Ecology, 00(0), 0000, pp. 000–000

Ultraviolet photodegradation facilitates microbial litter decomposition in a Mediterranean climate

Nameer R. Baker[1,3] and Steven D. Allison[1,2]

[1]*Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697 USA*
[2]*Department of Earth System Science, University of California, Irvine, California 92697 USA*

Abstract. Rates of litter decomposition in dryland ecosystems are consistently underestimated by decomposition models driven by temperature, moisture, and litter chemistry. The most common explanation for this pattern is that ultraviolet radiation (UV) increases decomposition through photodegradation of the litter lignin fraction. Alternatively, UV could increase decomposition through effects on microbial activity. To assess the mechanisms underlying UV photodegradation in a semiarid climate, we exposed high- and low-lignin litter to ambient and blocked UV over 15 months in a Mediterranean ecosystem. We hypothesized that UV would increase litter mass loss, that UV would preferentially increase mass loss of the lignin fraction, and that UV would have a negative effect on microbial activity. Consistent with our first hypothesis, we found that UV-blocking reduced litter mass loss from 16% to 1% in high-lignin litter and from 29% to 17% in low-lignin litter. Contrary to our second hypothesis, UV treatment did not have a significant effect on lignin content in either litter type. Instead, UV-blocking significantly reduced cellulose and hemicellulose mass loss in both litter types. Contrary to our third hypothesis, we observed a positive effect of UV on both fungal abundance and the potential activities of several assayed extracellular enzymes. Additionally, under ambient UV only, we found significant correlations between potential activities of cellulase and oxidase enzymes and both the concentrations and degradation rates of their target compounds. Our results indicate that UV is a significant driver of litter mass loss in Mediterranean ecosystems, but not solely because UV directly degrades carbon compounds such as lignin. Rather, UV facilitates microbial degradation of litter compounds, such as cellulose and hemicellulose. Thus, unexpectedly high rates of litter decomposition previously attributed directly to UV in dryland ecosystems may actually derive from a synergistic interaction between UV and microbes.

Key words: decomposition; extracellular enzymes; lignin; microbes; photodegradation; ultraviolet radiation.

Introduction

Litter decomposition is a key contributor to the global annual flux of ~68 Pg carbon (C) that enters the atmosphere from heterotrophic respiration (Raich and Schlesinger 2002). Much early work on litter decomposition was performed in mesic ecosystems, where temperature, moisture, and litter chemistry are primary drivers of decomposition rates (Meentemeyer 1978, Parton et al. 1987). However, models built around these three drivers consistently underestimate rates of decomposition in more xeric dryland ecosystems, such as semiarid Mediterranean grasslands and arid deserts (Whitford et al. 1981). Multiple hypotheses have been proposed for the unexplained mechanisms contributing to this discrepancy: foraging by subterranean microarthropods (Johnson and Whitford 1975), persistence of microbe-sustaining microclimates as a result of high overnight humidity (Whitford et al. 1981, Nagy and Macauley 1982, Dirks et al. 2010), and photodegradation by solar radiation (Pauli 1964, Moorhead and Reynolds 1989). Photodegradation in terrestrial ecosystems as a result of ultraviolet radiation (UV), in particular, has become the focus of a growing body of literature in the last decade (reviewed in King et al. 2012 and Song et al. 2013).

Photodegradation is thought to take on added importance in dryland ecosystems through a variety of mechanisms. First, litter in dryland ecosystems is subject to a greater intensity of solar radiation because there are fewer days of cloud cover and lower levels of shade than in more productive ecosystems (Pauli 1964). Second, in grassland ecosystems, litter is formed through the senescence of standing grass. This standing litter may be subject to photodegradation before it comes in contact with the soil microbial community (Austin and Vivanco 2006). Third, the presumed inhibition of microbial activity by dry climates should reduce the importance of microbial decomposition and increase the importance of abiotic drivers, such as photodegradation

Manuscript received 4 August 2014; revised 12 November 2014; accepted 2 December 2014; final version received 3 January 2015. Corresponding Editor: S. D. Frey.
[3] E-mail: nrbaker@uci.edu

(Gallo et al. 2009). On the other hand, the elevated intensity of photodegradation in more xeric ecosystems may lead to microbial communities that are adapted to the effects of UV (Caldwell et al. 2007). If photodegradation can facilitate microbial decomposition through its effects on litter chemistry, it could enhance decomposition of litter by microbial communities that are adapted to dryland climates (Gallo et al. 2006, Henry et al. 2008, Foereid et al. 2010).

Though solar radiation in general (Henry et al. 2008, Gallo et al. 2009) and UV, in particular ,have been found to increase rates of litter mass loss in previous studies (Austin and Vivanco 2006, Day et al. 2007, Brandt et al. 2010, Lin and King 2014), the exact mechanism has yet to be established. It is thought that lignin-like compounds in litter should be the most susceptible to photodegradation, due to the presence of aromatic rings that can absorb UV wavelengths. There has been some evidence for this mechanism in the lab (Brandt et al. 2009, Austin and Ballaré 2010, Lee et al. 2012) and in the field (Gehrke et al. 1995, Rozema et al. 1997, Day et al. 2007, Gallo et al. 2009), but recent field studies have shown mixed (Brandt et al. 2007, 2010) or nonexistent (Lin and King 2014) effects of UV on the lignin fraction in litter.

UV is thought to affect litter mass loss through two primary pathways that lead to depolymerization, direct photolysis, and indirect photolysis. During direct photolysis, a photosensitive organic molecule, such as lignin, absorbs photons and is fragmented or rearranged by the infusion of energy, potentially resulting in a less chemically complex compound that is easier to degrade or leach out of the system (King et al. 2012). Indirect photolysis is similar, except that after absorbance of photons by photosensitive compounds, the resulting energy is transferred to reactive intermediates, such as O, OH-, H_2O_2 or reduced metals, which can then alter organic compounds, such as cellulose (reviewed in Lanzalunga and Bietti 2000). Both direct and indirect photolysis could affect litter mass loss by making organic compounds in litter more bioavailable for microbial decomposers (King et al. 2012).

In dryland ecosystems in particular, extended dry periods should result in the buildup of microbially available substrates in litter (Hon and Feist 1981), potentially facilitating wet season decomposition (Henry et al. 2008). Foereid et al. (2010) found evidence for facilitation in a lab study, but field studies have yet to determine how UV affects microbial properties in litter. UV could have detrimental effects on microbial communities, as it is known to damage microbial DNA (Rohwer and Azam 2000) and suppress growth of terrestrial microbes (Hughes et al. 2003). On the other hand, UV facilitation of microbial communities could be especially important in semiarid Mediterranean ecosystems with marked seasonality. In the dry summer months, UV might alter litter chemistry and stimulate mass loss while inhibiting microbial activity. These changes in litter chemistry could then facilitate microbial decomposition during the wet winter months with lower UV radiation. Microbial communities in dryland ecosystems might also be adapted to UV radiation, and there is some evidence that UV exposure alters microbial community composition (Caldwell et al. 2007). Long-term exposure could select for microbes that are more capable of withstanding UV radiation or better able to use photodegraded litter compounds.

We tested three hypotheses in a litterbag experiment whereby UV exposure and litter chemistry were both manipulated at two levels. First, we hypothesized that UV photodegradation would enhance litter mass loss in a Mediterranean ecosystem, potentially as a result of direct or indirect photolysis of organic compounds in litter (King et al. 2012). Second, we hypothesized that UV would preferentially degrade the lignin fraction in litter, as its aromatic structure is known to absorb UV wavelengths (Austin and Ballaré 2010) and is thought to undergo chemical changes when exposed to solar radiation (Lanzalunga and Bietti 2000). Finally, we hypothesized that the net result of UV is inhibition of microbial activity, given previous observations that UV can damage microbial DNA (Rohwer and Azam 2000), slow the growth of microbial communities (Hughes et al. 2003), and result in altered microbial community composition (Caldwell et al. 2007).

Materials and Methods

Site description and field manipulation

To test our hypotheses, we used a litterbag study with a split-plot design. Twelve 1-m^2 plots were paired into six split-plots at the University of California, Irvine Arboretum in Irvine, California, USA (33°39′ N, 117°51′ W). The Arboretum is situated 30 m above sea level and has a mean annual temperature of 17°C and mean annual precipitation of 30 cm. Local vegetation consists of coastal sage scrub.

Each set of paired plots consisted of one ambient plot (hereafter referred to as the UV-pass treatment) and one plot covered with polyester UV-blocking film supported by a PVC frame (hereafter referred to as the UV-block treatment). This film blocked 68% of all UV while allowing 90% transmittance of visible light, as measured by a UV photometer on-site. PVC frames were 1 m on each side and set up 40 cm above the soil surface, with strips of UV-blocking film 20 cm wide used to cover the plot area under the frame. Gaps 1 cm wide between strips of film allowed precipitation to infiltrate to the plot area, and the distance between the frame and soil surface was chosen to limit the potentially strong greenhouse effects of film coverage found by Uselman et al. (2011). Ambient plots had no PVC frame or film covering.

Within each paired plot, two types of litterbags were deployed: four containing litter of *Avena* species (*A. barbata* and *A. fatua*) with 7.38% (±0.05%; mean ± SE) lignin by mass, and four containing litter of *Elymus* *condensatus* (Giant wild rye), a grass species with 13.05% (±0.08%) lignin by mass. *Avena* litter contained 4.79% (±0.10%) crude protein and 4.08% (±0.03%) ethanol soluble carbohydrates, while *Elymus* litter contained 3.81% (±0.09) and 2.44 % (±0.19), respectively. Both litter types were more similar in cellulose and hemicellulose content than they were in lignin content (Table 2). Hereafter, *Avena* litter is referred to as low lignin and *Elymus* litter is referred to as high lignin. Both litter types were collected in late June of 2012 as standing, senesced litter from Loma Ridge (33°44′ N, 117°42′ W, 365 m elevation), a Mediterranean grassland managed by the Irvine Ranch Conservancy 16 km northeast of the field site in Irvine. Litter of each type was collected by clipping standing litter at least 20 cm above the soil surface to minimize prior soil contact, then homogenized by clipping to <5 cm lengths and mixing. A subsample was weighed and oven-dried to determine moisture content. The equivalent of 1.9-g dry mass of litter (including ash content) was then added to litterbags for each litter type and deployed in the field on 18 July 2012. Each litterbag was made of two types of mesh: a 1.5-mm aluminum mesh used for the side exposed to the sun and a 0.5-mm nylon bridal mesh used for the side exposed to the soil surface.

Four litterbags of both litter types were deployed into each of the six paired plots, resulting in 96 total litterbags (4 × 2 × 6 × 2). One litterbag of each litter type was then collected randomly from each of the paired plots at the end of the first dry season (2 October 2012), the middle of the wet season (18 January 2013), the end of the wet season (4 June 2013), and the end of the second dry season (17 September 2013), for a total of five time points (including the initial deployment) over a period of 15 months.

Collected litter was weighed to determine mass loss before being ground into fragments <0.5 cm in length and subsampled for extracellular enzyme assays, a bacterial cell count assay, and a fungal hyphae staining assay. The remainder of the litter was weighed and oven-dried to determine moisture content before being sent off for near-infrared (or near-IR) analysis of litter chemistry.

Extracellular enzyme assays

Litter was assayed for potential activity of eight enzyme classes using fluorescently labeled substrates (for hydrolytic enzymes) or colorimetric assays (for oxidative enzymes) according to methods detailed in German et al. (2012). The enzyme classes assayed consisted of hydrolytic cellulose and starch degradation (β-glucosidase, cellobiohydrolase, and α-glucosidase; or BG, CBH, and AG, respectively), hydrolytic hemicellulose degradation (β-xylosidase; or BX), hydrolytic chitin-degradation (N-acetylglucosaminidase; or NAG), peptide degradation (leucine-aminopeptidase; or LAP), and oxidative degradation (peroxidase and phenol oxidase; or PER and PPO, respectively). Negative potential activities were converted to zero values for statistical analyses.

Bacterial cell density

Methods for estimating bacterial cell density were identical to those used in Allison et al. (2013). In brief, ground litter was suspended in a phosphate-buffered, 1% glutaraldehyde solution on the day of sample collection to fix bacterial cells for storage. Within two weeks, 0.1 M tetrasodium pyrophosphate was added to each sample, and samples were sonicated to dislodge bacterial cells. Filtered extracts of sonicated litter were stained with 1× SYBR Green (Life Technologies, Grand Island, New York, USA) and then analyzed with an Accuri flow cytometer (BD Biosciences, San Jose, California, USA) to determine cell counts from fluorescing bacterial cells.

Fungal hyphal length

Methods for measuring fungal hyphal length were identical to those used in Allison et al. (2013). In brief, ground litter was suspended in 0.395% (mass per volume) sodium hexametaphosphate and vigorously stirred before being vacuum-filtered and stained with acid fuchsin. Two filters were made for each litter sample and affixed to a glass slide. Hyphae were counted with a Nikon Eclipse E400 microscope (Nikon Instruments, Melville, New York, USA) at 100× magnification using the grid-intercept method (Newman 1966, Giovanetti and Mosse 1980) and 50 grids per filter. Hyphal counts were converted to estimates of hyphal length in m/g of dry litter using a modified procedure of Sylvia (1992).

Litter chemistry

Oven-dried litter was sent to Cumberland Valley Analytical Services for near-IR spectroscopy, whereby reflectance of near-infrared wavelengths of light from each sample are matched to a verified database of spectra for plant materials with known chemical composition as determined by wet chemistry (Shepherd et al. 2005). Relative amounts of the following organic compounds were determined as proportions of total dried litter mass: lignin, cellulose (acid detergent fiber minus lignin) hemicellulose (neutral detergent fiber minus acid detergent fiber), ash, and non-ash dry mass (1 minus ash fraction). The proportion of total litter mass attributable to different C compounds will be referred to as concentration in the text. The concentration of non-ash dry mass was multiplied by the recovered dry mass at each time point to determine mass loss from the organic portion of litter. The same calculation was used to determine mass loss for each carbon compound (lignin, cellulose, and hemicellulose). The total mass or mass lost from each carbon compound will be referred to as content in the text.

Statistical methods

Effects of UV treatment, litter type, and sampling date on non-ash dry mass, litter chemistry, litter moisture, and bacterial cell counts were analyzed using mixed-model ANOVA with the identity of each pair of

TABLE 1. *P* values from ANCOVA for each dependent variable with respect to UV treatment, litter type, time of sampling, and all possible interactions with litter moisture content as a covariate.

Variable	H_2O	UV	Litter	Time	UV : Lit	UV : T	Lit:T	UV : Lit:T
Non-ash dry mass	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.084	0.370	0.750	0.946
Lignin (g)	**<0.001**	0.090	**<0.001**	**<0.001**	**0.016**	0.064	**<0.001**	0.346
Cellulose (g)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.025**	**0.006**	**0.023**	0.912
Hemicellulose (g)	**0.068**	**<0.001**	0.963	**<0.001**	0.300	**<0.001**	**<0.001**	**0.016**
Bacterial cells	0.522	0.151	0.223	**<0.001**	0.533	0.297	**0.042**	0.508
Fungal hyphae	0.609	**0.024**	**0.004**	**<0.001**	0.115	**<0.001**	**<0.001**	**0.017**
BG activity	0.884	0.444	**<0.001**	**<0.001**	0.132	0.212	**<0.001**	0.217
CBH activity	0.143	0.406	**<0.001**	**<0.001**	0.107	**0.026**	**0.002**	**0.030**
AG activity	**0.036**	0.070	**<0.001**	**<0.001**	0.072	0.340	0.252	0.281
BX activity	**<0.001**	0.230	**<0.001**	**<0.001**	0.194	**<0.001**	**0.008**	0.113
PPO activity	**0.021**	0.822	**<0.001**	**<0.001**	0.918	**<0.001**	0.059	**<0.001**
PER activity	0.935	0.233	**<0.001**	**<0.001**	0.733	0.415	**0.001**	**0.041**
NAG activity	**<0.001**	0.085	**<0.001**	**<0.001**	**0.028**	0.061	**0.042**	0.170
LAP activity	**<0.001**	**0.017**	0.109	**<0.001**	**0.001**	**0.008**	**0.020**	0.343
N_{df}, D_{df}	1, 72	1, 72	1, 72	3, 72	1, 72	3, 72	3, 72	3, 72

Notes: Significant *P* values ($P < 0.05$) are in bold. N_{df} and D_{df} are the degrees of freedom for the numerator and denominator of *F*, respectively. BG is β-glucosidase, CBH is cellobiohydrolase, AG is α-glucosidase, BX is β-xylosidase, PPO is phenol oxidase, PER is peroxidase, NAG is N-acetylglucosaminidase, and LAP is leucine-aminopeptidase. Abbreviations are lit, litter; T, time.

split plots as a random factor. We originally compared this simple model with a more complex model whereby plot identity was a random factor within which UV, litter type, and time were nested, but Akaike's information criterion (AIC) comparison showed no significant differences between the two models. The simpler model had a lower AIC_c (Akaike's information criterion corrected for sample sizes; 51.7 vs. 60.3), AIC (42.6 vs. 47.5), BIC (Bayesian information criterion; 85.0 vs. 97.0), and log-likelihood (–3.3 vs. –2.7), allowing us to employ it with a high degree of confidence. Tukey contrasts were used to determine the effect of UV within litter types at each time point.

Because litter moisture content was found to be significantly affected by UV treatment (Appendix A: Fig. A1, Table A1) and is known to be a strong control on decomposition processes in Mediterranean ecosystems, the model was run for all variables as an ANCOVA with litter moisture content as the covariate. Data were checked for normality using the Shapiro-Wilk test, and nonnormal data were log-transformed or square-root-transformed to improve normality when possible. Litter non-ash dry mass, lignin content, bacterial cell abundance, and observed potential β-glucosidase activity met assumptions of normality after being log transformed. Potential activities for β-xylosidase, phenol oxidase, and N-acetylglucosaminidase met assumptions of normality after being square-root-transformed. Potential peroxidase activity exhibited improved normality after square-root transformation, although a significant but tolerable deviation from normality was still evident. Litter hemicellulose content and potential activities of cellobiohydrolase and leucine aminopeptidase were still not normally distributed after transformation, but visual inspection of residuals suggested that deviations from normality were tolerable for untransformed data. Litter cellulose content, hyphal lengths, and potential α-glucosidase activity met assumptions of normality without data transformation.

To analyze enzymatic controls over decomposition, we tested for correlations between potential activities of different enzyme classes and the concentrations of their target carbon compounds at each time point. We also tested for correlations between potential enzyme activities at one time point and the rate of change in the content attributable to their target carbon compounds by the next time point. If there was a significant correlation, then we used linear regressions to determine how much variation in the rate of change in the content of each carbon compound could be attributed to potential extracellular enzyme activity. These regressions assume that enzyme activity causes the change in mass of target substrate. All statistical analyses were conducted in the R software environment version 3.0.2 (R Development Core Team 2013).

RESULTS

Litter mass loss and moisture content

Litter mass loss was affected by both litter type ($P < 0.001$, $F_{1,72} = 112.1$) and by UV treatment ($P < 0.001$, $F_{1,72} = 60.9$; Table 1, Fig. 1). Low-lignin litter lost the most mass across both UV treatments, with an average of 23.2% mass loss by June 2013. High-lignin litter lost an average of 11.0% of original mass across both UV treatments over the same time period. There was no significant interaction between litter type and UV treatment. High-lignin litter lost 16.2% of original mass by June 2013 under UV-pass, but exhibited negligible (<1%) mass loss under UV-block. Low-lignin samples showed a similar pattern (29.0% mass loss in UV-pass samples vs. 17.4% mass loss in UV-block samples), but post hoc tests within dates were only marginally significant (Fig. 1). Mass loss was not significantly affected by UV treatment after the first dry season (July 2012–September 2012), for either high-lignin or low-

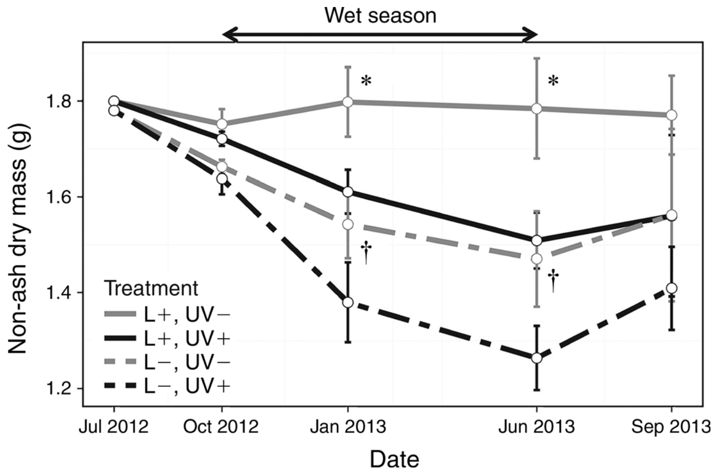

Fig. 1. Dry mass of the non-ash component of litter, in grams. Significant differences between UV treatments within litter types and sampling dates according to Tukey tests are denoted with asterisks ($P < 0.05$) or daggers ($P < 0.10$). Low-lignin samples (L−) are shown with dashed lines, and high-lignin samples (L+) with solid lines. UV-pass treatments (UV+) are shown in black, and UV-block (UV−) treatments are in gray. Error bars represent mean ± SE. The double-headed line above the plot indicates the duration of the wet season .

lignin samples, with differences in litter mass only appearing during or after the wet season (January 2013 and later time points). With the exception of the high-lignin UV-block treatment, all litter samples exhibited mass loss at each time point over the course of the experiment until the final September 2013 time point. September 2013 samples had higher concentration of ash, indicating that soil deposition over the course of the second dry season obscured mass loss from litter and likely introduced organic compounds into litterbags, with the net result being increased similarity between all treatments at the final time point.

Litter moisture content ranged from 7% to 14% of litter mass (Appendix A: Fig. A1). UV-block significantly reduced litter moisture content by 0.47 percentage points ($P = 0.04$, $F_{1,73} = 4.4$), and moisture content was significantly lower by 0.67 percentage points in low-lignin litter compared to high-lignin litter ($P < 0.001$, $F_{1,73} = 13.1$; Appendix A: Table A1, Fig. A1). Litter moisture also varied significantly over time with the lowest values in January 2013 ($P < 0.001$, $F_{3,73} = 94.3$).

Carbon fractions

High-lignin litter began the study with 13.05% ± 0.08% lignin by mass and low-lignin litter began the study with 7.38% ± 0.06%. Lignin content was significantly affected by litter type ($P < 0.001$, $F_{1,72} = 371.9$), but UV treatment had only a marginally significant effect ($P = 0.090$, $F_{1,72} = 2.96$). The significant increase in lignin content over time (Tables 1 and 2) is likely due to the deposition over time of particulate matter containing organic compounds, either microbial byproducts or plant detritus, that have a lignin-like near-IR signal. Litter cellulose content was significantly affected by litter type ($P < 0.001$, $F_{1,72} = 137.9$) and UV treatment ($P < 0.001$, $F_{1,72} = 74.5$). There was a significant interaction between UV treatment and litter type on cellulose content because UV had a stronger effect on cellulose content in high-lignin litter than in low-lignin litter (Tukey $P = 0.017$ for UV effect in low-lignin litter, $P < 0.001$ in high-lignin litter; Appendix B: Fig. B1). Litter hemicellulose content was not significantly affected by litter type, but was significantly affected by UV treatment ($P < 0.001$, $F_{1,72} = 25.5$; Table 1). Both litter types had reduced cellulose and hemicellulose content under UV-pass compared to UV-block (Table 2).

Bacterial cell counts

Neither litter type nor UV treatment had a significant effect on bacterial cell counts (Table 1; Appendix A: Fig. 2A). Bacterial abundance across and within all treatments was significantly higher on 4 June 2013 when compared to all other time points (Tukey $P < 0.001$).

Fungal hyphal length

In contrast to bacterial abundance, fungal hyphal length was significantly affected by both UV treatment ($P = 0.024$, $F_{1,72} = 5.3$) and litter type ($P = 0.004$, $F_{1,72} = 8.7$; Table 1, Fig. 2B). The UV-pass treatment did not, in

Table 2. Pearson coefficients and P values for the correlation (Corr.) between C fraction concentration and potential enzyme activity of the enzyme class that degrades that fraction.

	BG (cellulose)		CBH (cellulose)		BX (hemicellulose)		PER (lignin)		PPO (lignin)	
Treatment	Corr.	P	Corr.	P	Corr.	P	Corr.	P	Corr.	P
L–, UV+	**+0.475**	**0.011**	**+0.423**	**0.025**	+0.243	0.213	**+0.690**	**<0.001**	**+0.551**	**0.002**
L–, UV–	−0.043	0.823	−0.096	0.612	−0.096	0.612	+0.333	0.083	**+0.517**	**0.005**
L+, UV+	−0.017	0.927	−0.313	0.092	+0.198	0.293	**+0.510**	**0.004**	**+0.484**	**0.007**
L+, UV–	+0.018	0.925	−0.224	0.233	+0.092	0.628	+0.146	0.440	+0.236	0.210

Note: Bold text indicates significant correlations between enzyme activity and carbon fraction concentration.

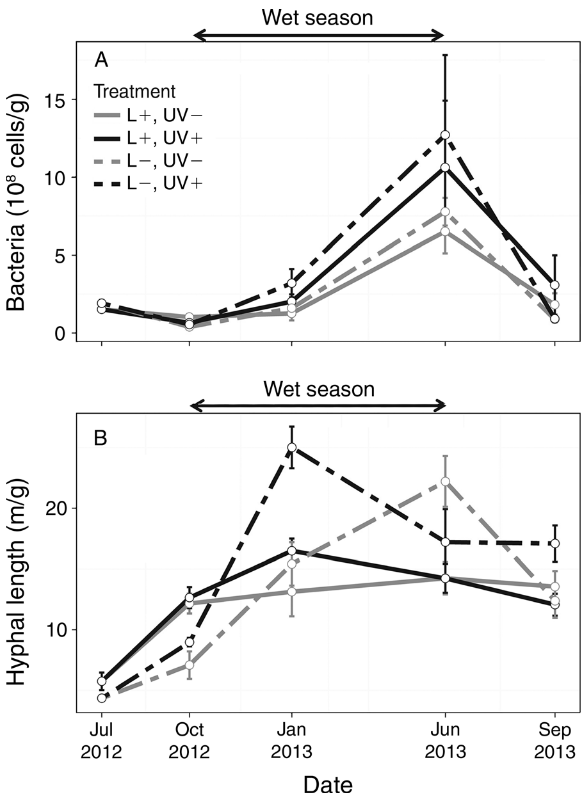

FIG. 2. Microbial abundance. (A) Bacterial cell counts measured by flow cytometry, in 10^8 cells/g of dry litter. (B) Length of fungal hyphae in m/g dry litter. Low-lignin samples are shown with dashed lines and high-lignin samples with solid lines. UV-pass treatments are shown in black and UV-block treatments are in gray. Error bars represent mean ± SE.

general, have a negative effect on fungal hyphal length across litter types when compared to UV-block, and UV treatment had no discernible effect on fungal hyphal length in high-lignin litter. We did find a significant three-way interaction between UV treatment, litter type, and sampling date ($P = 0.017$, $F_{3,72} = 3.6$), likely because fungal hyphal length in UV-pass samples was greater in low-lignin litter during the wet season compared to UV-block samples (25.0 ± 1.7 [mean ± SE] m/g hyphae in low-lignin litter under UV-pass vs. 15.4 ± 1.8 m/g under UV-block in January 2013; Tukey $P < 0.001$). In contrast, UV-block samples did not attain peak fungal abundance until the end of the wet season.

Potential extracellular enzyme activities

Potential extracellular enzyme activities varied with time, litter type, and occasionally by UV treatment (Table 1). In general, potential enzyme activities were lower in high-lignin litter compared to low-lignin litter, and lower during the dry season compared to the wet season (Fig. 3). The main effect of UV treatment was only significant for potential leucine aminopeptidase activity ($P = 0.017$, $F_{1,72} = 6.0$). There were significant interactions with UV for the four enzymes depicted in Fig. 3, in addition to peroxidase (trends similar to phenol oxidase) and N-acetylglucosaminidase (trends similar to leucine aminopeptidase). Leucine aminopeptidase exhibited significantly higher (Tukey $P < 0.001$) potential activity under UV-pass compared to UV-block across all time points in high-lignin litter only (Fig. 3D). UV-block had marginally significant negative effects on potential activity of α-glucosidase ($P = 0.070$, $F_{1,72} = 3.4$) and N-acetylglucosaminidase ($P = 0.085$, $F_{1,72} = 3.0$).

Potential activities of β-glucosidase and cellobiohydrolase were significantly positively correlated with percent mass of cellulose in low-lignin litter, but only under UV-pass. Potential activities of peroxidase and phenol oxidase were significantly positively correlated with percent mass of lignin in both litter types under UV-pass, but, with the exception of phenol oxidase in low-lignin litter, not under UV-block (Table 3).

Potential activities of three enzymes were significantly positively correlated with the rate of change in the content of their target carbon compounds, but only in litter under UV-pass. β-xylosidase was positively correlated with the rate of change in hemicellulose content in low-lignin litter, and phenol oxidase was positively correlated with the rate of change in lignin content in high-lignin litter. Peroxidase activity was positively correlated with the change in lignin content in both litter types (Table 4).

DISCUSSION

Our first hypothesis was that UV-block would reduce mass loss in both high- and low-lignin litter. Our results supported this hypothesis: reducing UV transmittance by 68% in the UV-block treatment significantly reduced mass loss in high-lignin litter and reduced mass loss to a marginally significant extent in low-lignin litter (Fig. 1). This effect was significant even after accounting for a slight but significant negative effect of UV-block on litter moisture. Several previous studies have shown that attenuating solar radiation through shading can reduce litter mass loss in arid (Gallo et al. 2009) and semiarid (Henry et al. 2008) ecosystems. A number of studies have also found, as we did, that reducing UV can reduce litter mass loss rates in semiarid ecosystems. Austin and Vivanco (2006), Day et al. (2007), Brandt et al. (2007, 2010), and Lin and King (2014) all found that blocking UV reduced litter mass loss in the field anywhere from 3% over 5 months (Day et al. 2007) to 33% over 18 months (Austin and Vivanco 2006). Taken together, these results confirm that UV can increase litter mass loss in dryland ecosystems.

Contrary to our second and third hypotheses, our study indicates that UV photodegradation does not result in enhanced mass loss from the lignin fraction, nor does it inhibit microbial decomposition. Instead, UV photodegradation appears to facilitate microbial decomposition by increasing the efficiency of extracellular enzymes produced by microbial communities. Lignin

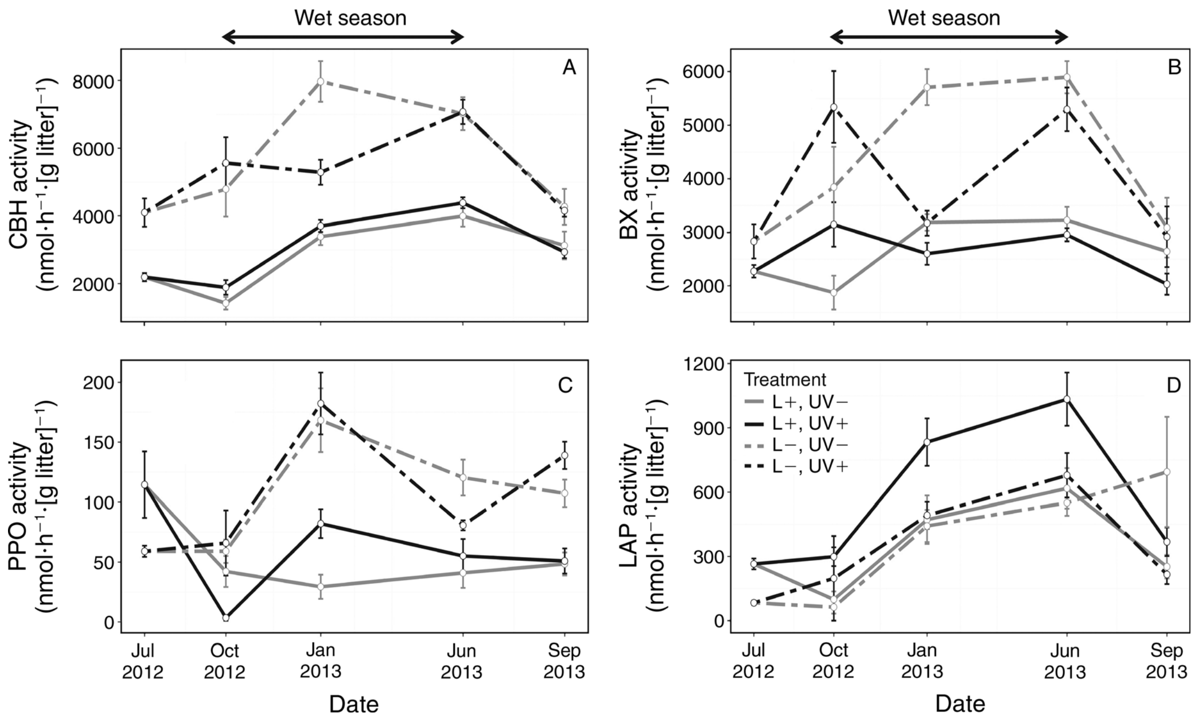

FIG. 3. Potential extracellular enzyme activities in nanomoles of substrate per hour per gram of dry litter for four representative enzymes: (A) cellobiohydrolase (CBH); (B) β-xylosidase (BX); (C) phenol oxidase (PPO); and (D) leucine-aminopeptidase (LAP).

mass loss in our litter was not affected by UV treatment, with UV-blocking instead reducing the loss of litter cellulose and hemicellulose (Tables 1 and 2). In addition, the net effect of UV-pass on litter microbial communities does not appear to be inhibitory; we found no effect of UV treatment on bacterial abundance (Fig. 2A), a potentially positive effect of UV-pass on fungal abundance (Fig. 2B), and no consistent effect of UV treatment on potential extracellular enzyme activity. Instead, we found that litter-degrading extracellular enzymes may be more effective under UV-pass. We found correlations between potential enzyme activity and both substrate availability and substrate degradation in litter under UV-pass and no such correlations under UV-block. Our results indicate that the functioning of Mediterranean grassland microbial communities may be dependent on ambient UV.

Though most studies of photodegradation have hypothesized that UV acts directly upon the lignin fraction in litter, it should be noted that these studies have not established a direct link between UV exposure and lignin degradation. Although lab studies suggest that lignin-like model compounds are photochemically active and absorb light in the ultraviolet range (Lanzalunga and Bietti 2000, Austin and Vivanco 2006), it is unclear to what extent photodegradation affects the physical and chemical structure of lignin. Kirschbaum et al. (2011) exposed grass litter and pine needles to UV equivalent to midday levels continuously for 60 days and found no direct effect of UV on either litter mass loss or concentration of lignin. Over the course of 10 weeks in the laboratory, Brandt et al. (2009) tested the effects of UV exposure on five different litter types with initial lignin concentrations varying from

TABLE 3. Carbon fraction content across all treatments presented as grams of dry litter mass at the beginning of the study and in June 2013.

	Lignin (g)		Cellulose (g)		Hemicellulose (g)	
Treatment	Initial	June 2013	Initial	June 2013	Initial	June 2013
L–, UV+	0.139 ± 0.001	0.167 ± 0.011	0.738 ± 0.006	0.587 ± 0.025	0.587 ± 0.005	0.284 ± 0.016
L–, UV–	0.139 ± 0.001	0.181 ± 0.015	0.738 ± 0.006	**0.670 ± 0.035**	0.587 ± 0.005	**0.338 ± 0.012**
L+, UV+	0.244 ± 0.001	0.230 ± 0.013	0.827 ± 0.003	0.678 ± 0.019	0.484 ± 0.004	0.267 ± 0.023
L+, UV–	0.244 ± 0.001	0.263 ± 0.023	0.827 ± 0.003	**0.803 ± 0.028**	0.484 ± 0.004	**0.348 ± 0.018**

Notes: Bold values indicate when means under UV-block were significantly different ($P < 0.05$, Tukey test) from means of the same litter type under UV-pass. Low-lignin samples are L–, and high-lignin samples are L+. UV block samples are UV–, and UV pass samples are UV+.

TABLE 4. Pearson coefficients and P values for the correlation (Corr.) between potential enzyme activity and the change (Δ) in the carbon content attributable to the compound degraded by that enzyme class.

	ΔHemicellulose (BX at $t-1$)			ΔLignin (PER at $t-1$)			ΔLignin (PPO at $t-1$)		
Treatment	Corr.	R^2	P	Corr.	R^2	P	Corr.	R^2	P
L–, UV+	**−0.452**	**0.163**	**0.040**	**−0.448**	**0.159**	**0.042**	−0.136	-	0.556
L–, UV–	−0.058	-	0.799	−0.058	-	0.799	−0.127	-	0.574
L+, UV+	−0.227	-	0.287	**−0.497**	**0.213**	**0.013**	**−0.616**	**0.351**	**0.001**
L+, UV–	−0.028	-	0.896	−0.167	-	0.437	−0.237	-	0.265

Note: R^2 values for the linear regression of change in C content as a function of potential enzyme activity are shown for significant correlations and indicated by bold text. The change in carbon compound content between time points (e.g., ΔHemicellulose) is a function of potential extracellular enzyme activity at the earlier time point (e.g., BX at t – 1).

6.2% to 24.6%. After standardizing for exposed surface area, they found that lignin concentration had no effect on CO_2 efflux from litter, though mass loss was significantly greater in litter exposed to UV than in controls.

Whereas evidence for a direct effect of UV on the lignin fraction has been elusive in the lab, some field studies indicate that UV may influence the lignin content of litter. Separate studies by Gehrke et al. (1995) and Rozema et al. (1997) found that artificially enhancing levels of UVB radiation in the field caused the lignin content in litter to accumulate more slowly over time. In a California annual grassland, Henry et al. (2008) found that the rate of decrease in the concentration of lignin in grass litter over the course of a summer was roughly twice as rapid as rates of total mass loss. This indicates that mass loss during the dry season was preferentially occurring through the lignin fraction, ostensibly as a result of photodegradation. Day et al. (2007) found a similar result when exposing *Larrea tridentata* litter with very high lignin concentrations to 85% ambient and 15% ambient UVB, with greater attenuation of UVB corresponding to higher lignin content in samples at the end of the study. While it is possible that UV degraded lignin directly in these studies, it is also possible that increased microbial activity under near-ambient radiation confounded their results, given that microbial byproducts can be classified as lignin-like compounds when analyzing litter chemistry (Berg and McClaugherty 1987, Berg and Laskowski 2005). Rather than direct photolysis, reactive intermediates resulting from indirect photolysis of litter compounds also may have contributed to lignin degradation observed in these studies (King et al. 2012).

In contrast to these field studies, we did not find that litter lignin was significantly affected by UV treatment. Instead, our results showed a significant, strong effect of UV treatment on litter cellulose and hemicellulose content. Notably, we found that this effect was greater in high-lignin litter. Cellulose can be cleaved through photoexcitation of the α-glycosidic bond linking cellulose chains to one another, producing simpler cellulose chains and releasing CO (Schade et al. 1999). Such direct photolysis of cellulose has not been extensively studied in the lab, but our results and others indicate that direct and indirect photolysis of nonlignin compounds, such as cellulose and hemicellulose, could be a significant mechanism through which UV affects litter decomposition. Gehrke et al. (1995), Rozema et al. (1997), and Day et al. (2007) found a significant reduction in cellulose concentration or combined cellulose and hemicellulose concentration in litter exposed to higher levels of UV. Brandt et al. (2007, 2010) also found no effect of UV treatment on the concentration of lignin in litter, but a significant, if small, negative effect of UV on the combined cellulose and hemicellulose concentration in their 2007 study and a highly significant effect of UV on hemicellulose concentration in their 2010 study. Our results also fall in line with a study by Lin and King (2014), where attenuated UV reduced losses of hemicellulose content by 29% without having a significant effect on lignin content. Likewise, Gallo et al. (2009) found that cottonwood litter mass loss was partially driven by photomineralization of cellulose.

In addition to the aforementioned mechanisms of direct and indirect photolysis of cellulose and hemicellulose, it is likely that the effects of UV on mass loss could also result from degradation of the lignocellulose matrix without significantly affecting lignin mass loss. UV breakdown of lignin shielding other C compounds could make previously occluded cellulose, hemicellulose, and soluble C available to microbial decomposers, facilitating enhanced microbial decomposition of litter (Gallo et al. 2006). Based on NMR analyses of litter that had been photodegraded in the field during their 2014 study, Lin et al. (Y. Lin, J. King, S. Karlen, and J. Ralph, *unpublished manuscript*) found that UV degraded interunit ether linkages of lignin polymers without causing lignin mass loss, suggesting a mechanism whereby UV could weaken the lignocellulose matrix. Our results are also consistent with this potential mechanism, as cellulose mass loss was more affected by UV treatment in high-lignin litter than it was in low-lignin litter.

In contrast to our original third hypothesis, we found little evidence for inhibition of microbial activity by UV.

Bacterial and fungal abundances did not increase in litter under UV-block. Instead, UV-block treatment negatively affected fungal abundance during the wet season in our low-lignin litter. In addition, potential activities of six of eight extracellular enzymes were not affected by UV treatment, indicating that ambient UV does not generally inhibit extracellular enzyme activity (Table 1, Fig. 3). These results are somewhat surprising given the known detrimental effects of UV on microbial DNA (Rohwer and Azam 2000) and microbial community growth (Hughes et al. 2003). However, UV might promote microbial decomposition through biochemical interactions. We observed significant correlations between potential enzyme activities and the concentrations of their target carbon fractions, but almost exclusively under UV-pass (Table 3). We also only found significant correlations between the mass loss of a carbon fraction and its associated enzyme activity at the previous time point under UV-pass, and mainly for oxidative enzymes that target more complex organic compounds (Table 4). In other words, investment in enzymes targeting the most complex compounds in litter only had a significant effect on the mass change of those compounds when litter was exposed to ambient levels of UV. This result falls in line with previous findings by Gallo et al. (2009) and Brandt et al. (2010) that the amount of potential enzymatic activity required to degrade a litter cohort is greater when UV is blocked.

There have been several other studies, in addition to our own, that indicate that facilitation of microbial decomposition by photodegradation may occur when microbial communities are allowed to interact with photodegraded litter. Foereid et al. (2010) found that litter exposed to light for 289 days had much higher rates of CO_2 efflux in lab incubations when compared to litter that had only been exposed to radiation treatment for 43 days. Henry et al. (2008) found that wet season decomposition was significantly greater when litter had been exposed to ambient radiation during the preceding summer dry period. Similarly, Lin and King (2014) found that shaded litter exposed to attenuated UV exhibited carbon fraction dynamics similar to shaded litter exposed to ambient UV, but with significantly slower litter mass loss rates, indicating that decomposition of shaded litter in contact with the microbial community may be facilitated by UV photodegradation and the resulting release of soluble C in the surface litter layer. The results of our study suggest a mechanism that could explain UV facilitation of litter decomposition in these studies. Photodegradation of cellulose, hemicellulose, or the lignocellulose matrix might allow extracellular enzymes to break down their substrates more effectively.

Conclusions

Our study shows that UV photodegradation has a positive effect on both litter decomposition rates and microbial decomposer activity. UV-blocking reduces litter mass loss, but does not have a significant direct effect on litter lignin content. Instead, UV-blocking significantly reduces the degradation of cellulose and hemicellulose, potentially by limiting the direct or indirect photolysis of cellulose or the lignocellulose matrix that would otherwise occur under ambient UV. UV-blocking does not appear to increase bacterial or fungal abundance, and may in fact be detrimental for microbial decomposition, as extracellular enzymes produced by the microbial decomposer community were more effective at degrading their target substrates under ambient UV. These results indicate that UV photodegradation is an important driver of litter decomposition through its effects on nonlignin compounds and facilitation of microbial activity. These mechanisms of litter decomposition will likely become more important in the American Southwest if this region experiences a more arid climate in the future.

Acknowledgments

This work was funded by the Office of Science (Biological and Environmental Research), U.S. Department of Energy programs in Microbial Communities and Carbon Cycling and Ecosystem Research. We thank Orange County Parks, the Irvine Ranch Conservancy, and the UC Irvine Arboretum for site access. We would also like to thank Jennifer King, Yang Lin, and two anonymous reviewers for providing comments that improved the manuscript.

Literature Cited

Allison, S. D., Y. Lu, C. Weihe, M. L. Goulden, A. C. Martiny, K. K. Treseder, and J. B. H. Martiny. 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94:714–25.

Austin, A. T., and C. L. Ballaré. 2010. Dual role of lignin in plant litter decomposition in terrestrial ecosystems. Proceedings of the National Academy of Sciences USA 107:4618–4622.

Austin, A. T., and L. Vivanco. 2006. Plant litter decomposition in a semi-arid ecosystem controlled by photodegradation. Nature 442:555–558.

Berg, B., and R. Laskowski. 2005. Litter decomposition: a guide to carbon and nutrient turnover. Academic Press, New York, New York, USA.

Berg, B., and C. McClaugherty. 1987. Nitrogen release from litter in relation to the disappearance of lignin. Biogeochemistry 4:219–224.

Brandt, L. A., C. Bohnet, and J. Y. King. 2009. Photochemically induced carbon dioxide production as a mechanism for carbon loss from plant litter in arid ecosystems. Journal of Geophysical Research: Biogeosciences 114:G02004.

Brandt, L. A., J. Y. King, S. E. Hobbie, D. G. Milchunas, and R. L. Sinsabaugh. 2010. The role of photodegradation in surface litter decomposition across a grassland ecosystem precipitation gradient. Ecosystems 13:765–781.

Brandt, L. A., J. Y. King, and D. G. Milchunas. 2007. Effects of ultraviolet radiation on litter decomposition depend on precipitation and litter chemistry in a shortgrass steppe ecosystem. Global Change Biology 13:2193–2205.

Caldwell, M. M., J. F. Bornman, C. L. Ballaré, S. D. Flint, and G. Kulandaivelu. 2007. Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. Photochemical and Photobiological Sciences 6:252–266.

Day, T. A., E. T. Zhang, and C. T. Ruhland. 2007. Exposure to solar UV-B radiation accelerates mass and lignin loss of

Larrea tridentata litter in the Sonoran Desert. Plant Ecology 193:185–194.

Dirks, I., Y. Navon, D. Kanas, R. Dumbur, and J. M. Grünzweig. 2010. Atmospheric water vapor as driver of litter decomposition in Mediterranean shrubland and grassland during rainless seasons. Global Change Biology 16:2799–2812.

Foereid, B., J. Bellarby, W. Meier-Augenstein, and H. Kemp. 2010. Does light exposure make plant litter more degradable? Plant and Soil 333:275–285.

Gallo, M. E., a. Porras-Alfaro, K. J. Odenbach, and R. L. Sinsabaugh. 2009. Photoacceleration of plant litter decomposition in an arid environment. Soil Biology and Biochemistry 41:1433–1441.

Gallo, M. E., R. L. Sinsabaugh, and S. E. Cabaniss. 2006. The role of ultraviolet radiation in litter decomposition in arid ecosystems. Applied Soil Ecology 34:82–91.

Gehrke, C., U. Johanson, T. V. Callaghan, D. Chadwick, and C. H. Robinson. 1995. The impact of enhanced ultraviolet-B radiation on litter quality and decomposition processes in *Vaccinium* leaves from the Subarctic. Oikos 72:213–222.

German, D. P., K. R. B. Marcelo, M. M. Stone, and S. D. Allison. 2012. The Michaelis-Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study. Global Change Biology 18:1468–1479.

Giovanetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84:489–500.

Henry, H. A. L., K. Brizgys, and C. B. Field. 2008. Litter decomposition in a California annual grassland: interactions between photodegradation and litter layer thickness. Ecosystems 11:545–554.

Hon, D. N. S., and W. C. Feist. 1981. Free-radical formation in wood: the role of water. Wood Science 14:41–48.

Hughes, K. A., B. Lawley, and K. K. Newsham. 2003. Solar UV-B radiation inhibits the growth of Antarctic terrestrial fungi. Applied and Environmental Microbiology 69:1488–1491.

Johnson, K. A., and W. G. Whitford. 1975. Foraging ecology and relative importance of subterranean termites in Chihuahuan desert ecosystems. Environmental Entomology 4:66–70.

King, J. Y., L. A. Brandt, and E. C. Adair. 2012. Shedding light on plant litter decomposition: advances, implications and new directions in understanding the role of photodegradation. Biogeochemistry 111:57–81.

Kirschbaum, M. U. F., S. M. Lambie, and H. Zhou. 2011. No UV enhancement of litter decomposition observed on dry samples under controlled laboratory conditions. Soil Biology and Biochemistry 43:1300–1307.

Lanzalunga, O., and M. Bietti. 2000. Photo- and radiation chemical induced degradation of lignin model compounds. Journal of Photochemistry and Photobiology B 56:85–108.

Lee, H., T. Rahn, and H. Throop. 2012. An accounting of C-based trace gas release during abiotic plant litter degradation. Global Change Biology 18:1185–1195.

Lin, Y., and J. Y. King. 2014. Effects of UV exposure and litter position on decomposition in a California grassland. Ecosystems 17:158–168.

Meentemeyer, V. 1978. Macroclimate and lignin control of litter decomposition rates. Ecology 59:465–472.

Moorhead, D. L., and J. F. Reynolds. 1989. Mechanisms of surface litter mass loss in the northern Chihuahuan desert: a reinterpretation. Journal of Arid Environments 16:157–163.

Nagy, L. A., and B. J. Macauley. 1982. Eucalyptus leaf-litter decomposition: effects of relative humidity and substrate moisture content. Soil Biology and Biochemistry 14:233–236.

Newman, E. I. 1966. A method of estimating the total length of root in a sample. Journal of Applied Ecology 3:139–145.

Parton, W. J., D. S. Schimel, C. V. Cole, and D. S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51:1173–1179.

Pauli, F. 1964. Soil fertility problem in arid and semi-arid lands. Nature 204:1286–1288.

R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org

Raich, J. W., and W. H. Schlesinger. 2002. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus 44:81–99.

Rohwer, F., and F. Azam. 2000. Detection of DNA damage in prokaryotes by terminal deoxyribonucleotide transferase-mediated dUTP nick end labeling. Applied and Environmental Microbiology 66:1001–1006.

Rozema, J., M. Tosserams, H. J. M. Nelissen, L. Van Heerwaarden, R. A. Broekman, and N. Flierman. 1997. Stratospheric ozone reduction and ecosystem processes: enhanced UV-B radiation affects chemical quality and decomposition of leaves of the dune grassland species Calamagrostis epigeios. Plant Ecology 128:285–294.

Schade, G. W., R.-M. Hofmann, and P. J. Crutzen. 1999. CO emissions from degrading plant matter. Tellus 51:889–908.

Shepherd, K. D., B. Vanlauwe, C. N. Gachengo, and C. a. Palm. 2005. Decomposition and mineralization of organic residues predicted using near infrared spectroscopy. Plant and Soil 277:315–333.

Song, X., C. Peng, H. Jiang, Q. Zhu, and W. Wang. 2013. Direct and indirect effects of UV-B exposure on litter decomposition: a meta-analysis. PLoS ONE 8:e68858.

Sylvia, D. M. 1992. Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi. Methods in Microbiology 24:53–65.

Uselman, S. M., K. A. Snyder, R. R. Blank, and T. J. Jones. 2011. UVB exposure does not accelerate rates of litter decomposition in a semi-arid riparian ecosystem. Soil Biology and Biochemistry 43:1254–1265.

Whitford, W. G., V. Meentemeyer, T. R. Seastedt, K. Cromack, D. A. Crossley, P. Santos, R. L. Todd, and J. B. Waide. 1981. Exceptions to the AET model: deserts and clear-cut forest. Ecology 62:275–277.

Supplemental Material

Ecological Archives

Appendices A and B are available online: http://dx.doi.org/10.1890/14-1482.1.sm