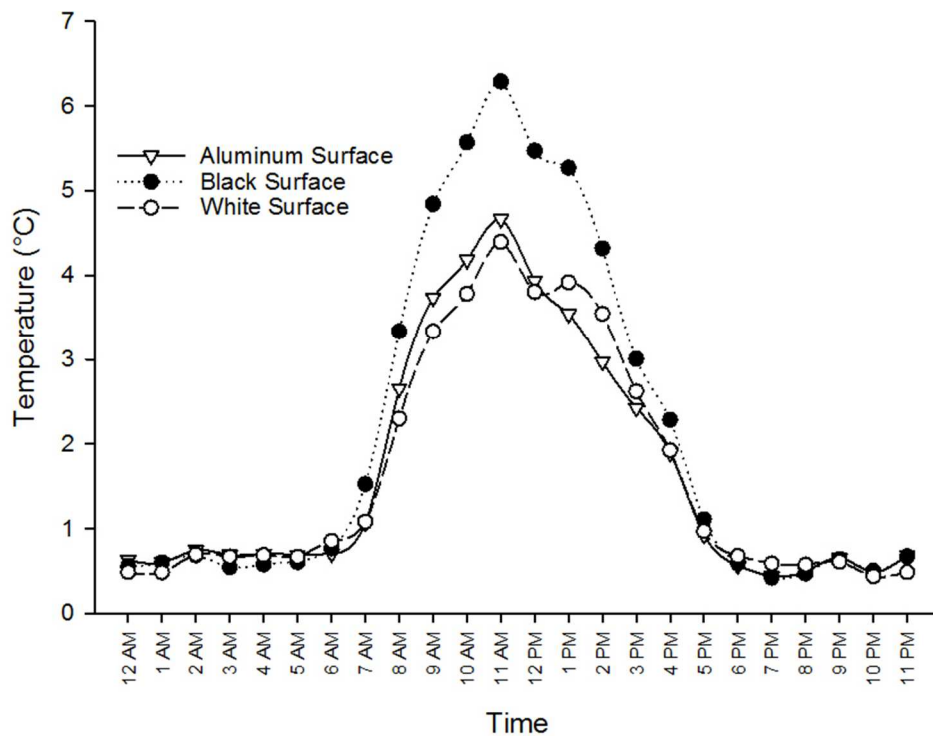


Figure 6. Temperature (°C) above ambient of study surfaces (aluminum, black and white), every hour, over 24 hours.

Salah *et al.* (2010) found that a temperature increase of 3°C increased decomposition significantly across 65% of the data collected. A possible explanation for this pattern is an increase in microbial activity as a result of the temperature increase. Witkamp (1966) found that microbial populations and respiration rates of the litter increased when temperatures

increased. Therefore, microbial decomposition likely contributed to the higher than expected mass loss of the litter collected from the black surface. Had the temperatures been constant across the surfaces, the mass loss values from the litter on the black surfaces would have likely been similar to the litter from the white surfaces.

Carbon concentrations did not show any support for the hypotheses as there were no significant differences between the three different surface types (Kruskal Wallis, $P > 0.05$)

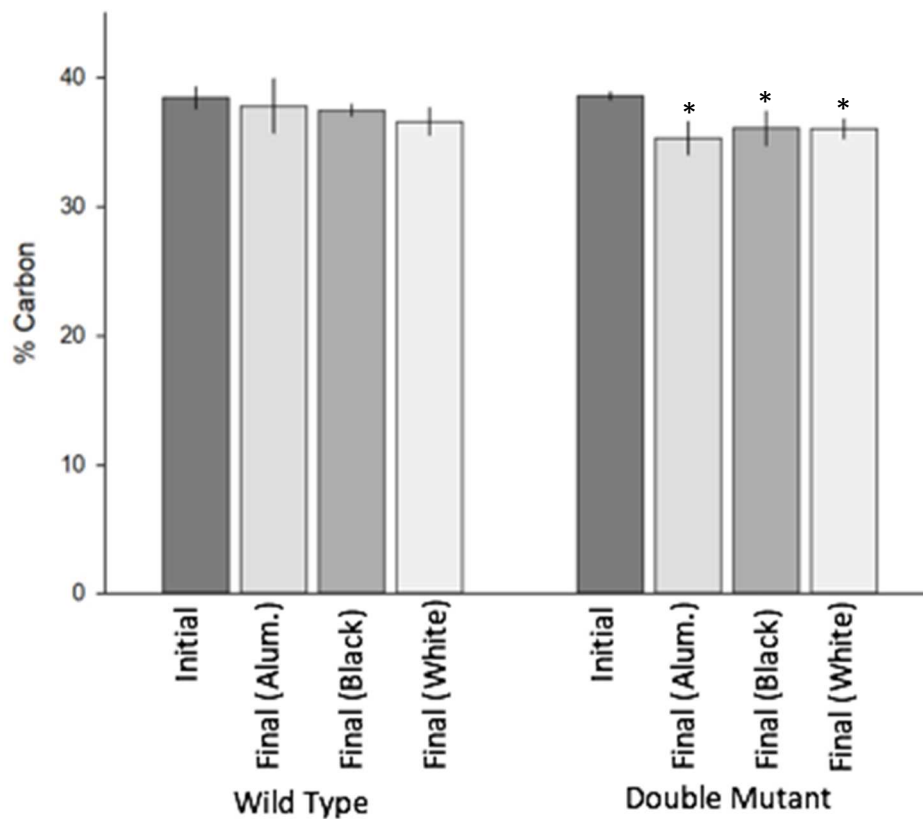


Figure 7. Initial percent carbon for *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) and final values based on litter collected from varying study surfaces (aluminum, black and white). Values are means of individual plants (n=9). Vertical error bars represent $\pm 1SE$ and an *asterisk* (*) indicates a significant difference between initial values and the other surfaces ($P < 0.05$).

Low nitrogen concentrations limit the amount of microbial decomposition acting on the litter due to microbes having to access N from outside of the litter. Nitrogen concentrations were generally not effected by the varying surface types between the initial and final

collections. An increase in nitrogen in the WT litter collected from the white surface was the only significant change. This nitrogen immobilization provides evidence that microbial decomposition likely played a significant role in the decomposition of this litter. This increase is likely a result of the conversion of inorganic nitrogen into organic nitrogen via microorganisms.

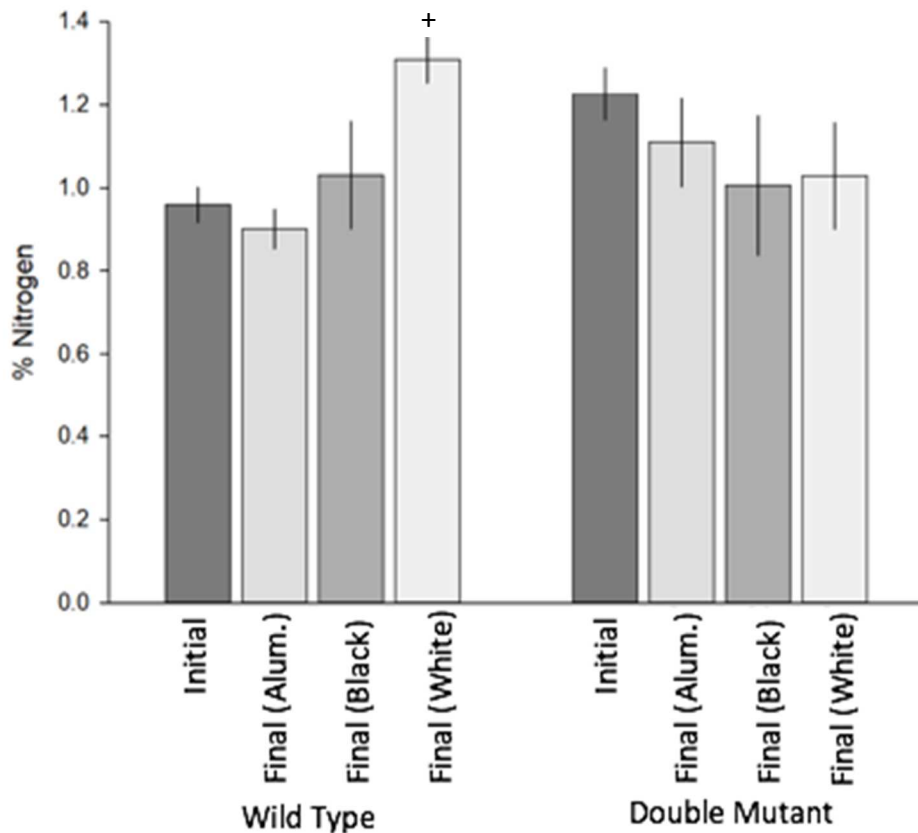


Figure 8. Initial percent nitrogen for *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) and final values based on litter collected from varying study surfaces (aluminum, black and white). Values are means of individual plants (n=9). Vertical error bars represent $\pm 1SE$ and an asterisk (+) indicates a significant difference between initial and final value, along with between white surface and aluminum and black surfaces ($P < 0.05$).

Carbon and nitrogen concentrations appeared to have an effect on the rate of decomposition as the WT litter, that had a higher C:N ratio, decomposed at a slower rate than the DM litter.

Previous studies have found that high initial C:N slows down the rate at which litter decomposes due to being nitrogen limited (Brandt *et al.* 2010; King *et al.* 2012; Day *et al.* 2015; Huang *et al.* 2017). Only having data from initial and final samples limited our ability to fully

understand the dynamics of carbon and nitrogen throughout the duration of this project. For future studies having data from each collection would help in better understanding the role that microbial decomposition played.

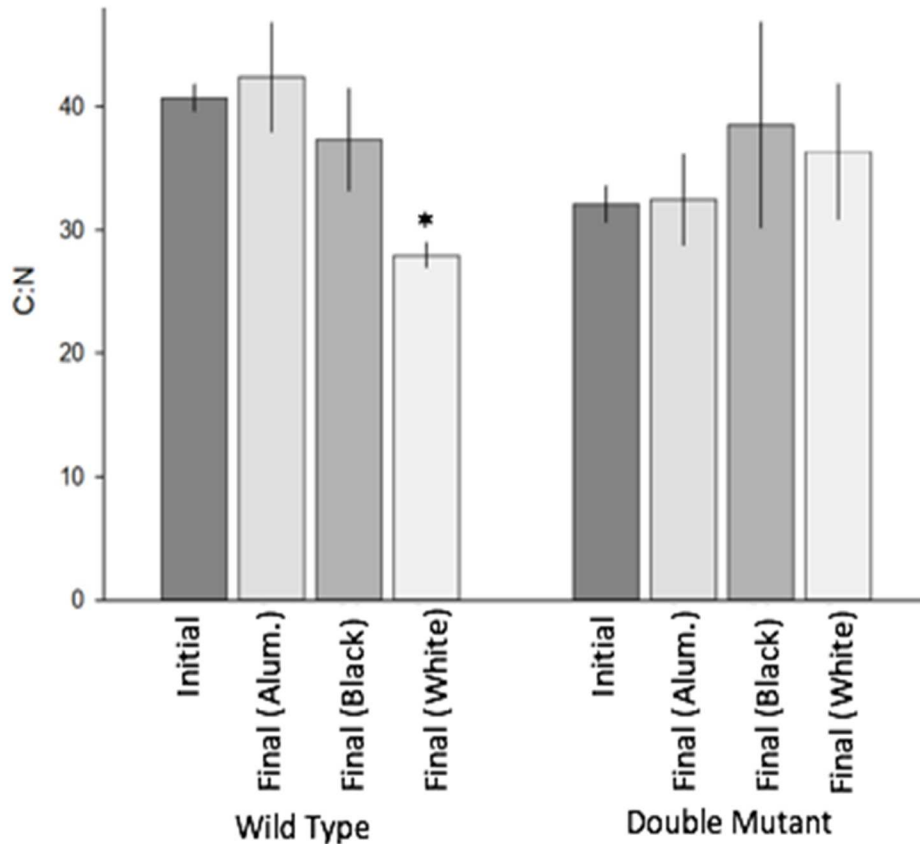


Figure 9. Initial C:N ratio for *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) and final values based on litter collected from varying study surfaces (aluminum, black and white). Values are means of individual plants (n=9). Vertical error bars represent $\pm 1SE$ and an *asterisk* (+) indicates a significant difference between initial and final value, along with between white surface and aluminum and black surfaces ($P < 0.05$).

We hypothesized that litter collected from the high albedo, aluminum surfaces ($\approx 90\%$ UV), would decompose at a faster rate than litter collected from the lower albedo white ($\approx 6\%$ UV) and black surfaces ($\approx 2\%$ UV). In support of our hypothesis, after 50-d, the WT and DM litter, collected from the aluminum surface, lost between 4-7% more mass than litter collected

from the white surfaces ($P < 0.05$). Also, after 200-d, the DM litter collected from the aluminum surface lost approximately 2% more mass than the black surface ($P < 0.05$).

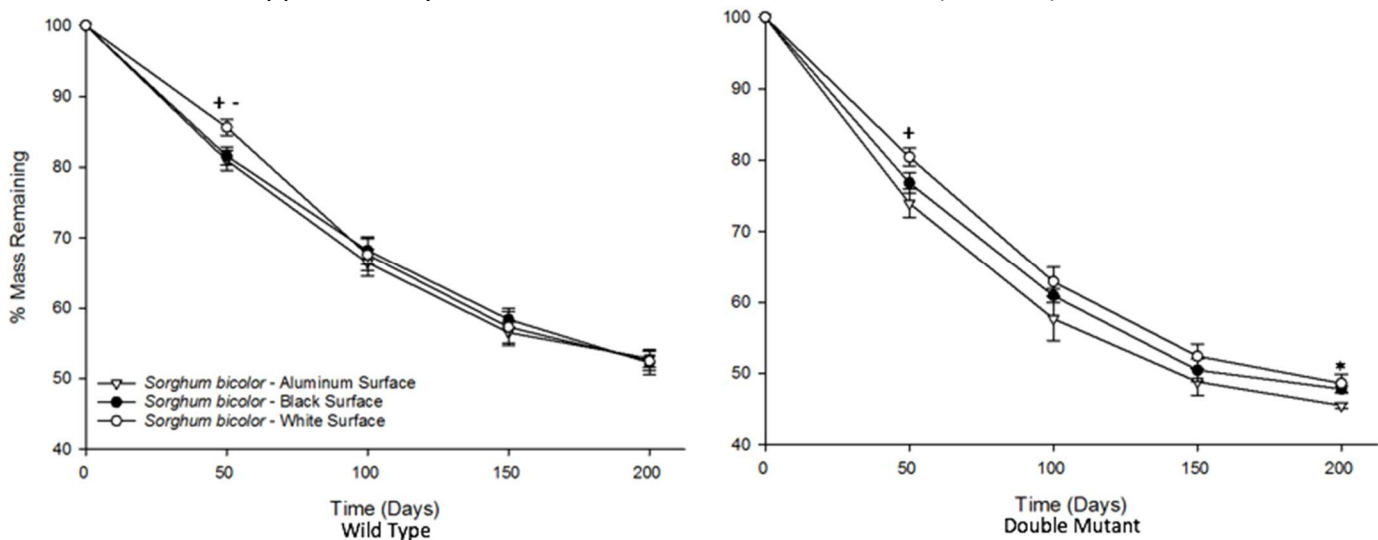


Figure 10. Mass (%) remaining over time of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) placed on varying study surfaces (aluminum, black and white). Values are means of individual plants ($n=6$). Vertical error bars represent $\pm 1SE$. An asterisk (*) indicates a significant difference between the aluminum and black surfaces ($P < 0.05$). A plus sign (+) indicates a significant difference between the aluminum and white surfaces ($P < 0.05$). A minus sign (-) indicates a significant difference between the black and white surfaces ($P < 0.05$).

In contrast to our hypothesis, after 50-d, WT litter on the black surface lost approximately 5% more mass than the white surfaces ($P > 0.05$). Results were not consistently significant, however, WT and DM litter collected from the aluminum surface consistently lost more mass over the length of the experiment. We speculate that the higher than anticipated results from the litter collected from the black surfaces came as a result of higher temperatures within the litterbags.

Increased UV-albedo had significant effects on hemicellulose fractions following the 50-d and 150-d collections. Through the use of UV-pass and UV-block filters, previous studies found that exposure to UV-radiation increased the rate at which hemicellulose is lost (Brandt *et al.* 2010; Lin & King 2014; Baker and Allison 2015). In these studies, the litter exposed to UV-radiation lost significantly more hemicellulose than the litter that was not, following each

collection. In our study we did not limit UV exposure through the use of filters, it was increased through the use of surfaces with varying albedos. The overall trends in our results were similar to previous studies, however differences were not consistently significant. After 50-d, the hemicellulose fraction remaining in the WT litter, on the aluminum surface, was 10.0% higher than the white surface and 14.1% higher than the black surface ($P < 0.05$).

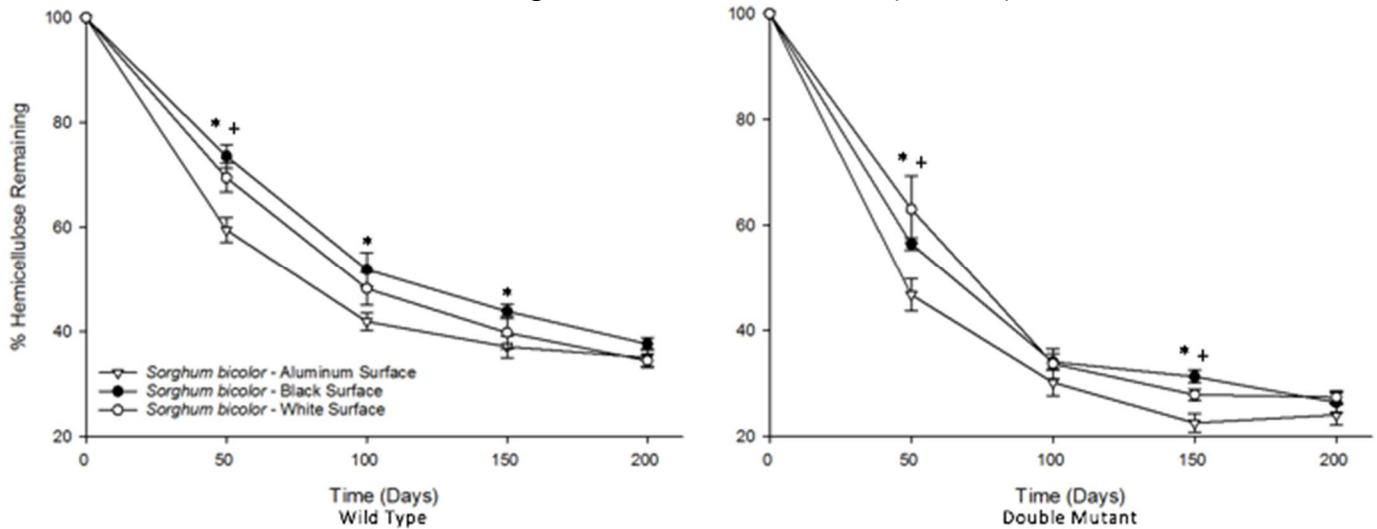


Figure 11. Hemicellulose concentrations (%) remaining over time of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) placed on varying study surfaces (aluminum, black and white). Values are means of individual plants (n=6). Vertical error bars represent $\pm 1SE$. An asterisk (*) indicates a significant difference between the aluminum and black surfaces ($P < 0.05$). A plus sign (+) indicates a significant difference between the aluminum and white surfaces ($P < 0.05$).

The hemicellulose fraction remaining in the DM litter, on the aluminum surface, was 16.3% higher than the white surface and 9.74% higher than the black surface ($P < 0.05$, Figure 9).

Similar results were also seen after 150-d, in the DM litter with the hemicellulose fraction in the litter collected from the aluminum surface being 5.32% higher than that from the white surface and 8.72% higher than the black surface ($P < 0.05$). Lin *et al.* (2015) found that the guaiacyl linkages that form cross linkages with hemicellulose are preferentially degraded when exposed to UV radiation. The breakdown of these linkages does not cause any measurable lignin loss, however, it makes hemicellulose more susceptible to photodegradation. This would explain

why we found that UV-radiation exposure played a significant role in the loss of the hemicellulose fraction, but not the lignin fraction. Our results were similar to what was seen in Lin and King (2014), who found that UV-radiation reduced losses of hemicellulose by 29% but did not significantly effect lignin concentrations. We speculate that our results were inconsistent compared to previous studies, due to litter being exposed to direct and reflected radiation. Had there been a way to limit direct radiation, then the results would have provided a better overall representation of the role that surface albedo plays in photodegradation.

Cellulose concentrations of both the WT and DM litter decreased at a consistent rate over the the length of the experiment, however there were no significant differences between the three surface types ($P > 0.05$).

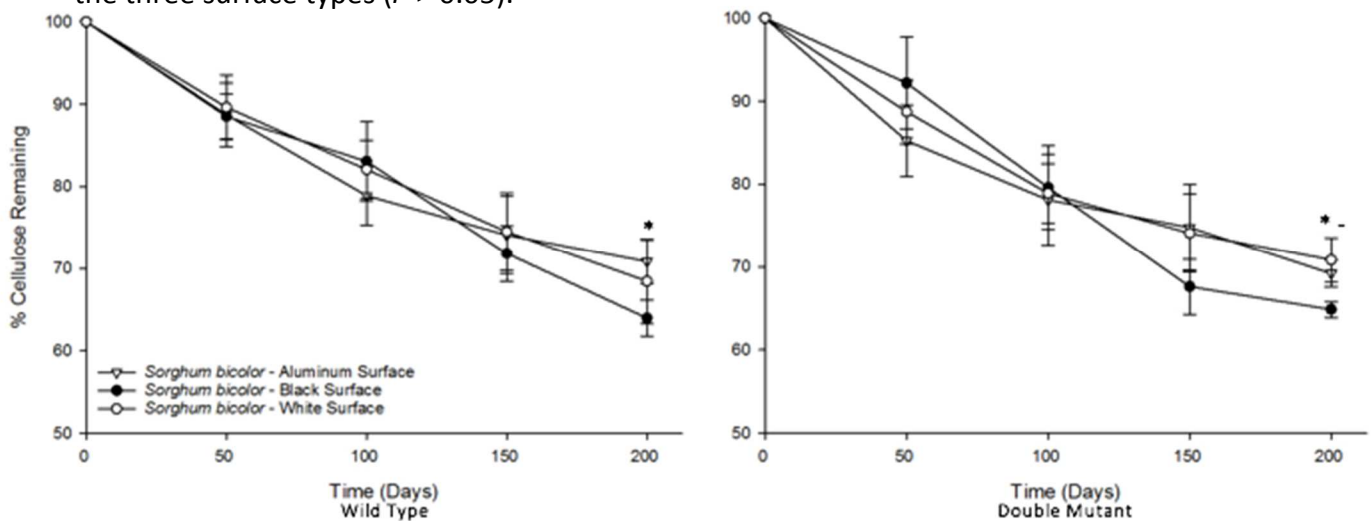


Figure 12. Cellulose concentrations (%) remaining over time of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) placed on varying study surfaces (aluminum, black and white). Values are means of individual plants ($n=6$). Vertical error bars represent $\pm 1SE$. An asterisk (*) indicates a significant difference between the aluminum and black surfaces ($P < 0.05$). A minus sign (-) indicates a significant difference between the black and white surfaces ($P < 0.05$).

Similarly, in Brandt *et al.* (2010), cellulose concentrations declined following each collection, however there were no significant differences between the different UV-block and UV-pass filters. A proportion of cellulose within the plant cell wall is free and unprotected making it

susceptible during the early phase of decomposition (Chesson 1997; De Santo *et al.* 2009). In a study done by Austin and Ballaré (2010), they found that cellulose itself is not capable of absorbing radiation and photodegrading when free of lignin. Therefore, the cellulose that is free and unprotected was likely not broken down through photodegradation, but instead through microbial decomposition. The remaining cellulose is protected by lignin, and the lignin must be broken down in order for the cellulose to be decomposed (Berg *et al.* 1982, 1984; Berg & McClaugherty 1987; Aber *et al.* 1990; Adair *et al.* 2017). Since there were no significant declines in lignin, the remaining cellulose likely was not susceptible to photodegradation. This would explain why exposure to varying levels of UV-radiation did not significantly impact the concentration of cellulose within the plant litter.

We expected that loss of the lignin fraction would have been significantly higher in litter collected from the aluminum surface due to the high surface reflectance. However, this was not the case as values remained inconsistent over the length of the experiment. The lignin fraction of both WT and DM litter remained at levels above 100% after every collection over the 200-d of the experiment. Similarly, Brandt *et al.* (2010), found lignin fractions that were above 100%. This increase in lignin percentage is believed to be the result of an increase in microbial by-products, that are not differentiated through the forage fiber technique (Couteaux *et al.* 1995; Brandt *et al.* 2010; Lin & King 2014; Bosco *et al.* 2016; Ruhland *et al.* 2018). Exposure to low-wavelength visible and UV-radiation is believed to accelerate the rate at which lignin is lost from plant litter (Rozema *et al.* 1997; Day *et al.* 2007, 2015; Henry *et al.* 2008; Austin & Ballaré 2010) Therefore, it is surprising that exposure to varying levels of UV-radiation did not impact lignin concentrations. However, with lignin concentrations being low (less than 4%), changes

may have been difficult to detect. Results in Adair *et al* (2017) indicated that the rate at which lignin photodegrades is relatively slow, at a rate of between 1.1-1.5% per year. With this study only being performed for 200-d, any changes in lignin concentrations would be extremely difficult to detect when it photodegrades at such a slow rate. Although there were no significant changes in lignin concentration, there have been several studies performed that have shown that photodegradation does increase the mass loss of lignin (Day *et al.* 2007; Henry 2008; Austin and Ballaré 2010; Austin *et al.* 2016) Therefore, the study period may be the limiting factor that does not allow detectable changes in lignin levels.

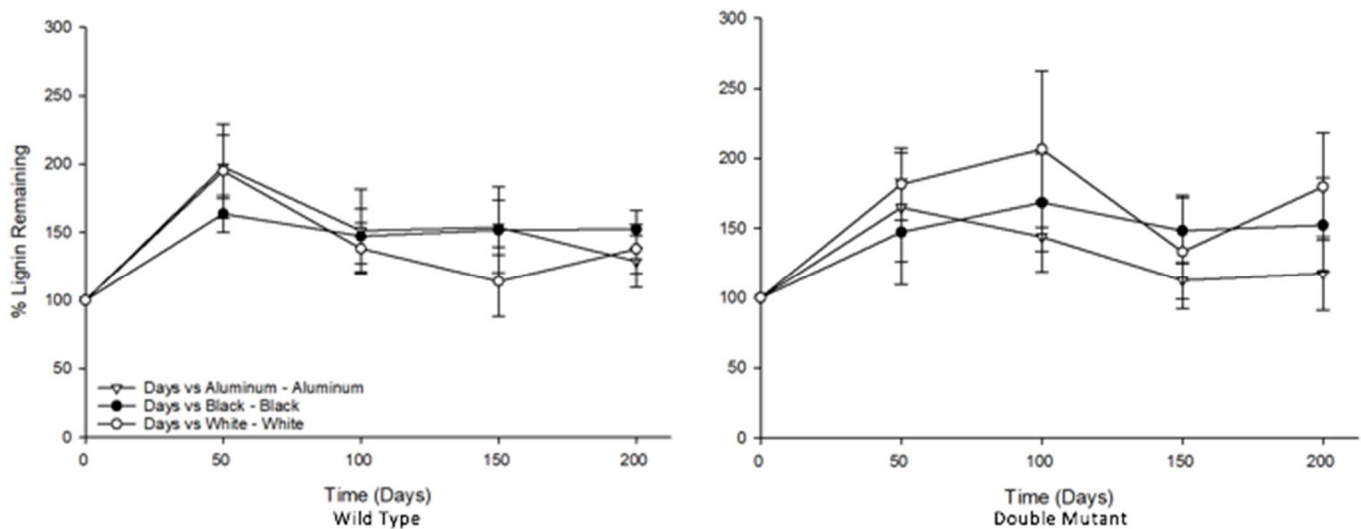


Figure 13. Lignin concentrations (%) remaining over time of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) placed on varying study surfaces (aluminum, black and white). Values are means of individual plants (n=6). Vertical error bars represent $\pm 1SE$.

Lignin results did not support our second hypothesis either, as photodegradation played a larger role in decomposition of the lower lignin, double-mutant *Sorghum bicolor* (2.37%) than the wild-type (3.33%) variety. Similarly, other recent studies have also found, that initial lignin concentration does not impact the magnitude of photodegradation (Brandt *et al.* 2010; King *et al.* 2012; Day *et al.* 2015). Although initial lignin concentrations were significantly different between the two *S. bicolor* litter varieties, they differed by less than 1% in initial lignin content,

therefore the impact that lignin had on the photodegradation process may have been limited. In Brandt *et al.* (2010), *A. gerardii* (8.1% lignin) and *B. gracilis* (6.6%) litter was used for the study and they found that photodegradation played a larger role in the decomposition of the high lignin, *A. gerardii* litter at two sites out of three. In comparison to the *S. bicolor* that we used, both of their litter types had initial lignin concentrations that were at least 3% higher. The difference in initial lignin concentrations between their two litter types was also higher, at 1.5% in comparison to 1% for ours. Although their results were not entirely consistent, they suggest that using litter with higher initial lignin concentration makes significant differences easier to detect. Lignin is assumed to be the primary compound that is susceptible to photodegradation (King *et al.* 2012). Therefore, by using a litter that has a higher percentage of initial lignin, the role of photodegradation on that litter should also be higher. If we were to have used different litter that had higher initial concentrations of lignin, the results may have better demonstrated what was hypothesized.

Bulk-soluble phenolics are believed to limit litters susceptibility to microbial decomposition and increase the susceptibility to UV photodegradation due to being strong UV absorbers (Day *et al.* 2007). Lignin itself is classified as a phenolic, however it has been hypothesized that there are other phenolics that are also photoreactive (King *et al.* 2012). Multiple recent lab studies have found that the abundance of phenolic units control the breakdown of polysaccharides and total C loss in litter (Bertrand *et al.* 2006; Grabber *et al.* 2009). By measuring bulk-soluble phenolics, we were better able to understand if they played a significant role in the rate at which litter photodegrades. Bulk-soluble phenolic concentrations

were not significantly different between the WT and DM litter at any point during our experiment ($P > 0.05$).

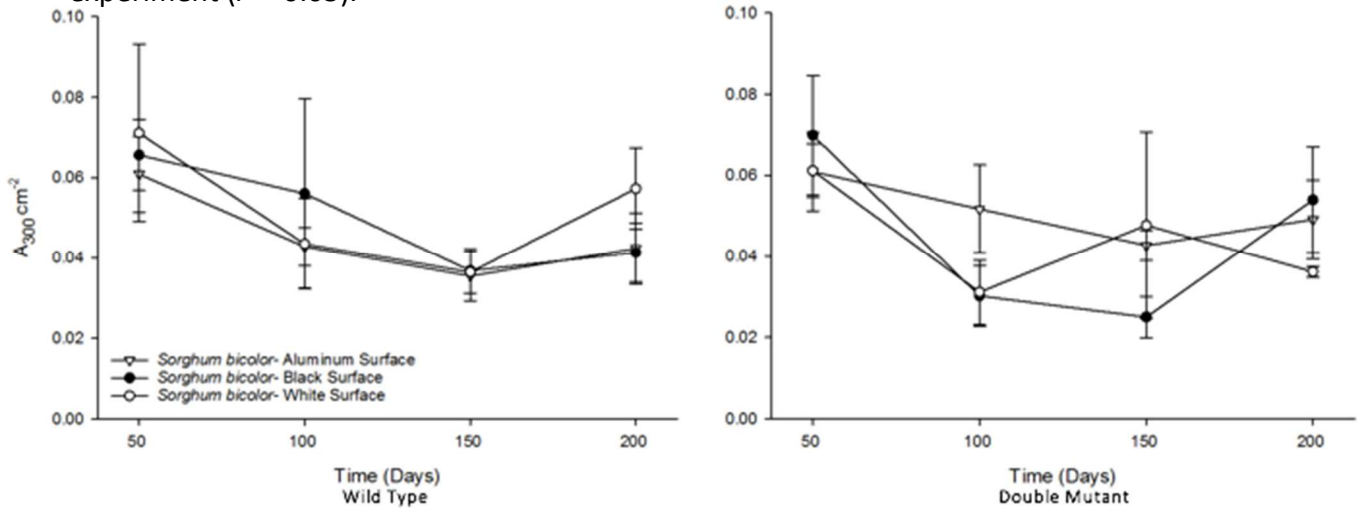


Figure 14. Bulk-soluble phenolic ($A_{300} \text{ cm}^{-2}$) concentrations remaining over time of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) placed on varying study surfaces (aluminum, black and white). Values are means of individual plants ($n=6$). Vertical error bars represent $\pm 1SE$.

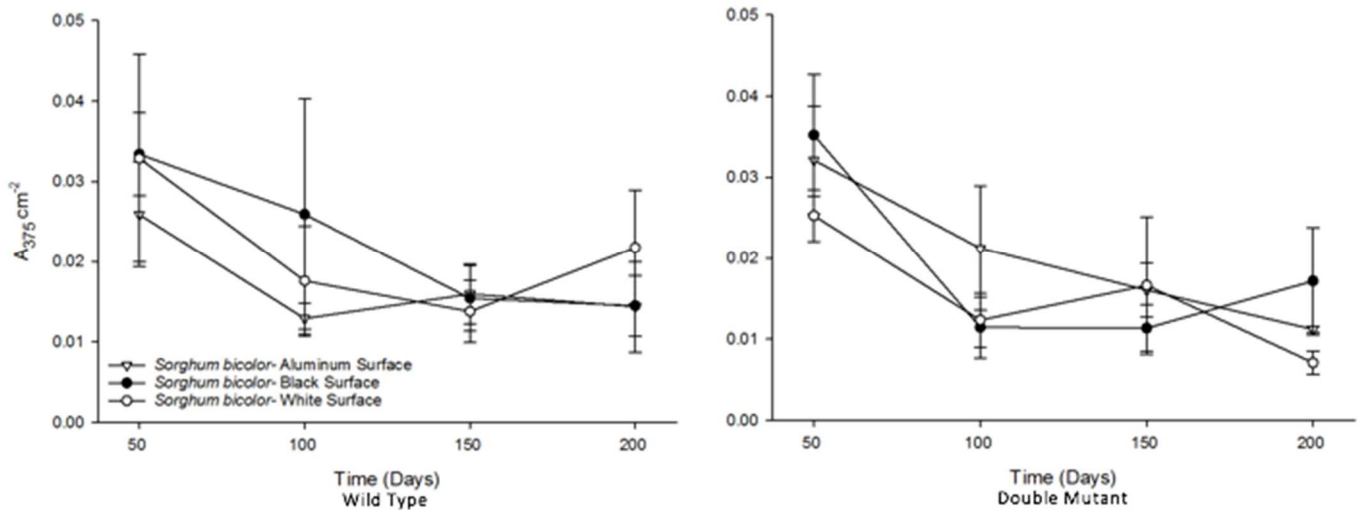


Figure 15. Bulk-soluble phenolic ($A_{375} \text{ cm}^{-2}$) concentrations remaining over time of *Sorghum bicolor* (wild type) and *Sorghum bicolor* (double mutant) placed on varying study surfaces (aluminum, black and white). Values are means of individual plants ($n=6$). Vertical error bars represent $\pm 1SE$.

Levels fluctuated over the length of the experiment, however did not demonstrate any noticeable trends. Had there been differences between the two litter types, variations in the amount of mass loss may have been attributed to initial phenolic concentrations.

Prior to this study, there has been limited research done on the role that surface albedo plays in photodegradation. Previous studies hypothesized that increased surface albedo would

increase the rate at which litter photodegrades (Rozema *et al.* 1999; King *et al.* 2012). However, these hypotheses were never tested. The results from our study were not consistently significant, however, the trends in the data appear to reveal that surface albedo plays at least a minor role in the rate at which litter photodegrades. Further research would be useful in gaining a better understanding of the role that surface albedo plays in photodegradation.

For future studies, increasing the number of replicates collected from each surface type would be beneficial. It would strengthen the overall power of the statistics and hopefully provide results that are more consistent and better support the hypothesis. Performing a similar study in a lab type setting would also be beneficial because it would eliminate several of the variables (precipitation, temperature, etc.) that likely skewed the results for this study. Finally, future studies will require a different cell wall constituent analysis technique due to the ANKOM fiber analysis technique providing consistently inaccurate results. This form of analysis should be avoided going forward.

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Appendix

| | | Collection 1 | 50 Days | | |
|----------|-------|--------------|-----------------|------------------------|-------------|
| | | Initials | Post Collection | Percent Mass Remaining | |
| Aluminum | WT22 | 2.0456 | 1.7206 | 0.841122409 | |
| | WT13 | 2.0538 | 1.5521 | 0.755721102 | |
| | WT59 | 1.9235 | 1.5759 | 0.819287757 | |
| | WT63 | 1.9472 | 1.5839 | 0.813424404 | |
| | WT37 | 1.9431 | 1.6455 | 0.846842674 | |
| | WT71 | 1.9821 | 1.5512 | 0.782604309 | |
| | DM22 | 1.9816 | 1.462 | 0.737787646 | |
| | DM7 | 1.9354 | 1.5166 | 0.783610623 | |
| | DM27 | 1.965 | 1.5386 | 0.783002545 | |
| | DM41 | 1.9635 | 1.4604 | 0.743773873 | |
| | DM58 | 1.9586 | 1.4523 | 0.74149903 | |
| | DM65 | 2.0305 | 1.3207 | 0.650430928 | |
| | Black | WT18 | 1.9416 | 1.6116 | 0.830037083 |
| | | WT55 | 1.9707 | 1.5839 | 0.803724565 |
| | | WT33 | 2.0278 | 1.7108 | 0.843672946 |
| WT36 | | 1.9226 | 1.6349 | 0.850358889 | |
| WT44 | | 2.0298 | 1.6327 | 0.804364962 | |
| WT61 | | 1.9334 | 1.4786 | 0.764766732 | |
| DM34 | | 1.9426 | 1.528 | 0.786574694 | |
| DM39 | | 1.9079 | 1.4259 | 0.747366214 | |
| DM62 | | 1.9568 | 1.3833 | 0.70691946 | |
| DM54 | | 1.945 | 1.553 | 0.798457584 | |
| DM6 | | 1.9375 | 1.512 | 0.780387097 | |
| DM20 | | 1.9184 | 1.5182 | 0.791388657 | |
| White | | WT56 | 1.942 | 1.6737 | 0.86184346 |
| | | WT7 | 1.9796 | 1.6989 | 0.858203678 |
| | | WT16 | 1.9391 | 1.6923 | 0.87272446 |
| | WT4 | 2.0071 | 1.7706 | 0.882168303 | |
| | WT26 | 1.9755 | 1.7016 | 0.861351557 | |
| | WT50 | 1.9221 | 1.5416 | 0.802039436 | |
| | DM35 | 1.9193 | 1.5612 | 0.81342156 | |
| | DM26 | 1.9476 | 1.4891 | 0.76458205 | |
| | DM11 | 1.9243 | 1.5876 | 0.825027283 | |
| | DM17 | 1.9142 | 1.5923 | 0.831835754 | |
| | DM50 | 1.9263 | 1.4797 | 0.76815657 | |
| | DM31 | 1.9891 | 1.6418 | 0.825398421 | |

| | | Collection 2 | 100 Days | Percent Mass | |
|----------|-------|--------------|-----------------|--------------|-------------|
| | | Initials | Post Collection | Remaining | |
| Aluminum | WT5 | 1.983 | 1.3742 | 0.692990419 | |
| | WT38 | 2.0551 | 1.2128 | 0.590141599 | |
| | WT10 | 1.9999 | 1.3688 | 0.684434222 | |
| | WT24 | 1.9415 | 1.3759 | 0.708678857 | |
| | WT70 | 1.9637 | 1.2193 | 0.620919692 | |
| | WT27 | 1.94 | 1.3406 | 0.691030928 | |
| | DM72 | 2.0469 | 0.9756 | 0.476623186 | |
| | DM68 | 1.9476 | 0.9456 | 0.485520641 | |
| | DM44 | 2.0014 | 1.2268 | 0.61297092 | |
| | DM9 | 1.9445 | 1.2652 | 0.650655696 | |
| | DM25 | 1.9419 | 1.1825 | 0.608939698 | |
| | DM52 | 1.9607 | 1.2309 | 0.627785995 | |
| | Black | WT45 | 1.9374 | 1.3089 | 0.67559616 |
| | | WT34 | 2.0038 | 1.3809 | 0.689140633 |
| | | WT66 | 1.9762 | 1.4068 | 0.711871268 |
| WT53 | | 1.9518 | 1.2822 | 0.656932063 | |
| WT21 | | 1.9821 | 1.4883 | 0.750870289 | |
| WT48 | | 1.9248 | 1.1731 | 0.609465919 | |
| DM46 | | 1.9067 | 1.0951 | 0.574343106 | |
| DM43 | | 1.9615 | 1.2507 | 0.637624267 | |
| DM63 | | 1.9442 | 1.215 | 0.624935706 | |
| DM33 | | 1.9406 | 1.1446 | 0.589817582 | |
| DM19 | | 1.9366 | 1.2064 | 0.622947434 | |
| DM53 | | 1.9885 | 1.2075 | 0.607241639 | |
| White | | WT14 | 1.9696 | 1.3842 | 0.702782291 |
| | | WT6 | 2.0985 | 1.5496 | 0.738432213 |
| | | WT43 | 1.9856 | 1.1783 | 0.593422643 |
| | WT67 | 1.9994 | 1.2785 | 0.639441833 | |
| | WT58 | 1.9574 | 1.291 | 0.659548381 | |
| | WT28 | 1.9598 | 1.4216 | 0.725380141 | |
| | DM12 | 1.9211 | 1.2694 | 0.660767269 | |
| | DM2 | 1.942 | 1.2721 | 0.655046344 | |
| | DM47 | 1.9505 | 1.3026 | 0.667828762 | |
| | DM38 | 1.9035 | 1.2247 | 0.643393748 | |
| | DM28 | 1.9366 | 1.0387 | 0.53635237 | |
| | DM14 | 1.9089 | 1.1693 | 0.612551731 | |

| | | Collection 3 | 150 Days | Percent |
|----------|-------|--------------|-----------------|-------------|
| | | Initials | Post Collection | Mass |
| | | | | Remainng |
| Aluminum | WT62 | 1.9428 | 1.1802 | 0.607473749 |
| | WT25 | 1.9231 | 1.0761 | 0.559565285 |
| | WT40 | 2.0048 | 1.0593 | 0.528381883 |
| | WT11 | 1.9847 | 1.1345 | 0.571622915 |
| | WT64 | 2.0024 | 1.0394 | 0.519077107 |
| | WT31 | 2.0567 | 1.2454 | 0.605533136 |
| | DM67 | 1.935 | 0.8926 | 0.46129199 |
| | DM64 | 1.9782 | 1.0606 | 0.536143969 |
| | DM23 | 1.9618 | 0.8506 | 0.433581405 |
| | DM8 | 1.92 | 1.0554 | 0.5496875 |
| | DM71 | 2.0812 | 0.9629 | 0.46266577 |
| | DM51 | 1.9521 | 0.9495 | 0.486399262 |
| | Black | WT19 | 1.9254 | 1.1196 |
| WT2 | | 1.9915 | 1.1774 | 0.591212654 |
| WT3 | | 2.0165 | 1.1264 | 0.558591619 |
| WT72 | | 2.0239 | 1.2072 | 0.596472158 |
| WT42 | | 2.0049 | 1.2529 | 0.624918949 |
| WT47 | | 1.9215 | 1.0589 | 0.551079886 |
| DM40 | | 1.9934 | 1.0261 | 0.514748671 |
| DM21 | | 1.9441 | 1.0792 | 0.555115478 |
| DM61 | | 2.0409 | 0.9184 | 0.44999755 |
| DM69 | | 1.9918 | 0.9348 | 0.469324229 |
| DM56 | | 1.9369 | 1.0422 | 0.538076308 |
| DM5 | | 1.9973 | 0.9999 | 0.500625845 |
| White | | WT17 | 2.0299 | 1.1629 |
| | WT9 | 2.0805 | 1.2523 | 0.601922615 |
| | WT49 | 1.9332 | 1.0366 | 0.536209394 |
| | WT39 | 1.9696 | 0.9366 | 0.475528026 |
| | WT65 | 1.9473 | 1.1385 | 0.584655677 |
| | WT29 | 1.9664 | 1.3128 | 0.667615948 |
| | DM13 | 2.0172 | 0.9889 | 0.490233988 |
| | DM3 | 2.0162 | 1.1288 | 0.559865093 |
| | DM48 | 1.9119 | 1.0742 | 0.561849469 |
| | DM16 | 1.9604 | 0.9169 | 0.467710671 |
| | DM37 | 1.9806 | 1.1121 | 0.561496516 |
| | DM30 | 1.959 | 0.9903 | 0.505513017 |

| | | Collection 4 | 200 Days | | |
|----------|-------|--------------|-----------------|-------------|-------------|
| | | Initials | Post Collection | | |
| Aluminum | WT51 | 1.926 | 0.9169 | 0.476064382 | |
| | WT57 | 1.9857 | 1.0232 | 0.515284283 | |
| | WT69 | 1.9593 | 1.0698 | 0.546011331 | |
| | WT12 | 1.9597 | 1.0792 | 0.550696535 | |
| | WT23 | 2.0196 | 1.1272 | 0.558130323 | |
| | WT32 | 1.9506 | 1.0228 | 0.524351482 | |
| | DM45 | 1.9143 | 0.8695 | 0.454213028 | |
| | DM70 | 2.0717 | 0.9342 | 0.450934016 | |
| | DM66 | 2.036 | 0.9578 | 0.47043222 | |
| | DM4 | 1.9814 | 0.8895 | 0.448925003 | |
| | DM59 | 1.9317 | 0.8721 | 0.451467619 | |
| | DM24 | 1.9145 | | | |
| | Black | WT54 | 1.9071 | 0.9167 | 0.480677468 |
| | | WT46 | 1.9019 | | |
| WT35 | | 1.9521 | 0.9969 | 0.510680805 | |
| WT60 | | 1.9247 | 1.0441 | 0.542474152 | |
| WT20 | | 2.0664 | 1.1955 | 0.578542393 | |
| WT1 | | 1.9796 | 0.9902 | 0.500202061 | |
| DM1 | | 1.9132 | 0.9388 | 0.490696216 | |
| DM60 | | 1.916 | 0.9148 | 0.477453027 | |
| DM42 | | 1.9459 | 0.9021 | 0.463590113 | |
| DM57 | | 1.975 | 0.9652 | 0.488708861 | |
| DM18 | | 1.9431 | 0.915 | 0.47089702 | |
| DM55 | | 1.9273 | | | |
| White | | WT8 | 2.1269 | 1.2077 | 0.567821712 |
| | | WT52 | 1.9806 | 1.0166 | 0.513278804 |
| | WT68 | 1.9991 | 1.106 | 0.553248962 | |
| | WT41 | 1.9071 | 1.0176 | 0.533585024 | |
| | WT30 | 1.9826 | 1.0003 | 0.504539494 | |
| | WT15 | 1.9664 | 0.9384 | 0.47721725 | |
| | DM32 | 1.9387 | 0.8748 | 0.451230206 | |
| | DM10 | 1.9113 | 0.9956 | 0.520902004 | |
| | DM36 | 1.9069 | 0.8594 | 0.450679113 | |
| | DM49 | 1.9627 | 1.0053 | 0.512202578 | |
| | DM15 | 1.945 | 0.9211 | 0.473573265 | |
| | DM29 | 1.9121 | 0.9717 | 0.508184718 | |

Hemicellulose

Aluminum WT

| | | | | | | |
|--------------|-------|-------|-------|-------|-------|-------|
| Collection 1 | 16.97 | 17.14 | 17.16 | 15.41 | 17.75 | 15.25 |
| Collection 2 | 13.94 | 14.04 | 16.13 | 13.61 | 14.91 | 13.63 |
| Collection 3 | 12.7 | 17.1 | 15.01 | 13.4 | 18.15 | 13.72 |
| Collection 4 | 14.37 | 14.44 | 14.8 | 17.11 | 14.46 | 12.17 |

Black WT

| | | | | | | |
|--------------|-------|-------|-------|-------|-------|-------|
| Collection 1 | 19.19 | 20.74 | 19.87 | 20.43 | 20.25 | 22.93 |
| Collection 2 | 20.15 | 16.91 | 17.74 | 16.74 | 18.07 | 14.18 |
| Collection 3 | 15.53 | 17.55 | 16.73 | 17.14 | 18.18 | 17.58 |
| Collection 4 | 15.73 | 17.52 | 16.9 | 18.01 | 14.28 | 16.07 |

White WT

| | | | | | | |
|--------------|-------|-------|-------|-------|-------|-------|
| Collection 1 | 18.98 | 18.94 | 20.31 | 17.88 | 18.37 | 16.43 |
| Collection 2 | 16.91 | 14.1 | 18.3 | 15.32 | 15.89 | 16.59 |
| Collection 3 | 17.15 | 16.36 | 16.76 | 14.94 | 15.92 | 13.18 |
| Collection 4 | 13.71 | 17.47 | 15.23 | 12.86 | 15.53 | 16.36 |

Aluminum DM

| | | | | | | |
|--------------|-------|-------|-------|-------|-------|-------|
| Collection 1 | 14.87 | 15.1 | 16.25 | 14.18 | 11.8 | 16.03 |
| Collection 2 | 10.64 | 12.8 | 12.28 | 12.41 | 14.4 | 10.54 |
| Collection 3 | 12.83 | 10.13 | 9.38 | 10.3 | | |
| Collection 4 | 12.6 | 15.89 | 10.26 | 12.5 | 10.62 | |

Black DM

| | | | | | | |
|--------------|-------|-------|--------|-------|-------|-------|
| Collection 1 | 17.76 | 17.43 | 17.09 | 16.85 | 16.92 | 17.09 |
| Collection 2 | 13.44 | 13.63 | 10.143 | 12.68 | 15.71 | 13.11 |
| Collection 3 | 14.26 | 16.13 | 11.8 | 16.16 | 14.84 | 14.15 |
| Collection 4 | 12.44 | 15.42 | 13.53 | 14.04 | 9.36 | |

White DM

| | | | | | | |
|--------------|-------|-------|-------|-------|-------|-------|
| Collection 1 | 15.91 | 14.86 | 16.36 | 18.28 | 17.85 | 26.45 |
| Collection 2 | 12.19 | 12.93 | 13.06 | 11 | 12.31 | 13.16 |
| Collection 3 | 13.45 | 13 | 12.8 | 14.19 | 12.8 | 12.81 |
| Collection 4 | 13.45 | 13 | 12.8 | 14.19 | 12.8 | 12.81 |

| | | | | | | |
|--------------|-------|-------|-------|-------|-------|-------|
| Cellulose | | | | | | |
| Aluminum WT | | | | | | |
| Collection 1 | 38.31 | 34.78 | 29.82 | 30.81 | 30.97 | 41.81 |
| Collection 2 | 37.5 | 35.98 | 39.99 | 48.33 | 46.54 | 41.33 |
| Collection 3 | 40.35 | 33.39 | 40.35 | 45.53 | 38.28 | 44.35 |
| Collection 4 | 42.32 | 42.33 | 45.42 | 38.7 | 40.5 | 40.52 |
| Black WT | | | | | | |
| Collection 1 | 35.03 | 34.45 | 36.69 | 35.25 | 38.55 | 29.73 |
| Collection 2 | 38.96 | 36.11 | 41.41 | 39.16 | 39.83 | 40.15 |
| Collection 3 | 39.01 | 40.68 | 31.7 | 33.68 | 45.1 | 44.2 |
| Collection 4 | 38.75 | 38.37 | 36.65 | 45.25 | 42.48 | 39.34 |
| White WT | | | | | | |
| Collection 1 | 36.15 | 26.29 | 32.95 | 34.42 | 35.72 | 33.27 |
| Collection 2 | 39.53 | 36.31 | 38.16 | 36.11 | 41.34 | 42.87 |
| Collection 3 | 42.37 | 35.9 | 43.85 | 43 | 40.06 | 40.83 |
| Collection 4 | 36.5 | 47.88 | 48.59 | 40.91 | 36.3 | 39.49 |
| Aluminum DM | | | | | | |
| Collection 1 | 39.62 | 24.25 | 29.24 | 36.22 | 36.14 | 37.97 |
| Collection 2 | 44.12 | 40.86 | 36.8 | 42.48 | 38.94 | 41.66 |
| Collection 3 | 47.32 | 44.79 | 42.95 | 47.2 | | |
| Collection 4 | 44.93 | 43.9 | 46.51 | 48.28 | 46.22 | |
| Black DM | | | | | | |
| Collection 1 | 41.06 | 31.4 | 34.9 | 39.22 | 32.12 | 38.3 |
| Collection 2 | 48.08 | 38.15 | 40.55 | 35.35 | 37.15 | 33.56 |
| Collection 3 | 41.5 | 44.35 | 36.76 | 37.81 | 39.15 | 43.27 |
| Collection 4 | 41.43 | 44.2 | 40.9 | 40.44 | 38.3 | |
| White DM | | | | | | |
| Collection 1 | 37.16 | 36.45 | 35.65 | 33.54 | 33.42 | 26.89 |
| Collection 2 | 39.87 | 38.69 | 44.48 | 34.9 | 37.76 | 46.25 |
| Collection 3 | 43.71 | 42.18 | 45.28 | 42.18 | 37.87 | 38.1 |
| Collection 4 | 48.4 | 48.41 | 49.19 | 40.23 | 43.1 | 39.71 |

| | | | | | | |
|--------------|------|------|------|------|------|------|
| Lignin | | | | | | |
| Aluminum WT | | | | | | |
| Collection 1 | 7.2 | 5.2 | 9.8 | 11.2 | 7.9 | 3.7 |
| Collection 2 | 6 | 8.6 | 6.9 | 3.2 | 4.1 | 12.5 |
| Collection 3 | 9.2 | 10.9 | 8.4 | 5.3 | 6.2 | 4.6 |
| Collection 4 | 7 | 8.5 | 8.1 | 11.8 | 4.9 | 11.8 |
| Black WT | | | | | | |
| Collection 1 | 5.3 | 7.9 | 5.5 | 6.8 | 6.2 | 8.3 |
| Collection 2 | 8.6 | 9.1 | 6.8 | 4.9 | 6 | 4 |
| Collection 3 | 7.1 | 6 | 11.5 | 15.3 | 4 | 7.6 |
| Collection 4 | 13.3 | 9.9 | 9.1 | 3.3 | 5.4 | 10.2 |
| White WT | | | | | | |
| Collection 1 | 5.5 | 13 | 7.6 | 6 | 4.1 | 9.3 |
| Collection 2 | 5.4 | 6.7 | 8.1 | 8.1 | 4.9 | 3.4 |
| Collection 3 | 6.7 | 14.4 | 4.3 | 5.6 | 4.3 | 4.7 |
| Collection 4 | 8.4 | 6.5 | 9.1 | 12.9 | 6.2 | 6.3 |
| Aluminum DM | | | | | | |
| Collection 1 | 3.4 | 11.5 | 10.7 | 3.9 | 4.6 | 3.3 |
| Collection 2 | 1.3 | 7.8 | 8.6 | 4.1 | 7.4 | 7.4 |
| Collection 3 | 4.6 | 5.8 | 4.9 | 6 | | |
| Collection 4 | 3.8 | 9.6 | 6.5 | 6.1 | 8.7 | |
| Black DM | | | | | | |
| Collection 1 | 2.5 | 8.4 | 3.8 | 2.4 | 8.8 | 2 |
| Collection 2 | 2 | 8 | 5.4 | 11.1 | 7.8 | 9 |
| Collection 3 | 6.4 | 3.5 | 9.6 | 10.4 | 8.2 | 4.1 |
| Collection 4 | 8.6 | 6.6 | 8.4 | 12.3 | 2 | |
| White DM | | | | | | |
| Collection 1 | 3.2 | 2.5 | 5.7 | 6.6 | 7.6 | 3.6 |
| Collection 2 | 2.9 | 9.2 | 1.6 | 14.4 | 6.8 | 2.3 |
| Collection 3 | 4.1 | 5.5 | 2.2 | 6.9 | 13.7 | 11.1 |
| Collection 4 | 5.4 | 1.7 | 4.5 | 10 | 10.6 | 15.7 |

| Collection 1 | | BSP- WT | | | | |
|--------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 300 | 0.04449892 | 0.096693516 | 0.046378613 | 0.060840607 | 0.056214809 | |
| 375 | 0.01854372 | 0.02726841 | 0.015599251 | 0.050821781 | 0.017332077 | |
| Collection 2 | | | | | | |
| 300 | 0.053768635 | 0.03108263 | 0.038303375 | 0.054624557 | 0.036508083 | |
| 375 | 0.012562275 | 0.0128088 | 0.009104729 | 0.010067463 | 0.019970417 | |
| Collection 3 | | | | | | |
| 300 | 0.013513088 | 0.02950716 | 0.057515621 | 0.036427021 | 0.045494556 | 0.030157089 |
| 375 | 0.017108917 | 0.017920017 | 0.013811588 | 0.031599522 | 0.011442661 | 0.003826141 |
| Collection 4 | | | | | | |
| 300 | 0.013905048 | 0.057842255 | 0.064241886 | 0.062155247 | 0.024552345 | 0.031630993 |
| 375 | 0.007012367 | 0.004458427 | 0.040859699 | 0.012795925 | 0.016300678 | 0.004871368 |
| WT Black | | | | | | |
| Collection 1 | | | | | | |
| 300 | 0.063620567 | 0.107190609 | 0.047391891 | 0.053912163 | 0.05452919 | 0.067427635 |
| 375 | 0.042702198 | 0.027676105 | 0.023489475 | 0.024672985 | 0.054890633 | 0.027079105 |
| Collection 2 | | | | | | |
| 300 | 0.121602535 | 0.015227795 | 0.058496952 | 0.029092312 | | |
| 375 | 0.010764599 | 0.021076679 | 0.004258156 | 0.06768465 | | |
| Collection 3 | | | | | | |
| 300 | 0.023219585 | 0.060180187 | 0.043730736 | 0.025839806 | 0.035416603 | 0.032215595 |
| 375 | 0.008079052 | 0.008483887 | 0.015432358 | 0.018837929 | 0.033624649 | 0.00806427 |
| Collection 4 | | | | | | |
| 300 | 0.022837639 | 0.047901154 | 0.037604809 | 0.057015896 | | |
| 375 | 0.016139507 | 0.023562431 | 0.012809753 | 0.00546217 | | |
| WT White | | | | | | |
| Collection 1 | | | | | | |
| 300 | 0.162356377 | 0.020945549 | 0.094286919 | 0.083659649 | 0.026436806 | 0.039250374 |
| 375 | 0.020781994 | 0.001955986 | 0.032395363 | 0.053355217 | 0.004286766 | 0.084611416 |
| Collection 2 | | | | | | |
| 300 | 0.054055214 | 0.022703648 | 0.026988029 | 0.070364475 | | |
| 375 | 0.038051605 | 0.009626389 | 0.009008884 | 0.013683796 | | |
| Collection 3 | | | | | | |
| 300 | 0.022806168 | 0.035816193 | 0.024412632 | 0.036653996 | 0.058325291 | 0.040215015 |
| 375 | 0.009916306 | 0.0328722 | 0.009982586 | 0.012877464 | 0.008150101 | 0.008908749 |
| Collection 4 | | | | | | |
| 300 | 0.072968483 | 0.028781891 | 0.06337595 | 0.043934345 | 0.095930099 | 0.038935184 |
| 375 | 0.007488251 | 0.043421268 | 0.008470535 | 0.027732372 | 0.00412941 | 0.039556503 |

| Collection 1 | | BSP-DM | | | | |
|--------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 300 | 0.045193195 | 0.086408138 | 0.061879635 | 0.031940937 | 0.09106493 | 0.049183846 |
| 375 | 0.025118828 | 0.0577178 | 0.014361858 | 0.039414406 | 0.038671494 | 0.017519474 |
| Collection 2 | | | | | | |
| 300 | 0.083099365 | 0.026143551 | 0.044542313 | 0.034144878 | 0.07073307 | |
| 375 | 0.036641121 | 0.009772301 | 0.011745453 | 0.00514555 | 0.042973995 | |
| Collection 3 | | | | | | |
| 300 | 0.038989544 | 0.040631294 | 0.05385685 | 0.037392139 | | |
| 375 | 0.007718086 | 0.017595291 | 0.015064716 | 0.023750305 | | |
| Collection 4 | | | | | | |
| 300 | 0.036221981 | 0.068180561 | 0.043088436 | | | |
| 375 | 0.010876179 | 0.01195097 | 0.010761738 | | | |
| DM Black | | | | | | |
| Collection 1 | | | | | | |
| 300 | 0.036718845 | 0.109506607 | 0.031299114 | 0.116520882 | 0.057294369 | 0.068020821 |
| 375 | 0.039043427 | 0.031055927 | 0.054414272 | 0.011061668 | 0.056315899 | 0.019462109 |
| Collection 2 | | | | | | |
| 300 | 0.025220394 | 0.013443947 | 0.021910667 | 0.03280735 | 0.057575226 | |
| 375 | 0.025804043 | 0.012593746 | 0.006750107 | 0.005766392 | 0.006320953 | |
| Collection 3 | | | | | | |
| 300 | 0.036702633 | 0.005274773 | 0.02244997 | 0.038430691 | 0.017642021 | 0.029188156 |
| 375 | 0.014883518 | 0.005359173 | 0.017847538 | 0.008152485 | 0.002382755 | 0.019496441 |
| Collection 4 | | | | | | |
| 300 | 0.07754755 | 0.038500786 | 0.030132294 | 0.093166828 | 0.030582905 | |
| 375 | 0.007014751 | 0.041199684 | 0.0058918 | 0.009456158 | 0.022225857 | |
| DM White | | | | | | |
| Collection 1 | | | | | | |
| 300 | 0.071726322 | 0.055926323 | 0.062568665 | 0.05734539 | 0.083758831 | 0.035754204 |
| 375 | 0.014429092 | 0.038332939 | 0.025580406 | 0.02623415 | 0.021972656 | 0.025183201 |
| Collection 2 | | | | | | |
| 300 | 0.014791965 | 0.032808781 | 0.022097588 | 0.064779282 | 0.039395332 | 0.01270628 |
| 375 | 0.006111145 | 0.018231392 | 0.025823116 | 0.006475925 | 0.011894226 | 0.005485058 |
| Collection 3 | | | | | | |
| 300 | 0.019837379 | 0.116228104 | 0.021602154 | 0.033260822 | | |
| 375 | 0.009133816 | 0.005603313 | 0.041885853 | 0.00983572 | | |
| Collection 4 | | | | | | |
| 300 | 0.033406258 | 0.037743092 | 0.037258148 | | | |
| 375 | 0.007761002 | 0.009174824 | 0.004354954 | | | |

| | | | | |
|---------|-----|-------------|-------------|-------------|
| Initial | | | | |
| WTA | C | 37.95 | 37.92 | 36.61 |
| | N | 0.8103 | 0.9016 | 0.9282 |
| | C:N | 46.8345 | 42.0586 | 39.4419 |
| WTB | C | 38.79 | 38.00 | 43.47 |
| | N | 0.9846 | 0.8269 | 1.250 |
| | C:N | 39.39670932 | 45.95477083 | 34.776 |
| WTW | C | 39.86 | 38.33 | 34.90 |
| | N | 0.8180 | 1.129 | 0.9855 |
| | C:N | 48.72860636 | 33.95039858 | 35.41349569 |
| DMA | C | 39.61 | 38.77 | 37.99 |
| | N | 1.122 | 1.482 | 1.141 |
| | C:N | 35.3030303 | 26.16059379 | 33.29535495 |
| DMB | C | 39.57 | 38.96 | 38.21 |
| | N | 1.107 | 1.460 | 1.397 |
| | C:N | 35.74525745 | 26.68493151 | 27.35146743 |
| DMW | C | 40.72 | 36.73 | 36.43 |
| | N | 1.378 | 1.024 | 0.9266 |
| | C:N | 29.55007257 | 35.86914063 | 39.31577811 |
| Final | | | | |
| WTA | C | 36.69 | 41.86 | 34.85 |
| | N | 0.8822 | 0.8310 | 0.9892 |
| | C:N | 41.5892088 | 50.37304452 | 35.23048928 |
| WTB | C | 38.29 | 36.81 | 37.27 |
| | N | 1.278 | 0.9733 | 0.8425 |
| | C:N | 29.96087637 | 37.81978835 | 44.23738872 |
| WTW | C | 34.50 | 37.80 | 37.39 |
| | N | 1.233 | 1.275 | 1.418 |
| | C:N | 27.98053528 | 29.64705882 | 26.36812412 |
| DMA | C | 35.09 | 33.22 | 37.62 |
| | N | 0.9802 | 1.319 | 1.031 |
| | C:N | 35.79881657 | 25.18574678 | 36.48884578 |
| DMB | C | 37.75 | 36.98 | 33.51 |
| | N | 0.6861 | 1.251 | 1.081 |
| | C:N | 55.02113395 | 29.56035172 | 30.99907493 |
| DMW | C | 36.74 | 34.56 | 36.85 |
| | N | 0.7784 | 1.115 | 1.195 |
| | C:N | 47.19938335 | 30.9955157 | 30.83682008 |

| Wavelength (nm) | % Reflectance Aluminum | % Reflectance Black | % Reflectance White |
|-----------------|---------------------------|------------------------|------------------------|
| 280 | 83.9 | 2.1 | 6.5 |
| 290 | 83.8 | 2.1 | 6.6 |
| 300 | 83.6 | 2.2 | 6.8 |
| 310 | 83.7 | 2 | 6.8 |
| 320 | 83.7 | 1.8 | 6.7 |
| 330 | 82.2 | 1 | 6.7 |
| 340 | 82.9 | 1.6 | 6.5 |
| 350 | 83.3 | 2 | 6.7 |
| 360 | 83.9 | 2.2 | 7.3 |
| 370 | 83.8 | 2.1 | 8.8 |
| 380 | 83.7 | 1.3 | 12.4 |
| 390 | 83.9 | 3 | 19.2 |
| 400 | 83.6 | 3 | 38.8 |
| 410 | 83.4 | 3 | 65.9 |
| 420 | 83.6 | 3.1 | 81 |
| 430 | 83.5 | 2.8 | 84.1 |
| 440 | 83.8 | 2.8 | 85 |
| 450 | 84 | 2.9 | 85.5 |
| 460 | 84.3 | 2.9 | 86 |
| 470 | 84.4 | 2.9 | 86.2 |
| 480 | 84.6 | 3 | 86.4 |
| 490 | 84.8 | 3 | 86.6 |
| 500 | 85 | 3 | 86.7 |
| 510 | 85.1 | 3 | 86.7 |
| 520 | 85.3 | 3 | 86.7 |
| 530 | 85.4 | 3 | 86.7 |
| 540 | 85.5 | 3 | 86.6 |
| 550 | 85.6 | 3 | 86.6 |
| 560 | 85.7 | 3 | 86.6 |
| 570 | 85.8 | 3 | 86.5 |
| 580 | 85.8 | 3.1 | 86.5 |
| 590 | 86 | 3.1 | 86.5 |
| 600 | 86.1 | 3.1 | 86.5 |
| 610 | 86.1 | 3.1 | 86.5 |
| 620 | 86.1 | 3.1 | 86.6 |
| 630 | 86.1 | 3.1 | 86.7 |
| 640 | 86.1 | 3.2 | 86.9 |
| 650 | 86 | 3.2 | 87.1 |

| | | | |
|-----|------|-----|------|
| 660 | 86 | 3.3 | 87.4 |
| 670 | 85.9 | 3.3 | 87.6 |
| 680 | 85.8 | 3.4 | 87.9 |
| 690 | 85.5 | 3.3 | 87.9 |
| 700 | 85.3 | 3.4 | 88.2 |
| 710 | 85.1 | 3.5 | 88.5 |
| 720 | 84.8 | 3.6 | 88.7 |
| 730 | 84.5 | 3.8 | 89 |
| 740 | 84.2 | 4.1 | 89.4 |
| 750 | 83.9 | 4.5 | 90 |
| 760 | 83.6 | 5.2 | 90.9 |