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1   **Using 2D NMR spectroscopy to assess effects of UV radiation on cell wall chemistry**
2   **during litter decomposition**

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Abstract

Litter chemistry is one of the most studied controls on decomposition in terrestrial ecosystems. Solar radiation has been shown to increase litter decomposition rates in arid ecosystems through the process of photodegradation. However, it remains unclear how photodegradation affects litter chemistry, especially the abundance and composition of lignin, which is thought to play a key role in photodegradation. Using two-dimensional nuclear magnetic resonance (2D NMR) spectroscopic methods, we quantified the molecular-level changes in litter chemistry associated with photodegradation. Litter of *Bromus diandrus* was exposed in the field to two levels of radiation (with and without ultraviolet (UV) wavelengths) and two durations of exposure (2.5 months during summer, and one year). Through fiber analysis by sequential digestion, we found that the litter hemicellulose fraction decreased significantly from 31.6% to 24.9% after one year of decomposition. In litter exposed for one year, the hemicellulose fraction was significantly lower in litter with UV exposure compared to litter without UV exposure (23.8% vs. 25.9%). These results indicate that UV photodegradation has a small but significant effect on litter chemistry compared to other decomposition processes. Even though fiber analysis showed no loss of total lignin, 2D NMR analysis demonstrated that UV exposure reduced the major lignin structural units containing β -aryl ether inter-unit linkages by 9% and decreased the relative abundance of lignin *p*-hydroxyphenyl units by 20%. The 2D NMR analysis also revealed that lignin guaiacyl units were preferentially lost after one year of decomposition relative to the reference material, but no effects of UV exposure on guaiacyl were observed. These results suggest that photodegradation causes partial degradation, not necessarily complete breakdown, of lignin structures. Our data also

36 demonstrate that applications of 2D NMR methods are valuable for acquiring detailed
37 information on lignin and polysaccharide chemistry during both biotic and abiotic
38 decomposition processes.

39 **Keywords**

40 photo-oxidation, photo-mineralization, photo-priming, cellulose, dryland, HSQC
41 (heteronuclear single-quantum coherence)

Introduction

Litter chemistry is perhaps the most studied control on litter decomposition in terrestrial ecosystems (e.g. Amin et al. 2014; Bertrand et al. 2006; Melillo et al. 1982; Talbot et al. 2011). Together with climatic variables (i.e. temperature, precipitation, and actual evapotranspiration), litter chemistry has been shown to reasonably predict litter decomposition rates (Aerts 1997; Moore et al. 1999). However, models based on litter chemistry and climate tend to under-estimate decomposition rates in arid ecosystems (Adair et al. 2008; Parton et al. 2007; Schaefer et al. 1985). This discrepancy may be explained in part by photodegradation, the process through which solar radiation contributes to organic matter decomposition (reviewed by King et al. 2012). Photodegradation *directly* breaks down plant litter through photochemical oxidation and releases gases such as CO₂, CO, and CH₄ (Brandt et al. 2009; Lee et al. 2012; Schade et al. 1999). Photodegradation also *indirectly* contributes to litter decomposition by affecting litter chemistry, because solar radiation can partially degrade litter and make it more vulnerable to microbial decomposition (Foereid et al. 2010; Frouz et al. 2011; Wang et al. 2015). Recently, photodegradation has been suggested to influence organic matter turnover in the surface soil (Mayer et al. 2012). For example, Feng et al. (2011) found that photodegradation increased the solubility of soil organic matter and potentially contributed to soil C loss through leaching. However, our understanding of the chemical mechanisms underlying photodegradation is still incomplete.

Photodegradation is generally assumed to increase the breakdown of lignin (Austin and Ballaré 2010; King et al. 2012; Song et al. 2013), which exhibits strong absorption of both ultraviolet (UV) and shortwave visible radiation (George et al. 2005). However,

contradictory results have been reported with regard to changes in lignin content during photodegradation of plant litter. Using the acid-detergent method (Van Soest 1963), Song et al. (2014) found that exposure to UV radiation increased loss of lignin, whereas Kirschbaum et al. (2011) and Lin and King (2014) found no significant change in lignin content following UV exposure. Focusing on lignin content alone does not advance our mechanistic understanding of the role of lignin during litter photodegradation. This knowledge gap further hinders our ability to predict the contribution of photodegradation to litter decomposition.

Recent studies have started to explore how lignin chemical composition and structure change during litter photodegradation. Feng et al. (2011) found that UV photodegradation increased the breakdown of aliphatic substances in corn (*Zea mays*) and loblolly pine (*Pinus taeda*) litter, but photodegradation did not affect lignin-derived phenols in water-extractable fractions. Frouz et al. (2011), on the other hand, found that photodegradation enhanced loss of lignin syringyl units of bushgrass (*Calamagrostis epigejos*) litter. These inconsistent changes in lignin chemistry in response to litter photodegradation emphasize the need for more in-depth investigation of the chemical mechanisms behind photodegradation.

New methods in solution-state nuclear magnetic resonance (NMR) spectroscopy have been developed in recent years to enable rapid evaluation of lignin and polysaccharide structures, even on (unfractionated) whole cell walls or whole plant material (Kim and Ralph 2010; Kim et al. 2008; Mansfield et al. 2012). The swelling of ball-milled cell wall material in organic solvent produces a gel that allows the use of two-dimensional (2D) ^1H – ^{13}C heteronuclear single-quantum coherence (HSQC) NMR spectroscopy for

88 relatively detailed characterization of lignin and polysaccharide (Kim and Ralph 2010;
89 Mansfield et al. 2012). In principle, this 2D NMR method provides compositional
90 information on whole lignin, not the lignin components released by degradative methods,
91 such as thioacidolysis, cupric oxidation, tetramethylammonium hydroxide
92 thermochemolysis, and hydrolysis. The 2D NMR method does not degrade or alter cell
93 wall chemistry beyond sonication and ball-milling (Kim et al. 2008). Furthermore, it
94 characterizes many key features of plant cell walls, including lignin units, lignin inter-
95 unit linkages, and hemicellulose, most of which cannot be inferred from one-dimensional
96 solid-state ^{13}C NMR experiments. Integration of 2D NMR contours generates highly
97 reproducible measurements (within 5%) of cell wall components and has been used in
98 comparative studies on genetic modification of cell walls and litter decomposition (e.g.,
99 Petrik et al. 2014; Talbot et al. 2011; Wilkerson et al. 2014; Yelle et al. 2013). This
100 method also provides estimates of lignin units that are comparable to other conventional
101 methods, including thioacidolysis, nitrobenzene oxidation, and derivatization followed by
102 reductive cleavage (Mansfield et al. 2012). The method tends to overestimate the absolute
103 abundance of the terminal end units (e.g., *p*-coumarate units) because of their long
104 relaxation times compared to those of the bulk polymers. In addition, cellulose
105 abundances are underestimated in the cell wall gels because the crystalline cellulose does
106 not swell in solvent. Nevertheless, the 2D NMR method is accurate in providing
107 comparative information between samples (Mansfield et al. 2012). It has been reported to
108 offer better resolution of hemicelluloses and provides information on natural acetylation
109 that is not available in a cell wall dissolution method based on acetylation (Kim et al.
110 2008). Therefore we chose to employ the 2D NMR method to examine changes in lignin

and hemicellulose for litter subjected to photodegradation and other decomposition processes.

The aim of this study was to quantify the molecular-level changes in litter chemistry with photodegradation. Samples of a common grassland litter were treated with either ambient or reduced UV radiation under field conditions for two durations, 2.5 months or one year. Changes in lignin units, lignin inter-unit linkages, and hemicelluloses were studied using 2D NMR spectroscopy. Differences in litter chemistry between the degraded samples and the reference samples were attributed to decomposition over time. We interpreted the differences in litter chemistry between UV treatments as the result of UV photodegradation, which includes both *direct* (abiotic photo-oxidation) and *indirect* (enhancement of microbial decomposition) effects of UV exposure.

Materials and methods

Litter samples of *Bromus diandrus* were exposed to two levels of UV radiation at the University of California's Sedgwick Reserve in Santa Ynez, California, USA (43°42'N, 120°2'W, approximately 35 km northwest of Santa Barbara, California). The site is dominated by European annual grasses, including *B. diandrus*. The site experiences a Mediterranean climate with alternating hot, dry summers from May to October and cool, rainy winters from November through April. *Bromus* species are commonly found in temperate climates across the world, and many of them are considered to be invasive in North America (D'Antonio and Vitousek 1992). Steel frames with plastic louvers that either pass or block UV radiation (UV-pass or UV-block treatments) were used to manipulate UV radiation received by grass litter. These frames were effective in

manipulating UV radiation and allowed penetration of rainfall. There was no difference in air temperature or relative humidity between UV-pass and UV-block treatments. A detailed description of the frames, including dimensions, placement, and optical characteristics, is provided by Lin and King (2014). Litter samples (leaves and stems) were exposed to UV treatments in the field for two durations (2.5 months and one year). For samples with 2.5 months of UV exposure, UV-pass and UV-block screens ($n = 10$) were placed above naturally senesced *B. diandrus* litter from mid August to late October, 2011 to capture short-term UV effects during the dry season. Only litter from the very top of the litter layer was collected because this litter was consistently exposed to solar radiation. Approximately 5 g of litter were collected from underneath each screen. For litter with one year of UV exposure, recently senesced litter was collected from the field site in July 2011, placed in aluminum mesh bags, and suspended 5 cm under the UV-pass and UV-block screens (above the litter layer; $n = 10$) from late August 2011 to early September 2012 to capture longer-term UV effects. Mass loss data of this set of litter samples were reported in Lin and King (2014). There were 5 g of litter in each mesh bag before the field exposure, and approximately 4 g remained after one year of field exposure. Although aluminum mesh bags were only used for litter with one year of UV treatment and not for the litter exposed for 2.5 months, one year of UV-pass treatment still resulted in 2.5-fold higher UV exposure than 2.5 months of UV-pass treatment (183 MJ/m² vs. 49 MJ/m²). We use these two sets of litter to represent two different dosages of UV radiation. For the reference material (time 0), approximately 5 g of recently senesced litter was collected in July 2011 from each of 10 randomly-selected plots in an open area,

adjacent to the UV treatment site. The reference material was stored in the dark under laboratory conditions until further analysis.

After UV treatments, litter samples were collected from the field, sorted to remove green plants, arthropods, and visible dust, oven-dried for two days at 55 °C, and ground using a mini Wiley mill with US standard #20 mesh (Thomas Scientific, Swedesboro, New Jersey, USA). To quantify litter fiber fractions, ground subsamples (~0.5 g) were analyzed by a sequential digestion procedure (Van Soest 1963; $n = 10$; Type 200 Fiber Analyzer, ANKOM Technology, Macedon, New York, USA), hereafter referred to as “fiber analysis.” The fiber fractions include the “cell solubles” (soluble carbohydrates, proteins, and lipids), hemicelluloses, cellulose, and lignin fractions. Because no mass loss data were available for litter with 2.5-months of exposure, we can not report changes in fiber fractions on a mass basis; instead, we report the proportions of fiber fractions in percentages.

The 2D ^1H – ^{13}C HSQC NMR spectroscopy was used to characterize the lignin composition and structure of litter cell walls following the protocol described in Kim and Ralph (2010) and Mansfield et al. (2012). In short, for a subset of samples ($n = 3$), 1 g of ground litter tissue was sequentially extracted with water, 80% (vol/vol) ethanol, and acetone. Each solvent extraction was repeated three times for a total of nine extractions. These extractions remove soluble compounds (e.g. starch, protein, and polyphenols) that may distort the examination of cell wall material. During our extraction procedure for the 2D NMR analysis, some soluble lignin and hemicellulose-derived compounds were removed, and their responses to time and UV treatments were not examined here. A subsample of the extracted cell wall material (~250 mg) was ground again using a ball

mill (Planetary Micro Mill Pulverisette 7 premium line, Fritsch, Idar-Oberstein, Germany) with 20 ml zirconium dioxide (ZrO₂) grinding jars and ten 10-mm ZrO₂ ball bearings in each jar for 45 min (5 min pause with every 5 min grinding; actual grinding time, 25 min). Then, 30 mg of ball-milled isolated cell wall material was transferred to a 5-mm NMR tube, followed by 500 µl of pre-mixed 4:1 dimethylsulfoxide (DMSO-*d*₆)/pyridine-*d*₅ (vol/vol). The NMR tubes were sonicated until cell wall material and solvent formed a gel. The 2D ¹H–¹³C HSQC NMR spectra were acquired on a Bruker AVANCE 500 Spectrometer (500 MHz; Rheinstetten, Germany) with a cryogenically-cooled triple-resonance inverse NMR probe. The detailed set-up of NMR experiments can be found in Mansfield et al. (2012). NMR spectral were processed using Bruker's Topspin 3.1 software.

Resonance assignments were confirmed with the “NMR database of lignin and cell wall model compounds” (Ralph et al. 2004) and additional references (Kim and Ralph 2010; Talbot et al. 2011; Yelle et al. 2013). Relative abundances of lignin syringyl (S), guaiacyl (G), and *p*-hydroxyphenyl (H) units were determined by integrating S-2/6, G-2, and H-2/6 C–H correlations in the aromatic region of the 2D NMR spectra, respectively (Fig. 1d-f; 7.0/100–8.3/150 ppm). Lignin methoxyl (OMe), the α-position of the lignin β-aryl-ether (L_{Aα}), and acetylated xylan units (2-*O*-Ac-β-D-Xylp and 3-*O*-Ac-β-D-Xylp) were also integrated in the aliphatic region of the 2D NMR spectra (Fig. 1a-c; 2.7/50–6.0/95 ppm). Integration regions for the above features can be found in Supplementary Table 1. Abundances of L_{Aα}, 2-*O*-Ac-β-D-Xylp, and 3-*O*-Ac-β-D-Xylp were evaluated by dividing their integrals by the integral of OMe, as OMe was found to be relatively stable during acid and enzymatic degradation (Lundquist and Lundgren 1972; Yelle et al. 2013).

A student's T-test was used to compare effects of UV treatments on fiber fractions and cell wall chemical features at each exposure duration (SPSS 20, IBM Corporation). Before using the T-test, the data were checked for equality of variances using Levene's test. If equal variances could not be assumed, the degrees of freedom of the T-statistic were adjusted using the Welch-Satterthwaite method.

Results and Discussion

Litter fiber composition was significantly altered over time (Table 1). The fraction of litter hemicellulose, averaged across the two UV treatments, decreased from 31.6% in the reference material to 28.6% and 24.9% after 2.5 months (T-test, $P < 0.001$, $df = 28$) and one year of decomposition (T-test, $P < 0.001$, $df = 28$), respectively. Across the two UV treatments, the fraction of cell solubles increased from 25.5% in the reference material to 28.6% and 33.1% after 2.5 months (T-test, $P < 0.001$, $df = 28$) and one year of decomposition (T-test, $P < 0.001$, $df = 28$), respectively. These changes over time were larger in magnitude than changes induced by UV exposure. For litter exposed for 2.5 months, there were no significant effects of UV treatment except for a marginally significant effect of UV exposure on the cellulose fraction (T-test, $P = 0.086$, $df = 18$). For litter exposed for one year, all four fiber fractions were affected by UV treatments, but the change in hemicellulose fraction was the greatest in magnitude. The hemicellulose fraction was smaller in the UV pass compared to the UV block treatment (23.8% vs. 25.9%; T-test, $P < 0.001$, $df = 18$); cell solubles, cellulose, and lignin fractions were all higher in the UV pass treatment.

Compared to the reference sample, degraded samples had broadened contours along the proton dimension of the 2D NMR spectra (Figure 1). This phenomenon could be induced by inclusion of dust and soil particles or association with metals; however, it is commonly indicative of degradation of plant samples caused by enzymes, hydrothermal treatments, and acids (Samuel et al. 2011; Yelle et al. 2013). Therefore, it is likely that the chemical complexity of litter cell wall material increased after field decomposition relative to the already complex but nevertheless well-defined and limited structural types in the native cell wall. Further studies are needed to verify these hypotheses.

Aromatic regions of the NMR spectra showed that lignin syringyl (S) and guaiacyl (G) units were much more abundant than *p*-hydroxyphenyl (H) units in this grass material (Fig. 1d). Integration of lignin units showed that the abundance of G units decreased from 60% in the reference material to 52% across the two UV treatments after one year (Table 1, Fig. 1e and f; T-test, $P = 0.005$, $df = 7$), which corresponded to increases in S units (T-test, $P = 0.005$, $df = 7$) and to marginal increases in H units (T-test, $P = 0.075$, $df = 7$). The UV treatments did not affect levels of S and G units; however, it marginally decreased abundance of H units after 2.5 months (T-test, $P = 0.058$, $df = 4$) and one year (T-test, $P = 0.066$, $df = 4$). These results suggest that lignin structure underwent significant changes during one year of decomposition, and effects of UV photodegradation were small relative to those that occurred over time.

Aliphatic regions of the NMR spectra showed that β -aryl ethers ($L_{A\alpha}$) were the dominant linkage among lignin units (Fig. 1a-c). A small phenylcoumaran (L_B) signal was present in the reference sample, but not in degraded samples, suggesting that L_B linkages were vulnerable to decomposition. Integration results showed that levels of $L_{A\alpha}$

247 did not change over time, but one year of UV-pass treatment had 9% lower $L_{A\alpha}$ linkages
248 than the UV-block treatment (Fig. 2a: T-test, $P = 0.047$, $df = 4$). This result suggests that
249 the β -aryl ether linkages are degraded upon exposure to UV radiation, which is consistent
250 with previous studies on photodegradation of wood lignin (Argyropoulos and Sun 1996;
251 Lanzaunga and Bietti 2000). It is unclear why the levels of 3-*O*-Ac- β -D-Xylp were
252 higher in degraded samples than in the reference material, but one year of the UV-pass
253 treatment had less 2-*O*-Ac- β -D-Xylp (Fig. 2b: T-test, $P = 0.014$, $df = 4$) and marginally
254 fewer 3-*O*-Ac- β -D-Xylp units (Fig. 2c: T-test, $P = 0.083$, $df = 4$) than the UV-block
255 treatment. These reductions in xylan features were consistent with the fiber analysis result
256 showing that UV exposure reduced the hemicellulose fraction (Table 1). These results
257 indicate that exposure to UV radiation induced degradation of lignin and hemicelluloses.

258 Both fiber analysis and 2D NMR spectroscopy showed that changes in litter
259 chemistry over time were more prominent than those induced by UV treatments (Table
260 1). This result is not surprising given that many previous field experiments have shown a
261 significant but small contribution of photodegradation to overall litter mass loss
262 (reviewed by King et al. 2012). In our study, microbial decomposition was likely
263 responsible for the majority of changes in litter chemistry over time, despite the fact that
264 litter samples were not in direct contact with soil. For example, microorganisms may
265 have come in contact with the litter through aeolian transport, and endophytes that live
266 within the litter could also have contributed to decomposition. Microbial decomposition
267 of litter could have been sustained by atmospheric water vapor in our Mediterranean
268 climate, without water input from soil (Dirks et al. 2010). Therefore, our results indicate

that UV photodegradation has a small effect on litter chemistry compared to microbial decomposition.

The 2D NMR spectroscopy data demonstrate that UV exposure degraded the dominant inter-unit linkages in β -aryl ethers and lignin H units (Table 1 and Fig. 2a). Although the fiber analysis suggests that the lignin fraction was higher with UV pass compared to UV block after one year (Table 1), this increase in lignin fraction could simply be a reflection of the loss of hemicellulose or accumulation of microbial by-products (Couêteaux et al. 1995). Together, these data suggest that photodegradation weakens the lignin structure but may not completely break down lignin molecules. This pattern of partial degradation of lignin structure might explain results in previous studies that reported no responses of lignin content to UV treatments (Baker and Allison 2015; Brandt et al. 2010; Kirschbaum et al. 2011). More importantly, this pattern represents an important mechanism through which solar radiation may increase exposure of cell wall compounds to extracellular enzymes and consequently increase litter biodegradability (i.e. photo-priming, Bornman et al. 2015; Foereid et al. 2010; Wang et al. 2015). Photo-priming may occur during radiation exposure (e.g. this study; Baker and Allison 2015) or after the incorporation of litter into the soil (Foereid et al. 2010). Overall, our 2D NMR spectroscopy data offer novel empirical evidence to support the photo-priming hypothesis (Bornman et al. 2015).

In addition to effects on lignin structure, both fiber analysis and 2D NMR spectroscopy results support the idea that UV exposure degraded hemicellulose structure (Table 1 and Fig. 2). This result falls in line with findings from several field experiments (Baker and Allison 2015; Brandt et al. 2010; Brandt et al. 2007). However, mechanisms

behind abiotic photo-oxidation of hemicellulose, such as acetylated xylan, are less studied (except Yamagishi et al. 1970). Previous studies of cellulose acetate offer key insights into the photodegradation processes of acetylated xylan, because these two compounds are structurally analogous. Both compounds can be degraded by microbial carbohydrate esterases (Biely 2012; Puls et al. 2011), and UV exposure has been found to increase enzymatic degradation of cellulose acetate (Ishigaki et al. 2002; Jang et al. 2007). Therefore, it is likely that UV radiation facilitated microbial degradation of acetylated xylan (Fig. 2). Thus, photo-oxidation of acetylated xylan would be a second mechanism through which UV radiation may increase litter biodegradability.

Results from 2D NMR spectroscopy demonstrate that the relative abundance of lignin G units decreased significantly after one year of field decomposition, which is consistent with several studies that reported preferential loss of G units during early stages of litter decomposition (Christmas and Oglesby 1971; Kögel 1986; Quideau et al. 2005). However, other studies have found slower loss of G units relative to other lignin units, especially during later stages in litter decomposition (Bahri et al. 2006; Bertrand et al. 2006; Talbot et al. 2011), suggesting that changes in lignin G units during decomposition likely depend on the stage of decomposition and also on the species of litter material.

In conclusion, we found significant loss of hemicelluloses and lignin G units of *B. diandrus* litter over one year of field decomposition, driven primarily by microbial decomposition. Exposure to UV radiation induced partial lignin degradation that was evidenced by loss of β -aryl ethers and lignin H units, but was not apparent as a change in lignin fraction. Our results indicate that UV photodegradation has a small but significant effect on litter chemistry compared to microbial decomposition. These data suggest that

degradation of lignin and hemicelluloses are important pathways through which UV radiation increases litter degradability. Our study also demonstrates the effectiveness of 2D NMR spectroscopy in obtaining detailed comparative information about litter chemical composition that can lead to a better understanding of decomposition processes and C cycling in general.

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Table 1. Effects of UV radiation exposure and time of exposure on litter fiber fractions ($n = 10$, percentages by mass, fiber analysis) and lignin units ($n = 3$, percentages by mass, 2D NMR spectroscopy).

		Exposure time					
		2.5 months			1 year		
		UV-pass	UV-block	<i>P</i> [†]	UV-pass	UV-block	<i>P</i>
Fiber fractions							
% Cell solubles	25.5 (0.6)	28.5 (0.7)	28.7 (0.7)	n.s.	33.4 (0.3)	32.7 (0.3)	0.099
% Hemicelluloses	31.6 (0.3)	28.5 (0.5)	28.7 (0.5)	n.s.	23.8 (0.3)	25.9 (0.3)	< 0.001
% Cellulose	39.7 (0.5)	39.8 (0.3)	39.0 (0.3)	0.086	38.8 (0.2)	37.9 (0.3)	0.018
% Lignin	3.2 (0.2)	3.3 (0.2)	3.6 (0.2)	n.s.	4.0 (0.2)	3.5 (0.2)	0.068
Lignin units [‡]							
% Syringyl (S)	34.5 (1.2)	33.2 (2.0)	33.4 (1.6)	n.s.	39.9 (1.6)	41.4 (0.6)	n.s.
% Guaiacyl (G)	60.0 (0.8)	60.8 (2.4)	59.4 (1.7)	n.s.	53.8 (2.0)	50.7 (1.0)	n.s.
% <i>p</i> -Hydroxyphenyl (H)	5.5 (0.6)	6.0 (0.4)	7.2 (0.1)	0.058	6.3 (0.4)	7.9 (0.5)	0.066

Notes: Values are means with S.E. in parentheses. Bold results indicate significant differences at $\alpha = 0.05$ level.

* See Methods for the definitions of reference litter.

[†] P values of T-tests between UV treatments in a given exposure time.

[‡] Refer to Supplementary Table 1 for the integration areas of the lignin units.

Figure Captions:

Figure 1. 2D ^1H - ^{13}C HSQC NMR spectra of cell wall gel of *Bromus diandrus* litter in 4:1 DMSO- d_6 /pyridine- d_5 (vol:vol) in the aliphatic region (**a-c**) and the aromatic region (**d-f**). Spectra are aligned vertically to represent samples from the following treatments: reference, one year of UV-block, and one year of UV-pass. Contours in the aromatic region are integrated to estimate S/G/H ratios. Contours in the aliphatic region are integrated to estimate lignin methoxyl, lignin inter-unit linkage types, and acetylated xylan.

Figure 2. Effects of UV treatments (UV-pass, UV-block) and exposure duration on the ratios to lignin methoxyl (OMe) of (**a**) lignin β -aryl ether ($\text{L}_{\text{A}\alpha}$), (**b**) acetylated xylan (2-*O*-Ac- β -D-Xylp), and (**c**) acetylated xylan (3-*O*-Ac- β -D-Xylp) in *Bromus diandrus* litter. Error bars indicate standard errors ($n = 3$). ** and * indicate statistical differences between UV-pass and UV-block in a given exposure time at $\alpha = 0.05$ and 0.10, respectively. Dotted line indicates the value of measured variable in reference samples that were not exposed to UV treatments ($n = 3$).

Aliphatic region

Aromatic region

484

485 Figure 1

Figure 2

489 Supplementary Table 1. 2D NMR contour integration regions for lignin methoxyl (OMe),
 490 β -aryl-ether ($L_{A\alpha}$), acetylated xylan units (2-*O*-Ac- β -D-Xylp and 3-*O*-Ac- β -D-Xylp), and
 491 syringyl ($S_{2/6}$ and $S'_{2/6}$), guaiacyl (G_2), and *p*-hydroxyphenyl ($H_{2/6}$) lignin units.

Structure	^{13}C ppm	^1H ppm
OMe	57.2-54.3	4.02-3.36
$L_{A\alpha}$	73.4-70.4	5.15-4.79
2- <i>O</i> -Ac- β -D-Xylp	74.8-72.4	4.78-4.50
3- <i>O</i> -Ac- β -D-Xylp	76.1-74.0	5.08-4.84
$S_{2/6}$	105.6-102.0	7.03-6.56
$S'_{2/6}$	107.6-105.7	7.41-7.04
G_2	112.6-108.8	7.25-6.80
$H_{2/6}$	129.1-126.9	7.35-7.10

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