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Photodegradation Stimulates Microbial Activity Through Enhanced Water Solubility of Grass Litter Carbon

A Thesis submitted in partial satisfaction of the

requirements for the degree Master of Arts

in Geography

by

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March 2020

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Abstract

Photodegradation Stimulates Microbial Activity Through Enhanced Water Solubility of Grass Litter Carbon

Trevor L. Romich

Solar radiation is an important contributing factor to decomposition in drylands. Research suggests that in the presence of water, previously irradiated plant litter experiences greater microbial decay than litter which was not exposed to radiation. It is unclear how exactly radiation alters litter to allow this photopriming of microbial decomposition. However, the relationship to water suggests that radiation may make litter more water-soluble, and therefore more accessible to decomposers once water enters the system. I tested the hypothesis that the abiotic impact of solar radiation on grass litter would (1) increase the production of dissolved organic carbon (DOC) when litter is subsequently extracted with water, and (2) produce DOC that stimulates more microbial activity compared to unexposed litter. Dried senesced grass litter from three species, *Bromus diandrus*, *Avena fatua*, and *Hordeum murinum*, were placed in sealed bags and subjected to abiotic decomposition in either an outdoor experiment or an indoor experiment. Treated litter was then soaked in water, and the extract was analyzed to determine the dissolved organic carbon concentration and its bioavailability. Exposure to radiation resulted in more DOC for all species in both the indoor and outdoor experiments, suggesting that solar radiation does enhance solubility of grass litter. During a microbial incubation, I observed a significant increase in CO2 production and a marginally significant increase in DOC consumption for samples exposed to more radiation in the indoor experiment. However, as a fraction of initial DOC available,

radiation reduced these measures of microbial activity. This indicates that photodegradation produces compounds which are relatively difficult for microbes to decompose, but photodegradation can still stimulate microbial activity by increasing the total amount of available dissolved carbon. Taken together, these results suggest a possible mechanism for observed increases in mass loss due to photopriming: litter carbon is made more soluble by radiation, and is mobilized in the presence of water, allowing for increased microbial decomposition. This insight into decomposition mechanisms could aid in developing more mechanistic models of carbon cycling that include photopriming.

TABLE OF CONTENTS

Introduction

Photodegradation can enhance the decay of plant organic matter and can contribute a substantial fraction of total decomposition in drier environments (King et al., 2012; Wang et al., 2015). In such locations, decomposition occurs more rapidly than would be expected from variables such as moisture and temperature that work well for predicting decomposition in wetter environments. Researchers have pointed to photodegradation as a possible explanation for this phenomenon (Parton et al., 2007). Models that include photodegradation are better at estimating dryland decomposition than models that rely solely on biological decomposition, with one study reporting that photodegradation models predict 12% greater decomposition annually (Adair et al., 2017). In order to develop effective models of decomposition in drylands, it is important to understand the mechanisms of photodegradation.

There are two components to photodegradation. First, absorption of visible and ultraviolet radiation can lead to the abiotic breakdown of litter. Studies also frequently observe that litter which was previously irradiated subsequently experiences higher microbial decomposition rates (e.g., Gliksman et al., 2017; Austin et al., 2016); this enhancement of microbial decay due to radiation is known as photpriming. However, observation of photopriming's importance is not universal. The above-mentioned modeling study by Adair et al. (2017) compared a basic exponential decay model of biotic decomposition to several different types of photodegradation models, including some that attempted to account for photopriming by transforming recalcitrant carbon into more bioavailable carbon. Some of the photopriming models they tested performed better than the no-photodegradation model, but the best match for observations was a model that only included abiotic photodegradation

(Adair et al., 2017). There are also field studies that do not detect photopriming (e.g., Brandt et al., 2009). These conflicting results suggest that photopriming may only be relevant in some conditions, and the mechanics behind it remain unclear (King et al., 2012). A better mechanistic understanding of the process of photopriming may help reconcile the results of Adair et al. (2017)'s modeling study with the inconsistent but frequent observation that photopriming does in fact occur.

Photodegradation can break down three major carbon fractions in litter – cell solubles, cellulose/hemicellulose, and lignin and other highly recalcitrant compounds (Huang et al., 2017). However, previous research indicates that lignin and cellulose/hemicellulose may experience greater photodegradation than other components of plant litter. Lignin absorbs strongly in the ultraviolet (UV) wavelength range, leading researchers to suspect it may be impacted by photodegradation (King et al., 2012; George et al., 2005). Austin and Ballaré (2010) observed that wavelengths of radiation which promote photodegradation match those which are absorbed strongly by lignin. They also assessed mass loss from litter due to photodegradation using artificial plant litter created from laboratory materials; artificial litter with more lignin experienced greater mass loss after exposure to radiation (Austin & Ballaré, 2010). Wang et al. (2015)'s meta-analysis found that UV radiation resulted in a greater fraction of lignin than total mass being lost from litter, suggesting that this relationship may hold for actual plant litter.

Not all studies see a positive relationship between litter lignin content and photodegradation (e.g., Brandt et al. 2010; Brandt et al., 2007). King et al. (2012) performed a meta-analysis and, unlike Wang et al. (2015), found either no relationship or a reduction in

photodegradation by UV radiation with increasing litter lignin content. There is also evidence that UV radiation preferentially affects cellulose-type compounds. Lin & King (2014) found that hemicellulose, but not cellulose or lignin, decayed more with radiation exposure than without. Similarly, Brandt et al. (2007) observed a radiation-induced reduction in the remaining pooled cellulose and hemicellulose fractions of litter after three years of exposure to differing UV radiation levels.

Much research has focused on UV radiation due to the relationship with lignin, but UV and visible wavelengths are both important to photodegradation. Research on wood has identified UV radiation as a source of radiation-induced decomposition due to the aforementioned impact on lignin (George et al., 2005). UV radiation has also been observed to enhance decomposition of other types of plant matter, including grasses found in dryland environments (Brandt et al., 2010; Brandt et al., 2007; Brandt et al., 2009). Experiments have also demonstrated that visible light can account for close to 50% of the total photodegradation effect, both for artificial litter (Austin & Ballaré, 2010) and for *B. gracilis* grass litter (Brandt et al., 2009; Austin & Ballaré, 2010). Given the body of research suggesting the importance of both wavelength ranges to photodegradation overall, and the fact that photodegradation is observed to have a greater impact on compounds such as cellulose and lignin that are not water-soluble, I would expect both UV and visible radiation to promote dissolved organic carbon (DOC) production through photodegradation.

In aquatic systems, research has shown a positive relationship between photodegradation and litter carbon bioavailability. Moran and Zepp (1997) reviewed existing literature and found that already dissolved organic carbon is broken into smaller carbon

compounds by radiation and that this degradation leads to increased microbial activity. Purely abiotic photodegradation can increase production of dissolved organic carbon from aquatic plant litter. The remaining litter is then more susceptible to microbial breakdown (Vähätalo et al. 1998). These results suggest a link between photopriming and the production of DOC, which may be applicable to terrestrial environments as well.

Some terrestrial studies do suggest a relationship between biotic decomposition, photodegradation, and water. According to Gliksman et al. (2017), dew moisture can stimulate photopriming in situations where radiation exposure does not increase biotic decay of dry litter. Though not specifically intending to investigate water-sunlight interactions, Lin & King (2014) found that litter covered by a surface litter layer still decays faster in sunlight. As this lower litter layer is not directly exposed to radiation, the enhanced decay may be caused by greater downward transport of DOC from the surface layer (Lin & King 2014).

Based on these results, I tested the following mechanism for photopriming. First, photodegradation by UV and visible radiation breaks plant compounds—preferentially affecting lignin and cellulose/hemicellulose—into smaller structures that are more water-soluble. Then, in the presence of water, these compounds dissolve and become more available to microbes, increasing microbial activity.

I tested this mechanism for photopriming by investigating the impact of different radiation exposure levels on (1) DOC production, (2) microbial consumption of carbon, and (3) chemical characteristics of DOC.

If this mechanism is correct, I would expect:

1. Radiation increases DOC production in plant litter
2. DOC produced from litter exposed to radiation stimulates more microbial activity than DOC from litter that was not irradiated
3. DOC produced by photodegradation is preferentially derived from lignin and cellulose

I also sought to identify possible differences in the importance of UV and visible light to this process. Because past studies have found both wavelengths to be important, I would expect both to contribute to DOC production with effects of equal order of magnitude.

Methods

Grass litter from three nonnative California grasses—*Bromus diandrus*, *Hordeum murinum*, and *Avena fatua*—was collected on July 15, 2018 from the University of California Natural Reserve System Sedgwick Reserve near Santa Barbara, CA. These grasses were chosen because they are three of the most common grasses in California (D'Antonio et al., 2007), which should increase the likelihood that this study's results are generally indicative of grassland photodegradation in the state. Prior studies report differences in litter lignin and cellulose content among the three grass species used, which may lead to differences in their response to radiation exposure (Table 1). However, if the same pattern is observed in multiple species, this should provide stronger evidence that the pattern is a general trend than if only one species were used.

Table 1: Litter carbon fractions as determined by past studies			
Litter Carbon Fraction	*Avena*	*Bromus* (soon after senescence)	*Hordeum vulgare*
Lignin	4.1%	3.2%	4.36%
Cellulose	36.41%	39.7%	33.9%
Hemicellulose	30%	31.6%	48.6% (reported as soluble carbohydrates)
Cell solubles	29.49%	25.5%	
Source	Esch et al., 2019	Lin et al., 2015	Pancotto et al., 2005

The Sedgwick Reserve experiences a Mediterranean climate with cool, wet winters and warm, dry summers. The field litter collection site is located on a hill dominated by *Bromus* and *Avena* grasses; litter collection occurred before the senesced grass had been exposed to rain (University of California Natural Reserve System, 2020). *Bromus* and *Avena* litter were collected from a hilltop location (34.694^{o}N, 120.030^{o}W). Because no *Hordeum* litter was found at this location, *Hordeum* was instead collected on the side of the hill, near the base, about 50 meters away (34.695^{o}N, 120.030^{o}W). Standing dead litter was removed by hand, put into paper bags, and brought to the laboratory for storage. Litter was allowed to air dry in a fume hood in the laboratory for several days and was stored in loosely closed paper bags to minimize radiation exposure.

Before being subjected to the radiation treatment, litter was cut into segments of < 10 cm length, and roots were removed. Additionally, seeds were removed from *Bromus* and *Avena* litter. For *Hordeum* litter, the entire seed head was clipped off where it met the stem, as the seeds were more difficult to separate from the rest of the plant in this species.

In order to minimize any confounding effects of sterilization procedures on litter chemistry, the litter itself was not sterilized, but the handling of litter and sample bags was

designed to minimize microbial activity. The litter was oven-dried at 45^{o}C for 24 hours before being transferred to an ethanol-sterilized laminar flow hood where sample bags were constructed. For each sample bag, 0.25 g of litter was heat-sealed into approximately 10 cm by 10 cm polyvinyl fluoride plastic film (DuPont Tedlar® TST20SG4) bags that were transparent to ~90% of both UV and visible light. Bags were wiped with ethanol prior to adding litter to reduce microbial population. Due to this, as well as the dry conditions in the bags, I expected that microbial activity during the treatment would be minimal. This should mean any radiation treatment differences are primarily due to abiotic photodegradation.

I set up two experiments to subject plant litter to different radiation conditions. One experiment was established outdoors, on the roof of Webb Hall at UC Santa Barbara. In this experiment, three different radiation treatments were created by filtering solar radiation. Each treatment consisted of two screens placed next to each other, and sample bags were placed approximately 10 cm beneath these screens, affixed to a wooden board to keep them in place. A high radiation treatment allowed 90% of both UV and visible light to reach the bags (hereafter referred to as the UV + Visible treatment). The screen for this treatment was made of the same type of Tedlar film used to make the sample bags. A treatment that allowed 90% of visible light but only 14% of UV radiation to reach the bags (hereafter called the Visible Pass treatment) was established using a 0.178 mm thick polycarbonate sheet (SABIC Lexan®). A third Radiation Block treatment, created by covering Tedlar film in aluminum foil, excluded over 90% of radiation in both the visible and UV wavelengths. Each treatment received 12 sample bags of each grass species, for a total of 108 samples (3 species x 3 treatments x 12 replicates), and received 4 empty bags as controls. This experiment ran from August 18, 2018 to November 10, 2018, with the location of each treatment rotated weekly to

minimize differences in radiation reaching the treatment screens caused by the shadows of adjacent structures on the roof. Because the sample bags were attached to a wooden board, they could not be turned over, so litter in the outdoor experiment was only exposed to radiation on one side of the bags (see Appendix 1, Figures A1-1 and A1-3 for photos of the outdoor setup and of all three types of treatment screen).

The second experiment was established inside a climate-controlled room to assess the effect of radiation under more controlled environmental conditions. Radiation in this experiment was provided by UV lamps, which were left on 24 hours a day to maximize UV exposure (see Appendix 1, Figure A1-2 for a picture of the indoor experiment setup). However, these lamps had relatively low visible light output. Three radiation treatments were established using the same three types of screen material as in the outdoor experiment, but with only one screen per treatment. Exact radiation dosage under the lamps varied with location, but samples were randomly moved to new locations every week to minimize the difference in radiation exposure between samples in the same treatment. Sample bags were also flipped every week at the same time they were moved, allowing the indoor samples to receive radiation on both sides of the bag. A total of 45 samples were included in the indoor experiment—5 samples of each species for each treatment. The indoor experiment ran from August 28, 2018 to February 10, 2019.

Effectiveness of the radiation treatment was assessed by measuring radiation transmission. For each treatment, and for both experiments, the amount of radiation in the UV-A, UV-B, and visible (400-700 nm) ranges was measured both with and without the treatment screen. Visible light measurements were made with a QMSS Quantum Meter (Apogee Instruments), which measured radiation between 400 and 700 nm. UV-A radiation

was measured using a UVP UVX-36 radiometer (UV Products, now Analytik Jena), which responds most strongly to radiation between about 320 and 380 nm. UV-B radiation was measured using a UVP UVX-31 radiometer with strongest response between about 280 and 340 nm (UV Products, now Analytik Jena). Measurements were taken for the start and for the conclusion of each experiment; for the outdoor experiment, a third measurement was also made about halfway through the treatment period. These data were used to generate rough approximations for the total UV and visible radiation received by samples in each treatment. For the outdoor treatments, varying day length was also included in the calculation, and day length values for the start and end of the experiment as well as the middle measurement date were obtained using a National Oceanic & Atmospheric Administration online Solar Calculator (Earth System Research Laboratory, 2020).

The sample collection, processing, and analysis procedures were the same for both the outdoor experiment and the indoor experiment, except as noted below. After the radiation treatment period, sample bags were collected and subjected to a dark incubation to assess possible microbial activity during the treatment. Each litter sample was removed from the bag and transferred to a 4 oz glass jar. The jars were stored in the dark, and carbon dioxide (CO2) accumulation was monitored for 2 days by drawing air from the headspace through a butyl rubber septum. The CO2 concentration of this air was measured on an infrared gas analyzer (Li-Cor 6252, Lincoln, NE) at the beginning of the incubation, approximately one day after the incubation started, and at the incubation's conclusion. Results of the incubation showed minimal CO2 accumulation in all jars, indicating that microbial activity was not likely a major contributor to decomposition during the radiation treatment period.

Following these measurements, litter samples were soaked in 30 mL of cold high purity water (MilliQ, MilliporeSigma) for two hours on an orbital shaker to collect extractable organic carbon. The resulting solution was filtered with a pre-combusted 1 µm glass fiber filter into a 40 mL centrifuge tube and frozen at -18 °C until chemical analysis. Four methods of analysis, described below, were used to assess the impacts of the radiation treatments on litter extractable carbon: measurement of extract DOC, microbial incubation to assess bioavailability, absorbance of extracts at 410 nm, and absorbance at 254 nm.

First, a portion of the filtered sample extract was analyzed for dissolved organic carbon (DOC) concentration using a Shimadzu TOC-V_{CSN} total organic carbon analyzer. The TOC analyzer was calibrated prior to each analysis using a 250 ppm carbon (C) solution. Depending on the analysis, this solution was either glucose dissolved in high purity water or a diluted 1000 ppm stock carbon standard consisting of phosphoric acid and potassium hydrogen phthalate dissolved in water. Samples were run in batches of 10 to 20 to ensure that no sample was at room temperature for longer than 6 hours prior to analysis. Between each batch of samples, and at the end of each run, a pair of standards of the same concentration were analyzed to check for drift; no substantial drift in measurements was observed. This analysis was also performed on a subsample of litter from each species that had been extracted around the time the experiments started (see Appendix 2).

Second, sample extracts were subjected to a microbial incubation to assess ability to stimulate microbial activity. Microbial inocula were created by mixing approximately 0.25 g of soil from the Sedgwick Reserve with 30 mL of water, and centrifuging for 3 minutes at 1000 rpm to settle out large soil particles. Soil was obtained at both the hilltop and hill-base

litter collection sites by removing surface organic matter (mostly standing dead litter with some fallen seeds) and then collecting the top 3-5 cm of the remaining soil.

For each sample, inoculum was added to 10 mL of sample extract, and this mixture was sealed inside a 27.25 mL glass jar. Approximately 10 µL of inoculum was added to each sample in the outdoor experiment. The small volume of inoculum in the outdoor experiment led to difficulty ensuring the inoculum actually ended up in the sample solution, so a larger inoculum volume of about 20 µL was added to samples in the indoor experiment. For both experiments, the DOC concentration of the inoculum mixture itself was an order of magnitude lower than the concentration of the samples. Together with the extremely low volume of inoculum added, this meant that the inoculum itself accounted for a negligible fraction of the DOC in the jars.

CO2 accumulation in the jars was measured as an indicator of the extract's capacity to enhance microbial activity. Measurements during the incubation were conducted once daily until concentration was observed to have changed noticeably since the last measurement, at which point they were made 2-3 times daily until the CO2 production rate had passed its peak and levelled off. Ten jars were randomly selected as 'indicator jars' and were measured three times daily for the entire incubation. These indicator jars were used to ensure that all jars were measured before headspace CO2 reached values in excess of 10,000 ppm. After the measurement of each batch of 10 jars, jars were flushed with compressed air for 10 minutes to reset their air concentration to a known value. Samples from the two experiments were run separately. The incubation for the outdoor experiment took place on 7/1/19 and lasted for 4 days, and the indoor experiment incubations started on 6/18/19 and lasted for 7 days. The DOC concentration of samples (filtered again with a 0.2 µm nylon

filter) was determined before and after the incubation and was used to calculate DOC consumption during the incubations.

For each of the two incubations, several control jars were created using filtration blanks mixed with inoculum. Additional control jars containing only water were also included. The filtration blanks contained water that had been filtered with a 0.2 µm nylon filter at the same time as the samples themselves. No control jar in either set showed a noticeable CO2 production peak during incubation.

Extract chemistry was assessed using two proxy methods for classes of carbon compounds based on absorbance of specific wavelengths of radiation. The first absorbance method, based on work by Lever (1972), uses sample absorbance at 410 nm as a proxy for reducing sugars content. This method involved adding 25 µL of sample to 125 µL of a reagent mixture containing para-hydroxybenzoic acid hydrazide (PAHBAH), sodium hydroxide, hydrochloric acid, calcium chloride, and trisodium citrate. The sample and reagents were initially mixed in a 96-well thermocycler plate, which was heated to 100^{o} C in a thermocycler for 5 minutes before being cooled by floating in an ice water bath for 5 minutes. The mixture was then pipetted into a 96-well flat bottom plate, which was inserted into a TECAN Infinite M200 Pro plate reader to measure absorbance at 410 nm. Litter extract used for this method had only been filtered through the glass fiber filter. This measurement is a proxy for reducing sugars in the sample, as such sugars react strongly with PAHBAH to change the absorbance of solution at 410 nm. There is a range of sugars which respond substantially, but the strongest response is from maltose, fructose, glucose, and mannose (Lever, 1972). The measured absorbance at this wavelength is therefore a proxy of

the amount of reducing sugars in the sample – these are compounds which could be produced by breakdown of a range of plant cell components, including lignin and cellulose.

The second method uses the specific UV absorbance (SUVA) of the sample as a proxy for the sample's degree of aromaticity (Weishaar et al., 2003). Weishaar et al. (2003) found a positive relationship between the absorbance of river water at this wavelength and the aromatic compound content of the water. As lignin is an aromatic compound, I expect that photodegradation of lignin may result in the production of smaller, more water-soluble aromatic compounds, leading to greater specific UV absorbance of extract from samples that were exposed to more radiation.

I obtained this data for the sample extracts by measuring the absorbance at 254 nm and dividing the result by the sample's DOC concentration, as measured on the TOC analyzer. The sample extract that was used in this measurement had been filtered through a 0.2 μm nylon filter, in addition to the initial filtration with the glass fiber filter. A 75 μL volume of each sample was directly added to UV-transparent 96-well flat bottom plates, and their absorbance at 254 nm was measured using the same plate reader used for the 410 nm measurement.

All analyses were normalized to the initial litter mass for that sample prior to analysis. For specific UV absorbance at 254 nm, the data was also normalized to the DOC concentration. For incubation data, analysis was performed on the data normalized to initial litter mass only and on the data normalized to both initial litter mass and extract concentration at the start of the incubation. For the latter incubation analysis, I also expected to see a greater portion of DOC consumed or respired as CO2 for samples exposed to greater

radiation. This would indicate that radiation increased bioavailability per molecule of the DOC, which is to be expected if recalcitrant compounds are being broken down into less recalcitrant ones by radiation.

Statistical analyses were conducted in MATLAB. An n-way ANOVA was performed on the data after determining that it met the assumptions of normality and equal variance. This tested the significance of both species and radiation treatment as factors. Initially, the ANOVA model was run including interaction terms for all factors and was rerun progressively removing an interaction that was not significant each time. For absorbance data, since multiple plates were used, the plate number was also included as a third factor. In most cases all interactions were found not to be significant. Aside from the outdoor experiment's SUVA at 254 nm data, absorbance data did not meet the requirements for the ANOVA model; as a result, all absorbance metrics were tested using the Kruskal-Wallis one-way ANOVA for non-normal data instead. CO_2 production data from the indoor incubation did not have equal variance across species when normalized by initial DOC concentration, so the normalized CO_2 data for both experiments were also tested with the Kruskal-Wallis ANOVA.

Results

1 - Radiation exposure in different treatments

In both experiments, screens were effective in establishing the intended different UV radiation treatments (Figure 1). For the indoor experiment, the UV + Visible treatment received about an order of magnitude more UV radiation than the Visible Pass treatment, and the Visible Pass treatment received about two orders of magnitude more UV radiation than the Radiation Block treatment. A similar though less pronounced difference was observed with the outdoor experiment. The UV + Visible treatment received roughly 6 times more UV radiation than the Visible Pass treatment, and the Visible Pass treatment received roughly 40 times more UV radiation than the Radiation Block treatment.

The indoor experiment received about 3.6 times as much UV radiation as the outdoor experiment in the UV + Visible treatment and around 1.8 times as much UV radiation in the Visible Pass treatment. This likely resulted both from the greater length of the indoor experiment and from the use of UV lamps which were turned on for 24 hours a day to provide radiation (Table 2). In contrast, the Radiation Block treatment received less UV radiation in the indoor experiment than in the outdoor experiment. I suspect this is because the lamps used in the indoor experiment were always situated above the opaque screen, which was nearly flush against the wall on two sides during measurements. In the outdoor experiment, solar radiation would have been more likely to indirectly reach the sample bags at times when the sun was at a low angle (see Appendix 1 for photos of each experimental setup).

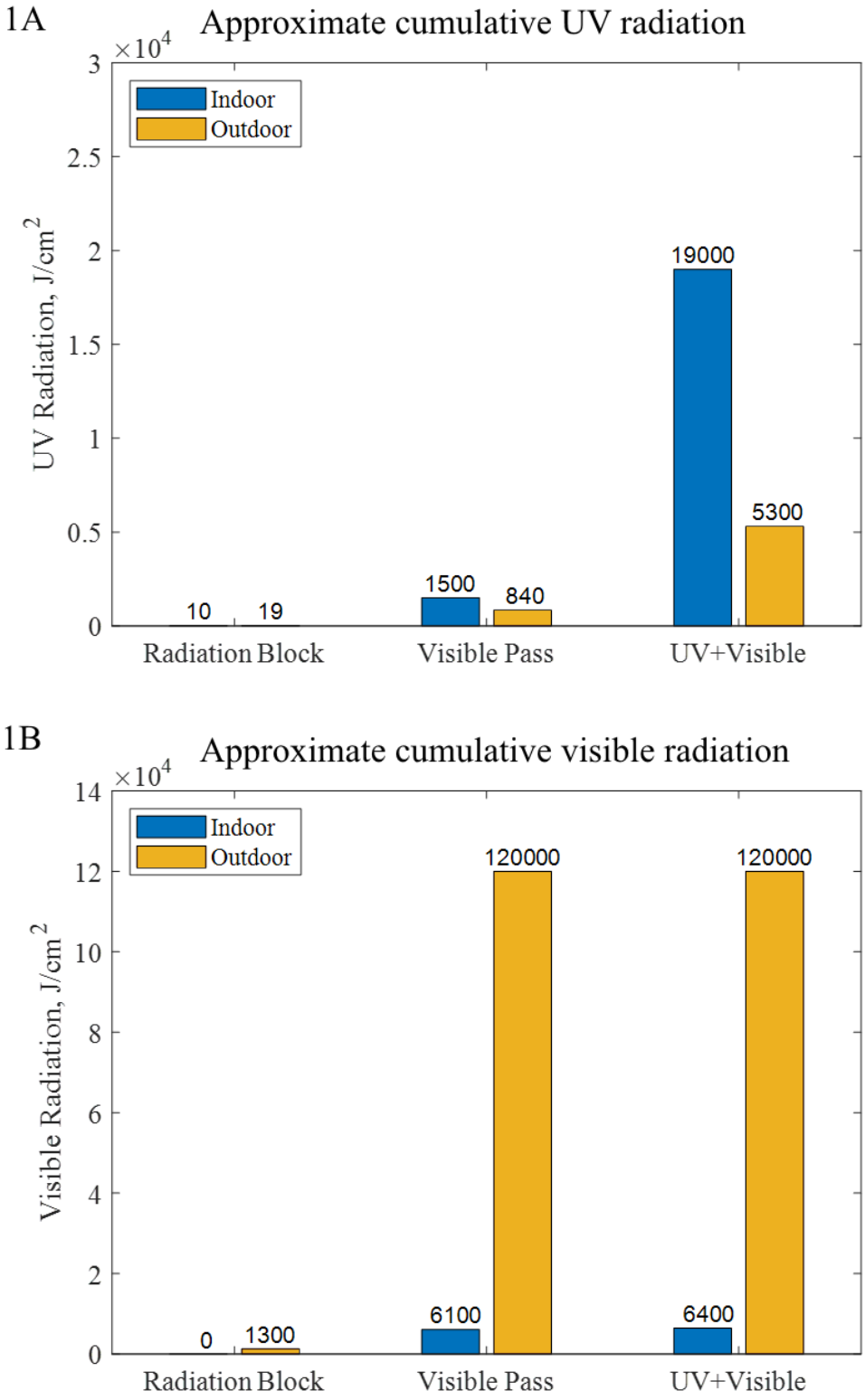

Figure 1: Approximate cumulative dosage of UV (1a) and visible (1b) radiation dosages for each treatment in both experiments. These are rough estimates for the radiation received by the litter samples, meaning they include both the reduction in radiation by the sample bag and the impact of the treatment screen.

Table 2: Approximate UV and visible radiation dosage per day (J/square cm/day)

	UV radiation			Visible radiation		
	Radiation Block	Visible Pass	UV + Visible	Radiation Block	Visible Pass	UV + Visible
Outdoor	0.2	10	63	16	1400	1500
Indoor	0.06	9	107	0	35	37

Screens also effectively established the desired treatment difference for visible light. In the outdoor experiment, the UV + Visible treatment received roughly the same amount of visible light as the Visible Pass treatment, while the Visible Pass treatment received almost two orders of magnitude more visible light than the Radiation Block treatment. Similarly, in the indoor experiment, the UV + Visible treatment received a comparable visible light dosage to the Visible Pass treatment (Figure 1).

The indoor experiment's Radiation Block treatment was measured as receiving 0 μmol photons/m^2/s at every location tested for each measurement time. This was a limitation of the sensor, which could not report decimal values, and is not an indication that the treatment received absolutely no radiation. If the sensor had reported 1 instead of 0 on each measurement, the calculated total visible light dosage for this treatment would have been 370 J/cm^2; this value can serve as an upper bound on cumulative visible light input.

In the outdoor experiment, where radiation was provided by the sun, total visible light dosage was higher than the UV dosage for all treatments. In contrast, samples in the indoor experiment received less visible light than UV radiation in both the Radiation Block and UV + Visible treatments. For the treatments exposed to visible light, the indoor samples also received two orders of magnitude less visible light than outdoor samples in the same

treatment. These outcomes were expected, as this experiment used UV lamps to provide radiation, and the lamps had low visible light output.

2 - Radiation impact on DOC

Samples in a treatment with more radiation produced more DOC than samples in treatments with lower radiation exposure (Figure 3, Tables 3). These results were statistically significant at 95% confidence in both experiments.

Table 3: Dissolved Organic Carbon Extracted Post-Treatment - mg C/(L*g litter)

	Radiation Block		Visible Pass		UV + Visible	
	Mean	SE	Mean	SE	Mean	SE
Outdoor						
Total**	558.1	3.3	779.0	4.7	932.7	6.5
Bromus	615.2	5.6	836.9	7.0	1013.8	15.5
Avena	569.8	6.7	879.5	13.4	1009.2	22.1
Hordeum	489.3	13.5	625.8	6.6	790.7	10.9
Indoor						
Total**	586.5	9.6	709.0	7.7	1071.2	10.4
Bromus	658.1	24.7	750.7	27.6	1154.0	20.0
Avena	658.2	24.1	707.3	15.8	1097.3	33.9
Hordeum	443.1	13.3	668.8	26.5	962.4	30.3

Table 3: Dissolved organic carbon extracted after the radiation treatment, as measured by the TOC analyzer. Average and standard error for both experiments are given by treatment, for (1) all samples in the treatment and (2) each species used in the study. Two asterisks (**) next to the total effect indicate a significant treatment difference for the experiment at 95% confidence.

Indoor experiment (n = 45) ANOVA results:
Species: 2 df, $F = 7.39$, $p = 0.0019$
Treatment: 2 df, $F = 63.43$, $p < 0.001$

Outdoor experiment (n = 96) ANOVA results:
Species: 2 df, $F = 23.15$, $p < 0.001$
Treatment: 2 df, $F = 75.57$, $p < 0.001$

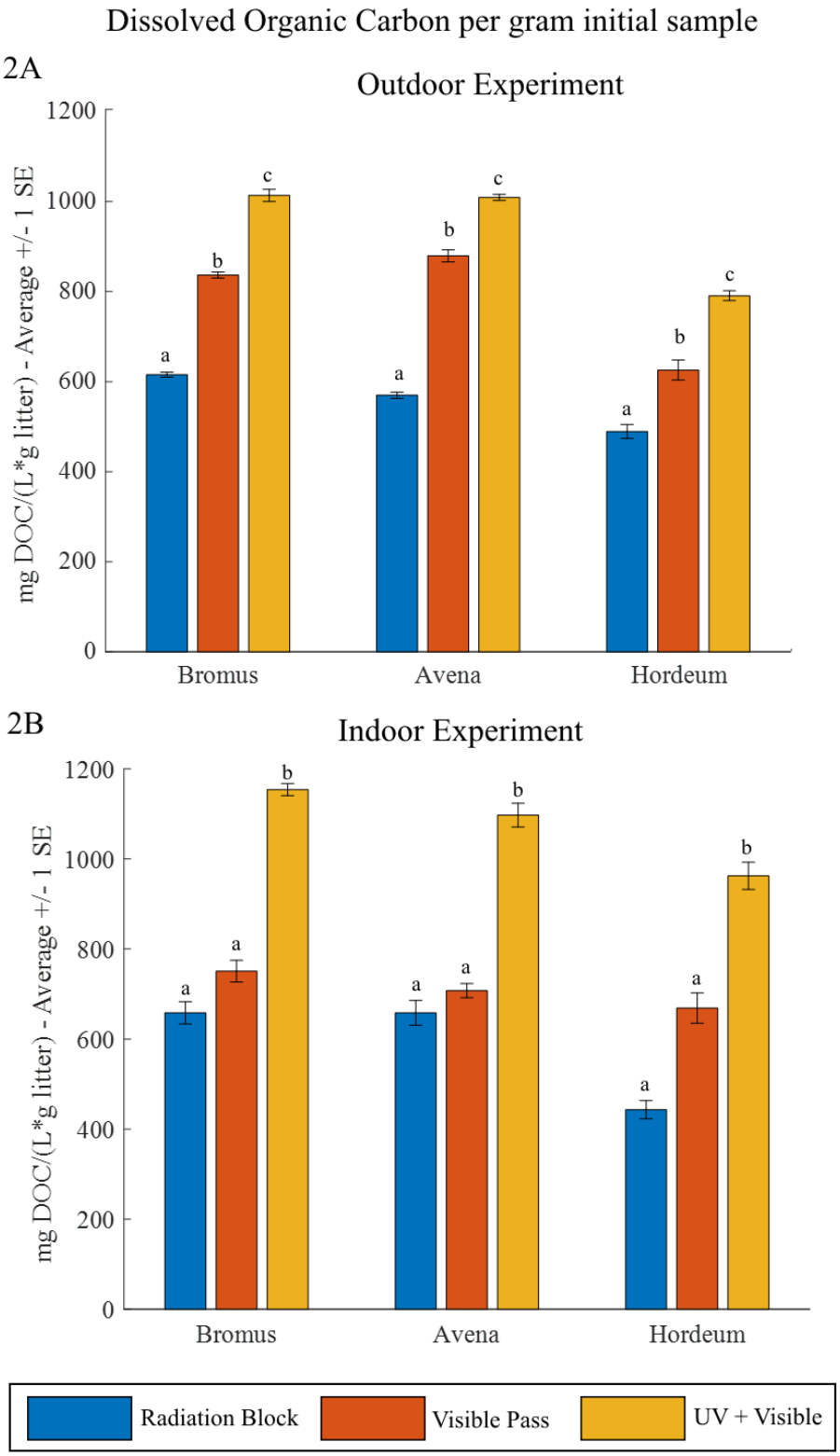

Figure 2: Dissolved organic carbon per gram initial sample as measured at the end of the radiation exposure treatment, for each species in (a) the outdoor and (b) the indoor experiment. Within species, different letters indicate a treatment difference that was significant at 95% confidence. Indoor n = 45; outdoor n = 96.

Outdoor Experiment

For the outdoor experiment, results of a two-factor ANOVA indicated a significant effect of both species ($p < 0.001$) and treatment ($p < 0.001$). Interaction between species and treatment was not found to be significant ($p = 0.1633$) and was removed before obtaining these values. DOC in the Visible Pass treatment was 40% greater than in the Radiation Block treatment, and DOC in the UV + Visible treatment was 20% greater than in the Visible Pass treatment (Table 3). Trends within species mirror the overall trend of increasing DOC production in treatments with greater radiation. For all three species, the difference between each radiation treatment was significant (Figure 2a).

Indoor Experiment

For the indoor experiment, the results of a two-factor ANOVA indicated a significant effect of both treatment ($p < 0.001$) and species ($p = 0.0019$). Interaction between species and treatment was not found to be significant ($p = 0.569$) and was removed before obtaining these values. Treatments with greater radiation resulted in extract with more DOC, with the Visible Pass samples producing 21% more DOC than the Radiation Block samples and the UV + Visible samples producing 51% more DOC than the Visible Pass samples (Table 3). For each of the three species, there was a significant difference between post-treatment DOC from the UV + Visible and post-treatment DOC from the Visible Pass treatment, but not between the Visible Pass and Radiation Block treatments (Figure 2b).

3 - Bioavailability of dissolved carbon: DOC consumption during incubation

DOC consumption during incubation generally increased with greater radiation exposure, as expected (Figure 3a and 3c; Table 4). However, high variability meant that results were not statistically significant at the species level or in the outdoor experiment, and were only marginally significant for the indoor experiment.

Contrary to expectations, DOC consumption as a fraction of the initial DOC *decreased* with increasing radiation exposure. This trend was consistent in both experiments and for most (but not all) species in each experiment (Figure 3b and 3c; Table 5).

Table 4: Amount of DOC consumed during incubation - mg C/(L*g litter)

	Radiation Block		Visible Pass		UV + Visible	
	Mean	SE	Mean	SE	Mean	SE
Outdoor						
Total	174.4	1.9	200.2	1.9	191.7	3.0
Bromus	167.1	3.5	169.8	2.2	182.3	6.3
Avena	138.1	3.0	177.5	6.6	152.3	10.9
Hordeum	221.2	7.4	250.4	3.0	246.7	4.6
Indoor						
Total*	185.8	5.0	215.6	5.0	256.9	7.3
Bromus	202.3	10.3	208.6	13.2	215.3	20.6
Avena	167.3	17.2	193.6	6.4	254.6	23.3
Hordeum	192.5	15.8	244.7	22.5	310.5	15.4

Table 4: Dissolved organic carbon consumed during the microbial incubation. Average and standard error for both experiments are given by treatment, for (1) the entire treatment and (2) each species used in the study. A single asterisk next to the total effect for an experiment indicates a significant treatment difference at 90% confidence.

Indoor experiment (n = 40) ANOVA results:
Species: 2 df, F = 1.53, p = 0.2306
Treatment: 2 df, F = 2.68, p = 0.0829

Outdoor experiment (n = 92) ANOVA results:
Species: 2 df, F = 18.05, p < 0.001
Treatment: 2 df, F =1.62, p = 0.2034

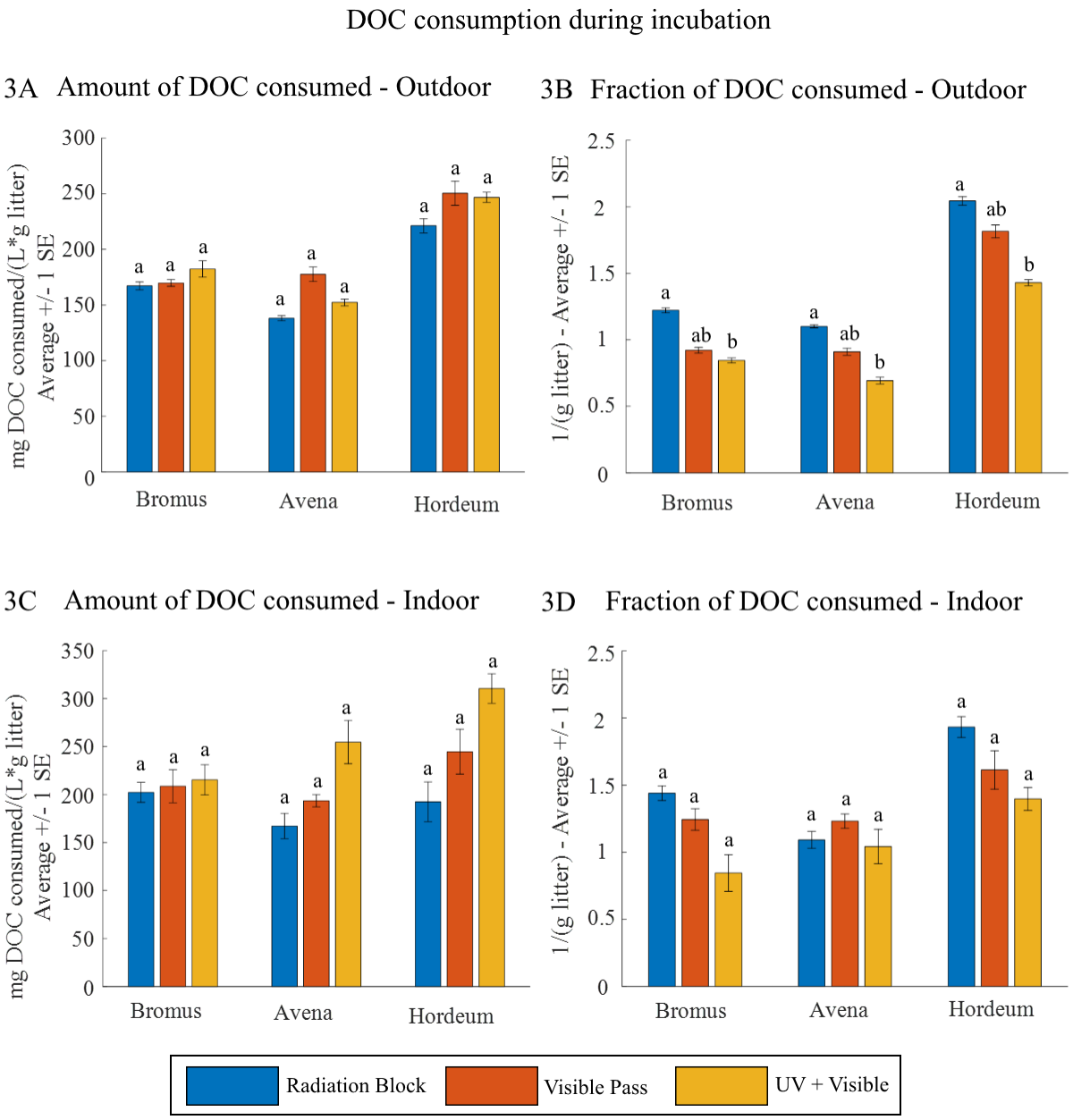

Figure 3: Dissolved organic carbon consumption during the microbial incubation for each experiment. The amount of DOC consumption (3A, 3C) is the difference between the DOC concentration at the end of the incubation and the concentration at the start of the incubation, divided by the initial mass of the litter sample. The fraction of DOC consumed (3B, 3D) is additionally divided by the initial DOC concentration at the start of the incubation. Within species, different letters indicate a treatment difference that was significant at 95% confidence. Outdoor n = 92 (3A and 3B); indoor n = 40 (3C and 3D)

Outdoor Experiment

The results of a two-factor ANOVA did not indicate a significant treatment effect ($p = 0.2034$) on the change in DOC during the incubation for the outdoor experiment. However, there was a significant species effect ($p < 0.001$). Interaction between species and treatment was not significant ($p = 0.8154$) and was removed. At the species level, no treatment was determined to have a significant difference from any other. Though differences were not statistically significant, the data for *Bromus* and *Hordeum* follow the general trend of increasing DOC consumption with greater radiation exposure. Overall data and data for *Avena* litter show lower DOC consumption in the radiation block treatment than the other two treatments, but show the highest DOC consumption for the Visible Pass treatment (Figure 3a, Table 4).

The outdoor experiment showed a decreasing trend in fraction of DOC consumed during incubation with increased radiation exposure. This result was statistically significant with high confidence ($p < 0.001$), in contrast to the incubation results for amount consumed. Interaction between species and treatment was not significant ($p = 0.4204$) and was removed. UV + Visible samples saw a 21% reduction in the fraction of DOC consumed relative to Visible Pass samples, and Visible Pass samples had a 15% reduction in fraction of DOC consumed relative to Radiation Block samples (Table 5). The trend was exhibited for all three species, and the difference between the Radiation Block and UV + Visible treatments was significant at 95% confidence for each (Figure 3b).

Table 5: Fraction of initial DOC consumed during incubation - (1/(g litter)

	Radiation Block		Visible Pass		UV + Visible	
	Mean	SE	Mean	SE	Mean	SE
Outdoor						
Total**	1.45	0.01	1.22	0.02	0.97	0.02
Bromus	1.22	0.02	0.92	0.01	0.85	0.03
Avena	1.10	0.02	0.91	0.03	0.69	0.05
Hordeum	2.04	0.02	1.82	0.03	1.43	0.02
Indoor						
Total*	1.46	0.04	1.36	0.03	1.08	0.04
Bromus	1.44	0.05	1.24	0.06	0.84	0.08
Avena	1.09	0.08	1.23	0.05	1.04	0.14
Hordeum	1.93	0.14	1.61	0.13	1.40	0.09

Table 5: Fraction of initial dissolved organic carbon consumed during the microbial incubation. Average and standard error for both experiments are given by treatment, for (1) the entire treatment and (2) each species used in the study. Two asterisks (**) next to the total effect indicate a significant treatment difference for the experiment at 95% confidence, and a single asterisk indicates a significant treatment difference at 90% confidence.

Indoor experiment (n = 40) ANOVA results:
Species: 2 df, $F = 6.85$, $p = 0.0031$
Treatment: 2 df, $F = 3.18$, $p = 0.0539$

Outdoor experiment (n = 92) ANOVA results:
Species: 2 df, $F = 102.74$, $p < 0.001$
Treatment: 2 df, $F = 23.14$, $p < 0.001$

Indoor Experiment

For the indoor experiment, a two-factor ANOVA indicated a marginally significant difference in DOC loss during incubation based on treatment ($p = 0.0829$), but no significant difference based on species ($p = 0.2306$). Interaction between species and treatment was not found to be significant ($p = 0.7023$) and was removed. DOC loss during incubation was greater in treatments with greater radiation exposure. The UV + Visible light treatment increased DOC loss by 19% over the Visible Pass treatment, and the Visible Pass treatment

increased DOC loss by 16% relative to the Radiation Block treatment. A similar trend is observed for each species, though differences between treatments were not statistically significant at the species level (Figure 3c, Table 4).

For the indoor experiment, greater radiation exposure led to a marginally significant reduction in the fraction of DOC consumed ($p = 0.0539$). Interaction between species and treatment was not significant ($p = 0.6204$) and was removed. Visible Pass samples had a 7% lower fraction consumed than Radiation Block samples, and UV + Visible samples experienced a 21% lower fraction of DOC consumed than Visible Pass samples (Table 5). Within species, this general trend was observed for *Bromus* and *Hordeum* extract, but not for *Avena* extract, where the Visible Pass treatment led to a greater fraction of DOC lost than either of the other two treatments. However, differences were not significant at the species level (Figure 3d).

4 - Bioavailability of dissolved carbon – CO2 production during incubation

As expected, extract from treatments with greater radiation showed greater CO2 production during incubation in both experiments. However, the result was not significant at the species level or in the outdoor experiment (Figures 4a and 4c; Table 6).

As with DOC consumption, when normalized to the initial DOC concentration as well as sample mass, CO2 production decreased with increasing radiation exposure. This difference was significant in the outdoor experiment (Figure 4b, Table 7), but not in the indoor experiment (Figure 4d, Table 7).

From here on, I refer to the measurement of CO2 production normalized to both initial sample mass and initial incubation DOC concentration as "normalized CO2 production," even though the CO2 data was normalized to initial sample mass for both analyses.

Table 6: CO2 production during incubation - µmol/g litter

	Radiation Block		Visible Pass		UV + Visible	
	Mean	SE	Mean	SE	Mean	SE
Outdoor						
Total	0.141	0.002	0.154	0.001	0.161	0.001
Bromus	0.140	0.003	0.139	0.002	0.147	0.003
Avena	0.118	0.002	0.142	0.003	0.153	0.005
Hordeum	0.163	0.007	0.179	0.004	0.183	0.004
Indoor						
Total**	0.123	0.003	0.141	0.003	0.167	0.003
Bromus	0.124	0.002	0.134	0.006	0.157	0.009
Avena	0.129	0.011	0.131	0.005	0.188	0.016
Hordeum	0.110	0.017	0.157	0.014	0.165	0.007

Table 6: CO2 production during the microbial incubation. Average and standard error for both experiments are given by treatment, for (1) the entire treatment and (2) each species used in the study. Two asterisks (**) next to the total effect indicate a significant treatment difference for the experiment at 95% confidence.

Indoor experiment (n = 39) ANOVA results:
Species: 2 df, F = 0.19, p = 0.8298
Treatment: 2 df, F = 3.32, p = 0.0481

Outdoor experiment (n = 94) ANOVA results:
Species: 2 df, F = 7.43, p = 0.001
Treatment: 2 df, F =1.89, p = 0.1564

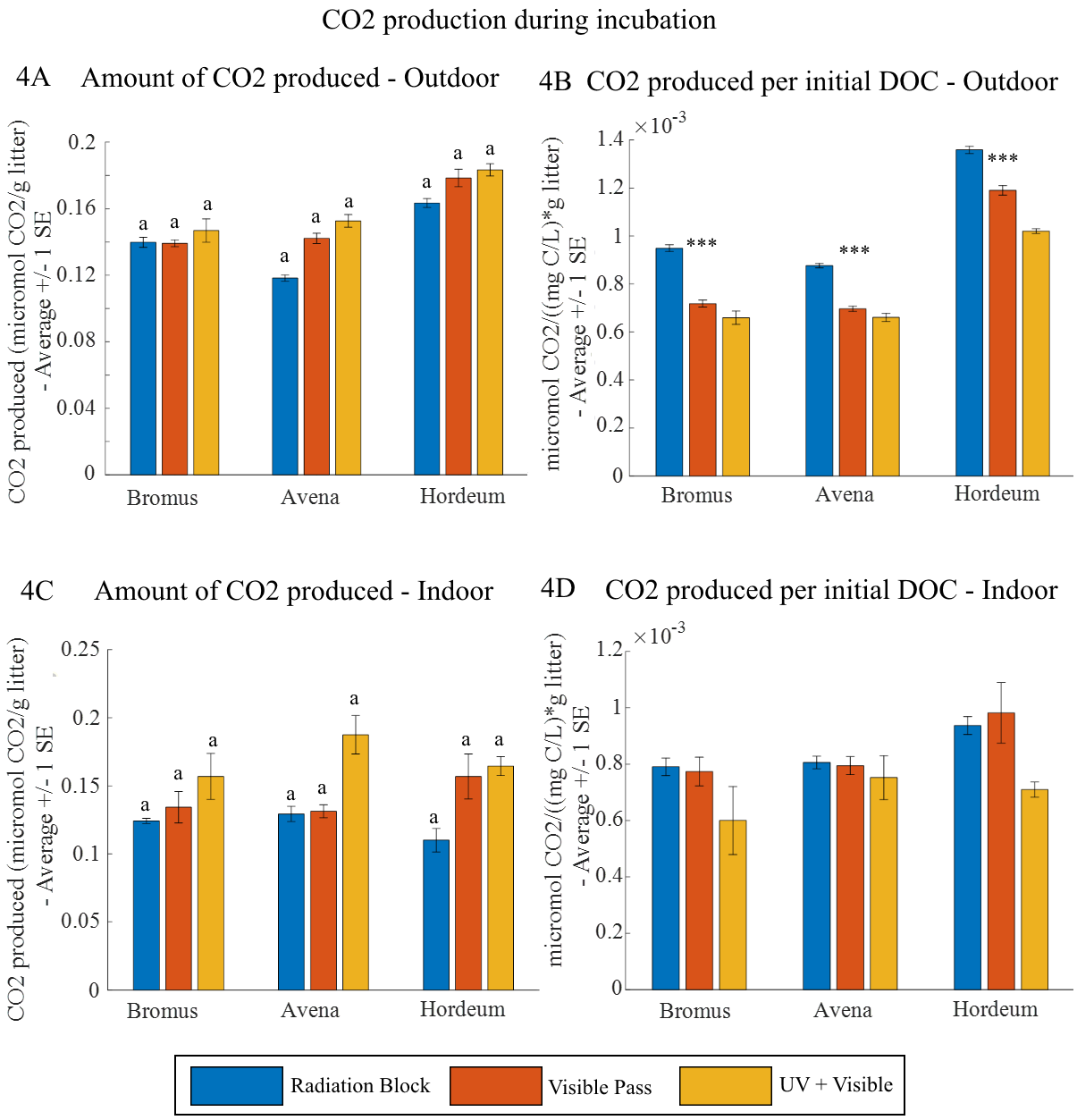

Figure 4: CO2 production during the microbial incubation, for each experiment. The amount of CO2 production (4A, 4C) was analyzed using a two-factor ANOVA. For these data, within species, different letters indicate a treatment difference that was significant at 95% confidence. The fraction of DOC consumed (4B, 4D) is additionally divided by the initial DOC concentration at the start of the incubation, and was separately analyzed using a Kruskal-Wallis one-way ANOVA for each species, with treatment as the factor. For these data, three asterisks (***) above a species group indicates a significant treatment effect for that species at 95% confidence; no asterisks means the result was not significant at 95% confidence. Outdoor n = 94 (4A) and 93 (4B); indoor n = 39 (4C and 4D).

Table 7: Normalized CO2 Production during incubation - µmol/(g litter * mg C/L)

	Radiation Block		Visible Pass		UV + Visible	
	Mean	SE	Mean	SE	Mean	SE
Outdoor						
Total**	0.00106	0.00001	0.00087	0.00001	0.00078	0.00001
Bromus	0.00095	0.00001	0.00072	0.00001	0.00066	0.00001
Avena	0.00088	0.00001	0.00070	0.00001	0.00066	0.00002
Hordeum	0.00136	0.00003	0.00119	0.00002	0.00102	0.00001
Indoor						
Total*	0.00083	0.00002	0.00085	0.00002	0.00067	0.00002
Bromus	0.00079	0.00003	0.00077	0.00002	0.0006	0.00003
Avena	0.00081	0.00005	0.00079	0.00003	0.00075	0.00011
Hordeum	0.00094	0.00012	0.00098	0.00008	0.00071	0.00003

Table 7: Normalized CO2 production during the microbial incubation. Average and standard error for both experiments are given by treatment, for (1) the entire treatment and (2) each species used in the study. This data was statistically tested using the Kruskal-Wallis one-way ANOVA with treatment or species as the factor. Two asterisks (**) next to the total effect indicate a significant treatment difference for the experiment at 95% confidence, and a single asterisk indicates a significant treatment difference at 90% confidence. Statistical test results for species are below, as well as detailed results for treatment.

Indoor experiment (n = 39) ANOVA results:
Species: 2 df, chi-square = 1.3, p = 0.5213
Treatment: 2 df, chi-square = 5.35, p = 0.0689

Outdoor experiment (n = 93) ANOVA results:
Species: 2 df, chi-square = 47.26, p < 0.001
Treatment: 2 df, chi-square = 14.33, p = 0.0008

Outdoor Experiment

For the outdoor experiment's microbial incubation, 5% more CO2 was produced in the UV + Visible treatment than in the Visible Pass treatment, and 9% more was produced in the Visible Pass treatment than in the Radiation Block treatment. This result was not statistically significant (p = 0.1564), but a significant species difference (p = 0.001) in CO2 production was observed. Interaction between species and treatment was not significant (p = 0.8566) and was removed (Table 6). At the species level, no significant difference in CO2 production was found, though the general increasing trend was still present (Figure 4a).

For the outdoor experiment, significantly more CO_2 was produced per unit initial DOC from samples exposed to more radiation ($p = 0.0008$). A significant species difference in this metric was also found ($p < 0.001$). Normalized CO_2 production was 17% lower in the Visible Pass treatment than in the Radiation Block treatment, and was 11% lower in the UV + Visible treatment than in the Visible Pass treatment. Significance was tested using the Kruskal-Wallis one-way ANOVA for non-normal data, despite being a two-factor setup, so the statistical test result for the overall data should be taken with caution (Table 7). This general trend was also observed for each of the three species, and the Kruskal-Wallis ANOVA indicated a significant treatment difference for each species (Figure 4b).

Data was also tested using the two-factor ANOVA, and results of this test indicated a significant effect of both species ($p < 0.001$) and treatment ($p < 0.001$), similar to the results of the Kruskal-Wallis ANOVA. Interaction between species and treatment was not significant ($p = 0.7183$) and was removed.

Indoor Experiment

For the indoor experiment, the two-factor ANOVA indicated that treatment ($p = 0.0481$) had a significant impact on CO_2 production, but that species ($p = 0.8298$) did not. Interaction between species and treatment was not found to be significant ($p = 0.7462$) and was removed. The UV + Visible treatment produced 19% more CO_2 than the Visible Pass treatment, and the Visible Pass treatment produced 15% more CO_2 than the Radiation Block treatment (Table 6).

Each species did exhibit the same trend as the overall data, with sizeable differences between the average CO_2 production in the Radiation Block and UV + Visible treatments. However, variability in the data was high, and treatment differences were not statistically significant at the species level (Figure 4c).

Exposure to more radiation in the indoor experiment also resulted in less normalized CO_2 production. This treatment difference was marginally significant ($p = 0.0689$), and species did not have a significant effect on CO_2 production ($p = 0.5213$). The treatment difference was not significant at the species level for any of the three species (Figure 4d). Overall, the UV + Visible treatment had 21% lower normalized CO_2 production than the Visible Pass treatment, which had 2% *higher* normalized CO_2 production than the Radiation Block treatment (Table 7). The minimal difference between the Visible Pass and Radiation Block treatments as well as the larger difference between Visible Pass and UV + Visible are both apparent at the species level despite the lack of statistical significance (Figure 4d). As with the outdoor experiment normalized CO_2 data was analyzed with the Kruskal-Wallis one-way ANOVA and should be treated with caution for the overall result.

5 - Differences in composition of DOC – Absorbance at 410 nm

Absorbance at 410 nm is a proxy for reducing sugar content in the sample, with greater absorbance indicating a greater presence of sugars. For half of the species/experiment combinations, absorbance at 410 nm was significantly higher for treatments with more radiation than it was for treatments with less radiation. This result indicates that radiation led

to a greater presence of sugars in extract (Table 8). This was significant for two species in the indoor experiment but only for one in the outdoor experiment (Figures 5a and 5b).

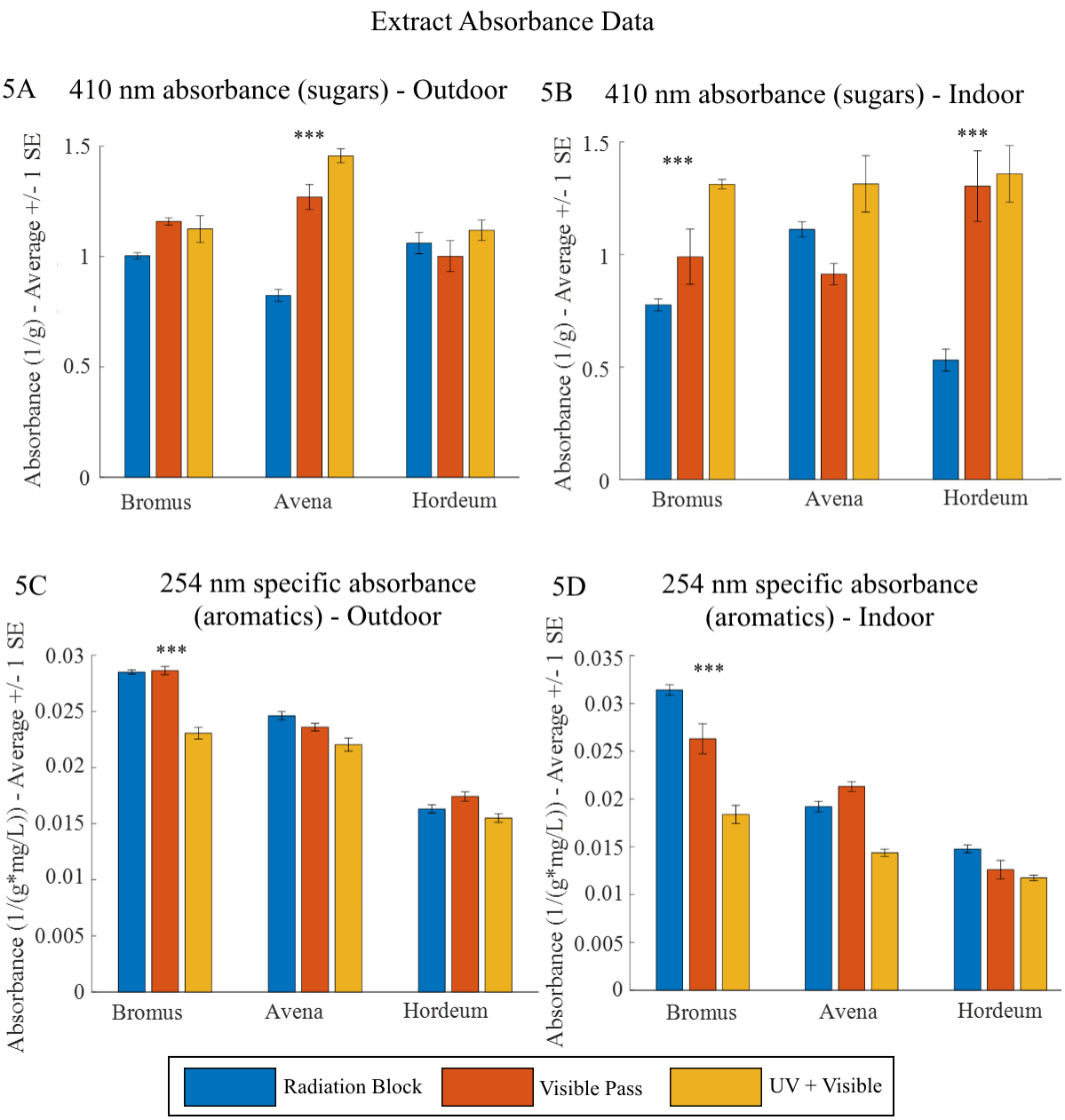

Figure 5: Absorbance at 410 nm (5A, 5B) and specific absorbance at 254 nm (5C, 5D) for each experiment. Absorbance at 410 nm is a proxy for reducing sugars in the sample extract, and specific absorbance at 254 nm is a proxy for aromatic compounds in the extract; the latter may result from lignin photodegradation. All data was analyzed using a Kruskal-Wallis one-way ANOVA for each species, with treatment as the factor. For these data, three asterisks (***) above a species group indicates a significant treatment effect for that species at 95% confidence; no asterisk means the group did not exhibit a significant treatment difference at this confidence level. Indoor n = 45 (5B) and 42 (5D); outdoor n = 94 (5A) and 93 (5C).

Table 8: Absorbance at 410 nm (reducing sugars proxy) - 1/g litter

	Radiation Block		Visible Pass		UV + Visible	
	Mean	SE	Mean	SE	Mean	SE
Outdoor						
Total**	0.965	0.013	1.143	0.014	1.229	0.019
Bromus	1.003	0.015	1.159	0.027	1.124	0.048
Avena	0.824	0.017	1.269	0.057	1.456	0.070
Hordeum	1.060	0.061	1.001	0.032	1.119	0.046
Indoor						
Total**	0.806	0.028	1.069	0.027	1.329	0.037
Bromus	0.776	0.026	0.990	0.034	1.313	0.049
Avena	1.112	0.123	0.913	0.047	1.314	0.157
Hordeum	0.530	0.021	1.304	0.126	1.358	0.126

Table 8: Absorbance of extracts at 410 nm. Average and standard error for both experiments are given by treatment, for (1) the entire treatment and (2) each species used in the study. This data was statistically tested using the Kruskal-Wallis one-way ANOVA with treatment, species, or plate used in the plate reader as the factor. Two asterisks (**) next to the total effect indicate a significant treatment difference for the experiment at 95% confidence, and a single asterisk indicates a significant treatment difference at 90% confidence. Detailed statistics for treatment are below, as well as for species and plate.

Indoor experiment (n = 45) ANOVA results:
Plate: 6 df, chi-square = 8.63, p = 0.1958
Species: 2 df, chi-square = 0.34, p = 0.8433
Treatment: 2 df, chi-square = 9.68, p = 0.002

Outdoor experiment (n = 94) ANOVA results:
Plate: 14 df, chi-square = 19, p = 0.1648
Species: 2 df, chi-square = 1.44, p = 0.4867
Treatment: 2 df, chi-square = 12.55, p = 0.0019

Outdoor Experiment

The outdoor experiment saw greater radiation exposure lead to more absorbance at 410 nm (p = 0.0019). UV + Visible treatment samples had 8% greater absorbance than Visible Pass samples, and Visible Pass samples had 18% greater absorbance than Radiation Block samples (Table 8). No significant effect of species (p = 0.4867) or of the plate used to measure samples in the plate reader (p =0.1648) was found. Significance of these overall outcomes was tested using the Kruskal-Wallis one-way ANOVA for non-normal data,

despite being a two-factor setup, so the statistical test result for the overall data should be taken with caution.

For each species (i.e. reduced to a single factor as the statistical test is designed for), treatments with more radiation led to significantly higher absorbance at 410 nm by *Avena* litter extract ($p = 0.0099$). There was no significant difference by treatment for *Bromus* extract ($p = 0.1338$) or *Hordeum* extract ($p = 0.3971$; Figure 5a).

Indoor Experiment

For the indoor experiment, a significant difference was observed between treatments ($p = 0.002$), with more radiation exposure leading to more absorbance at 410 nm. The UV + Visible treatment had 24% greater absorbance than the Visible Pass treatment, which had 33% greater absorbance than the Radiation Block treatment. No significant difference for plate ($p = 0.1958$) or species ($p = 0.8433$) was observed (Table 8). As noted above, the Kruskal-Wallis one-way ANOVA used for this data is for data with a single factor, but the experiment had a two-factor setup, so the statistical test result for the overall data should be taken with caution.

Within species, treatments with higher radiation dosage saw significantly more absorbance at 410 nm in *Bromus* extract ($p = 0.0079$) and *Hordeum* extract ($p = 0.0132$). The treatment difference for *Avena* extract was not significant ($p = 0.6126$; Figure 5b).

Table 9: Specific absorbance at 254 nm (aromatics proxy) - 1/(g litter * mg/L)

	Radiation Block		Visible Pass		UV + Visible	
	Mean	SE	Mean	SE	Mean	SE
Outdoor						
Total*	0.0232	0.0002	0.0230	0.0002	0.0202	0.0002
Bromus	0.0285	0.0002	0.0286	0.0004	0.0231	0.0004
Avena	0.0246	0.0004	0.0236	0.0003	0.0220	0.0004
Hordeum	0.0163	0.0005	0.0174	0.0006	0.0155	0.0004
Indoor						
Total**	0.0229	0.0007	0.0196	0.0004	0.0148	0.0003
Bromus	0.0314	0.0005	0.0263	0.0005	0.0184	0.0004
Avena	0.0192	0.0016	0.0213	0.0005	0.0144	0.0010
Hordeum	0.0148	0.0009	0.0126	0.0004	0.0118	0.0003

Table 9: Specific absorbance of extracts at 254 nm. Values for each sample extract were calculated by dividing the absorbance at 254 nm by the sample's initial DOC value used in the incubation. Average and standard error for both experiments are given by treatment, for (1) the entire treatment and (2) each species used in the study. This data was statistically tested using the Kruskal-Wallis one-way ANOVA with treatment, species, or plate used in the plate reader as the factor. Two asterisks (**) next to the total effect indicate a significant treatment difference for the experiment at 95% confidence, and a single asterisk indicates a significant treatment difference at 90% confidence. Detailed statistics for treatment are below, as well as for species and plate.

Indoor experiment (n = 42) ANOVA results:
Plate: 2 df, chi-square = 1.95, p = 0.3777
Species: 2 df, chi-square = 21.8, p < 0.001
Treatment: 2 df, chi-square = 7.63, p = 0.0221

Outdoor experiment (n = 93) ANOVA results:
Plate: 5 df, chi-square = 8.57, p = 0.1275
Species: 2 df, chi-square = 46.26, p < 0.001
Treatment: 2 df, chi-square = 5.02, p = 0.0814

6 - Differences in composition of DOC – Specific absorbance at 254 nm

Specific UV absorbance is a proxy for aromatic compounds in the sample, with greater absorbance indicating that more aromatic compounds are present. I am interested in this measurement as it may be influenced by the breakdown of lignin (an aromatic compound) into smaller aromatic compounds that are more water-soluble. Specific UV

absorbance results were similar between the two experiments. The indoor experiment saw significantly *reduced* specific UV absorbance with increased radiation exposure, and the same pattern was observed in the outdoor experiment, with marginal significance (Table 9). This result is the opposite of what I had initially predicted. For both experiments, a significant difference was detectable at the species level only for *Bromus* litter (Figures 5c and 5d).

Outdoor Experiment

Specific UV absorbance at 254 nm was reduced in treatments with more radiation exposure relative to treatments that received less radiation ($p = 0.0814$), with samples from the UV + Visible light treatment absorbing 12% less than samples from the Visible Pass treatment. Samples from the Visible Pass treatment had an average specific absorbance that was only 1% less than for the Radiation Block treatment. A significant species difference in specific UV absorbance ($p < 0.001$) was identified, but there was no significant plate effect ($p = 0.1275$; Table 9). As with the other absorbance metrics, this analysis used the Kruskal-Wallis one-way ANOVA despite a two-factor experimental design, so the overall result should be treated with caution.

For *Bromus* litter specifically, the UV + Visible light treatment extract had significantly lower specific UV absorbance than the other two treatments ($p = 0.0051$). No significant differences between treatments were observed for either *Avena* ($p = 0.2495$) or *Hordeum* ($p = 0.7596$), though the general trend of lower absorbance with greater radiation exposure is still visually apparent for *Avena* samples (Figure 5c).

Specific UV absorbance data for the outdoor experiment was deemed sufficiently normal to also analyze using a regular n-way ANOVA for normal data, though the test results are not directly comparable to the indoor experiment. This analysis indicated a significant difference in absorbance with treatment ($p = 0.0006$), species ($p < 0.001$), and the plate used to measure the sample's absorbance ($p = 0.0012$). No interactions were identified between any of these three factors.

Indoor Experiment

There was a significant treatment difference in specific UV absorbance at 254 nm ($p = 0.0221$) for the indoor experiment. UV + Visible treatment samples has 24% lower specific absorbance than Visible Pass samples, which had 14% lower specific absorbance than Radiation Block samples. A significant species effect was also identified ($p < 0.001$), and there was no significant plate effect ($p = 0.3777$; Table 9). Overall results should be interpreted with caution as they were obtained using the Kruskal-Wallis one-way ANOVA.

By species, only *Bromus* exhibited significantly lower specific absorbance at 254 nm ($p = 0.0039$), although the trend was visually apparent for *Hordeum* ($p = 0.1723$) as well as *Avena* ($p = 0.1142$). As with the overall result, this is consistent with the results of the outdoor experiment.

Discussion

Radiation impact on DOC

These results clearly demonstrate that radiation promotes DOC production in grass litter, with strongly significant and sizeable treatment differences apparent in both experiments and every species. This is in line with previous observations that DOC production is enhanced by exposure to radiation in other species (e.g., Vähätalo et al. 1998).

In the indoor experiment, UV radiation was the main driver of DOC production, as differences between the Visible Pass and Radiation Block treatments were not significant for any species. Radiation in the indoor experiment was provided by UV lamps with low visible light output, so this does not reflect the relative importance of each wavelength range for field conditions. However, the outdoor experiment saw a greater increase in DOC between the Visible Pass treatment and Radiation Block treatment than between the Visible Pass and UV + Visible treatments, suggesting that visible light might contribute more to DOC production than UV radiation in the field. For *Bromus* and *Hordeum* litter, each wavelength range led to about the same amount of extra DOC, so the greater overall visible light impact appears to largely result from greater visible light impact on *Avena* DOC. Taken as a whole, the results of both experiments agree with prior observations that both UV and visible light are of similar importance for photodegradation (e.g., Austin & Ballaré, 2010), indicating that this is true for DOC production as well as for litter mass loss.

Bioavailability of DOC

The outdoor experiment found weak evidence for my second hypothesis, that DOC from photodegraded litter stimulates more microbial activity. For both DOC consumption and CO2 production, trends were apparent but weak and not significant. Support for this hypothesis was stronger from the indoor experiment, though still less clear than for my first hypothesis. I suspect the lack of consistent evidence was due to variability of the incubation results, rather than the lack of an actual positive relationship between photodegradation and DOC bioavailability. There are several reasons for this. First, an increasing trend is visually apparent for most of the species, but with high variability relative to the treatment differences. Second, the indoor experiment was carried out for longer and under more controlled conditions than the outdoor experiment, and the treatment difference in the indoor experiment had greater statistical support. If my hypothesis about the relationship between bioavailability and radiation exposure is false, I would expect the longer, more controlled experiment to provide *weaker* evidence for the hypothesis or stronger evidence *against* it. However, several replicates had to be removed before analysis of the indoor experiment bioavailability data due to a likely failure to receive inoculum, and the incubation of indoor experiment samples took place before the outdoor one, suggesting that the outdoor samples may have experienced more consistent conditions during the incubation. It is likely that photodegraded litter DOC stimulates more microbial activity, but additional research, possibly with a longer treatment length, is necessary to identify this effect under field conditions.

Taken as a whole, my research indicates that DOC from photodegraded litter does stimulate more microbial activity. However, when the data is also normalized by the DOC concentration at the start of the incubation, the impact of greater radiation exposure is reversed. More radiation led to more CO2 produced and more DOC consumed, but the amount produced or consumed was smaller relative to the initial DOC concentration. While photodegradation resulted in DOC that stimulated more microbial activity, it did not do so because the compounds present were more accessible to microbes, but because the amount of DOC had increased. In fact, photodegradation seems to have *reduced* the average bioavailability of the compounds themselves.

DOC chemistry changes

Insight into changes in DOC composition can be gained from the absorbance proxy results. Absorbance at 410 nm is a proxy for the amount of sugars in the extract; therefore, the observed general trend of increasing absorbance at 410 nm with greater radiation exposure indicates that photodegradation is producing soluble sugars. No species showed a significant response in both experiments, and only one species showed a significant response in the outdoor experiment. Thus, it is possible that species differences in litter chemistry play a role in this response.

Specific absorbance at 254 nm is a proxy for aromaticity of the sample. It is of interest since lignin, one of the main compounds affected by photodegradation in past studies, contains aromatic rings and might be expected to produce soluble aromatic compounds if broken down. For both experiments, the observed response was the opposite of my initial expectation. Photodegradation reduced rather than increased the aromaticity of

litter extract, despite increasing the total amount of DOC. This may be because aromatic compounds were more strongly impacted by radiation than I expected. I had anticipated that lignin would be broken down into smaller aromatic compounds, but based on my results it is possible that lignin was degraded into non-aromatic compounds, or that lignin breakdown into aromatics was outpaced by the destruction of soluble aromatic compounds. There are several possible explanations for this observation, and my experiment was not designed in a way that allows me to determine which is correct, but this result nonetheless highlights the importance of litter chemistry to photodegradation.

Taken together, these proxy measurements clearly suggest that photodegradation causes noticeable chemical changes to litter DOC. Reduced abundance of aromatic compounds suggests a strong impact of radiation on aromatic linkages; although lignin is largely insoluble, this implies that it may also have been heavily impacted by radiation. The increased sugar content could result from breakdown of lignin, cellulose, or other plant compounds. However, if aromatic compounds were undergoing substantial breakdown due to radiation, then it seems likely that lignin breakdown contributed some of the additional sugars. This study thus provides some evidence of lignin breakdown, and less clear evidence that cellulose may also have been a source of DOC from photodegradation. These absorbance proxies are indirect approaches to understanding chemical changes and cannot conclusively confirm or reject my third hypothesis. Future photodegradation research with a more direct focus on litter and extract chemistry is needed.

There are a few noteworthy species differences in the response of the absorbance metrics to radiation exposure. In the outdoor treatment, only *Avena* had a clearly significant

increase in absorbance at 410 nm, indicating a greater production of reducing sugars with higher radiation exposure. This increase is largely driven by visible light. Similarly, *Bromus* litter was the only litter type for which aromaticity responded significantly to light, and for both experiments the majority of the reduction in aromaticity appears to result from UV radiation. As mentioned above, each of the three species used in the experiment differs in litter chemistry, and these absorbance metrics are likely an indication that this translates into a difference in photodegradation-produced DOC chemistry. Further complicating this analysis, time since senescence affects litter chemistry. Lin et al. (2015) also report changes in the relative abundance of different C fractions in *Bromus* litter during radiation exposure, with lignin making up a greater percentage of the total after longer exposure. Field observations indicated that *Bromus* and *Avena* litter used in this study had been senesced for longer than *Hordeum* litter. It is possible that the greater pre-collection radiation exposure of *Bromus* litter contributed to *Bromus* litter's strong aromaticity response in this study by increasing the relative proportion of lignin.

Conclusions

From this study, it seems clear that photodegradation makes litter carbon more water-soluble. Although it appeared to be more recalcitrant on a per-molecule basis, DOC from more photodegraded litter did lead to more total microbial activity. This means that production of DOC is a possible mechanism for photopriming. I did not directly test whether the DOC-enhanced microbial activity leads to greater subsequent decomposition of litter, but I expect that it would, based on prior observations of links between water and photopriming.

If water serves to facilitate photopriming by making litter carbon more soluble, this has important implications for the functioning of dryland environments. Arid ecosystems receive little rainfall, and in some cases precipitation is highly seasonal. However, I observed that only a couple hours of exposure to moisture following radiation treatments led to detectable differences in DOC production and bioavailability. It is possible that sources of moisture other than precipitation, such as fog or dew, could also facilitate photopriming through this mechanism. This result is in line with Gliksman et al. (2017) who observed that photopriming in an arid system only took place when dew-sourced moisture was present, but not in treatments that remained dry.

This research serves as a first step in a mechanistic differentiation between photodegradation and photopriming that could be incorporated into future photodegradation models. As mentioned above, Adair et al. (2017) found that their best-performing photodegradation model did not include photopriming. This is at odds with field and laboratory studies that have observed photopriming to occur, and in some cases to contribute a substantial fraction of the total photodegradation effect. Notably, Adair et al. (2017)'s models also included inhibition of microbial activity by radiation, but did not include any mechanism to temporally separate photopriming and microbial inhibition. Other studies indicate this time distinction is critical to photopriming. Lin et al. (2018) observed that alternating periods of light exposure and darkness facilitated photopriming even in cases where continuous light exposure did not. Gliksman et al. (2017) further observed that dew formation was needed for photopriming to happen; dew formation is most likely to happen at night when UV inhibition would not take place or in early morning when any inhibiting effect is minimal. My results suggest dew-induced photopriming happens because

photodegradation leads more litter carbon to dissolve in dew water, resulting in more microbial activity. Therefore, there is reason to believe that a model which separates inhibition and photopriming temporally, and accounts for this study's observation that photodegradation enhances water solubility of litter carbon, would more closely reflect the importance of photopriming observed in field experiments.

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Appendix 1: Experimental Setup Photos

Figure A1-1: An example of a sample bag containing *Avena* litter, affixed by hook-and-loop fasteners to the wood backing boards used in the outdoor experiment. Sample bags in the outdoor experiment were not removed from these backing boards until the end of the experiment, as separating the hook and loop fasteners repeatedly would likely have caused physical damage to the litter in the bags. As a result, the side of the bag (and litter within) that was facing upwards at the start of the experiment is the side that faced upwards for the entire experiment. Samples in the indoor experiment were not affixed in place by hook-and-loop fasteners, and were flipped each week.

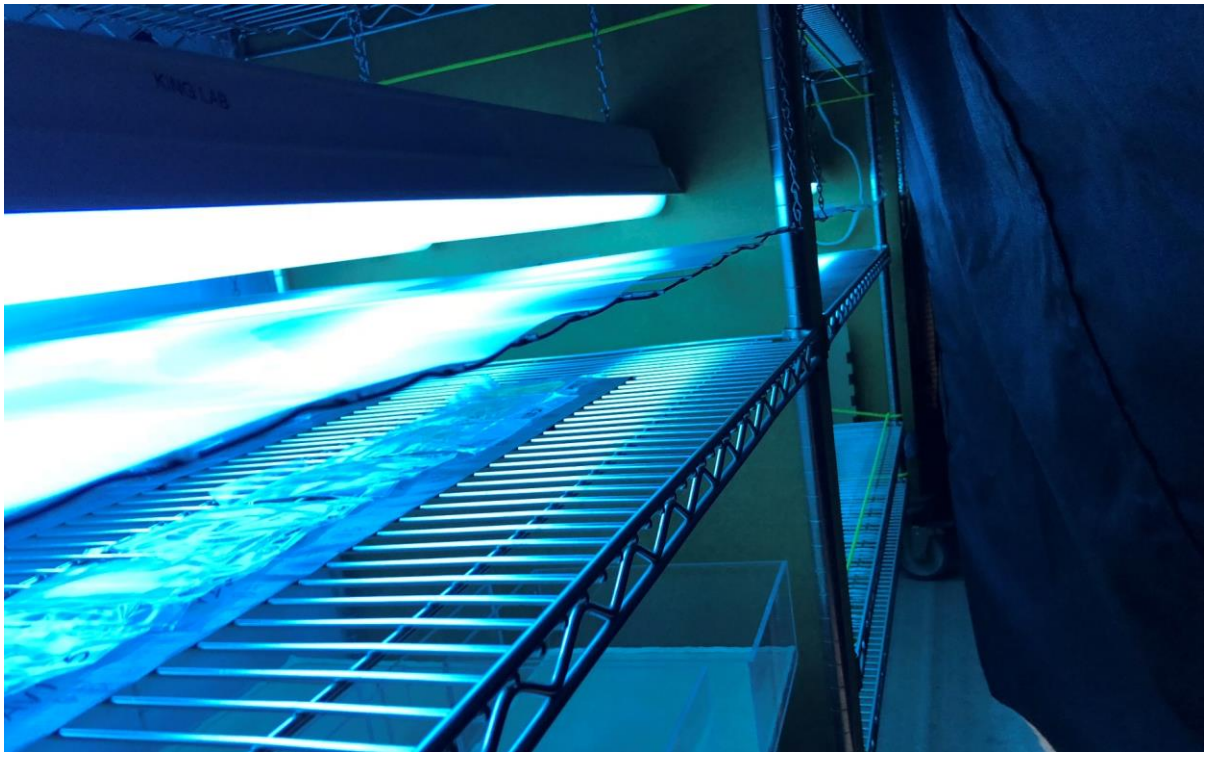

Figure A1-2: The indoor experiment setup. Visible in this picture are two of the three radiation treatments in the indoor experiment. The Visible Pass treatment screen, as well as one of the two rows of sample bags for this treatment, are visible on the left of the image. Slightly to the right of center, behind the cardboard divider, a portion of the UV + Visible treatment is visible. A second cardboard divider beyond the UV + Visible treatment obscures the Radiation Block treatment from view entirely. On the right side of the image is a shade cloth that was draped over the shelves where the experiment was set up.

Figure A1-3: The outdoor experiment, during morning. All three radiation treatments are visible; each consists of two screens adjacent to each other, and sample bags are affixed to the wooden boards beneath the screens. See figure A1-1 for a close-up view of a sample bag affixed to one of these boards. The treatments, in order from closest to farthest, are the Radiation Block treatment, the UV + Visible treatment, and the Visible Pass treatment. Bricks were used to weigh down the screens and keep them in place. In the morning, the

treatment closest to the camera in this photo is in shade for the longest time, and the treatment closest to the far wall enters the shade earliest in the evening. However, each treatment (including all the samples as well as both screens) was moved between these three locations weekly to ensure the greatest possible similarity between treatments of shadow influence. Screens were cleaned with water weekly in order to remove dust.

Appendix 2 - Dissolved Organic Carbon Pre-Treatment

Table A2-1: Approximate pre-treatment extract DOC concentration for each species				
Species	*Avena*	*Bromus*	*Hordeum*	Extraction Blanks
Dissolved organic carbon concentration Avg. +/- Standard Error	138.7 +/- 13.7 mg/L (n = 6)	152.7 +/- 6.5 mg/L (n = 6)	96.0 +/- 9.8 mg/L (n = 6)	3.779 +/- 0.5 mg/L (n = 4)

Table A2-1: Approximate pre-treatment extract DOC concentration, for each species. Values were measured on a TOC analyzer on October 2nd – 3rd, 2018, but the litter extract used had been extracted around the time the two experiments started and frozen until measurement. Extraction blanks were water that had been passed through a pre-combusted glass fiber filter at the same time as these extracts were filtered.