

The effects of heating, rhizosphere, and depth on root litter decomposition are mediated by soil moisture

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Abstract The breakdown and decomposition of plant inputs are critical for nutrient cycling, soil development, and climate-ecosystem feedbacks, but uncertainties persist in how the rates and products of litter decomposition are affected by soil temperature, rhizosphere, and depth of input. We investigated the effects of soil warming (+ 4 °C), rhizosphere, and depth of litter placement on the decomposition of *Avena fatua* (wild oat grass) root litter in a Mediterranean grassland ecosystem. Field lysimeters were

subjected to three environmental treatments (heating, control, and plant removal) and three ¹³C-labeled root litter addition treatments (to A horizon, to B horizon, and no-addition disturbance control) for each of two harvest time points. We buried root litter in February 2014 and measured loss of ¹³C in CO₂ from the soil surface and in leachate as dissolved organic carbon (DOC) over two growing seasons. At the end of each growing season we recovered the ¹³C remaining in the soil. Loss of root litter C occurred almost entirely via heterotrophic respiration, with an estimated < 2% lost as DOC during the initial decay period. The added roots were broken down and incorporated into bulk soil material very quickly; only ~ 30% of added root was visible after 6 months. In the first growing season, decomposition occurred faster in the B than in the A

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horizon, the latter having greater moisture limitation. Subsequently, there was almost no further decomposition in the B horizon. After two growing seasons, less than 20% of the added root litter C remained in the A or B horizons of all environmental treatments. Heating did not stimulate decomposition, likely because it exacerbated the moisture limitation. However, while plots without plants dried down more slowly than plots with plants, their decomposition rate was not significantly greater, possibly due to the lack of rhizosphere processes such as priming. We conclude that in this Mediterranean grassland ecosystem, soil moisture, which is affected by season, depth, heating, and rhizosphere, plays a dominant role in mediating the effect of those factors on root litter decomposition, which after two seasons did not differ by depth or by treatment.

Keywords Root litter · Decomposition · ^{13}C labeling · Warming · Soil moisture · Soil depth · Rhizosphere

Introduction

A significant portion of soil organic carbon (SOC) is derived from root litter (Rasse et al. 2005; Schmidt et al. 2011; Lehmann and Kleber 2015), particularly in grasslands where root-derived inputs can account for 70–80% of the annual net primary production (Scurlock et al. 2002). Climate and land-cover change may affect the vertical distribution of root litter inputs (Iversen 2010; Kell 2011); if root litter decomposition varies with soil depth or proximity to an active rhizosphere, climate and land-cover change may affect soil carbon storage even in the absence of any change in total belowground productivity.

While the depth-dependence of litter decomposition has been the subject of several recent studies, the relative control of abiotic and biotic factors on the vertical pattern of litter decomposition remains unclear. Soil depth affects root litter decomposition rates in some studies (Rovira and Vallejo 1997; Gill and Burke 2002; Garcia-Pausas et al. 2012; Berhe 2012), but not all (Weaver 1947; Sanaullah et al. 2011; Solly et al. 2015). Compared to topsoil, subsoil has a lower microbial biomass concentration and fungi:bacteria ratio (Stone et al. 2014), lower root density and

less rhizosphere volume, reduced connectivity between SOC moieties, and greater protection of organic matter by aggregates and mineral particles (Schrumpf et al. 2013), all of which tend to reduce microbial activity. However, given that subsoils are insulated from temperature and moisture extremes (Hanson et al. 2011), they may provide better conditions for decomposition (Sanaullah et al. 2011). The overall effect of soil depth on the rate of root litter decomposition depends, however, on site-specific biotic and abiotic factors (Rumpel and Kogel-Knabner 2011; Billings et al. 2015). For example, in a shortgrass steppe (Central Plains, USA) the rate of root litter loss over 33 months decreased with depth, attributed to differences in microbial community (Gill and Burke 2002); while in a temperate agroecosystem in southwest France, root-derived C and N losses over three years were similar across three depths, in spite of higher SOC content and greater microbial activity in the topsoil than in the subsoil (Sanaullah et al. 2011). In Mediterranean ecosystems, summer drought has been shown to limit decomposition, especially in surface horizons (Rovira and Vallejo 1997), although wetting–drying cycles, which have the potential to stimulate decomposition (Birch 1964; Borken and Matzner 2009), are more prevalent at the soil surface. In short, there is no consensus on the effect of depth on litter decomposition.

Soil temperature has long been recognized to influence the rate of litter decomposition (Gholz et al. 2000; Chapin et al. 2011). Although soil warming is expected to stimulate root litter decomposition through its direct effect on microbial metabolism (Kirschbaum 1995), it can also affect decomposition through its indirect effects on soil moisture and nutrient availability (Shaver et al. 2000). Moreover, the presence of live roots can affect decomposition via rhizosphere priming (Kuzyakov 2002; Zhu and Cheng 2011). For example, live roots can enhance litter decomposition by releasing labile compounds that stimulate microbial enzyme production, or they can suppress litter decomposition by either outcompeting microbes for soil moisture and nutrients or exuding labile organic compounds that microbes prefer to use over litter (Cheng and Kuzyakov 2005; Bird et al. 2011; Zhu et al. 2014). While the mechanisms linking warming and rhizosphere processes to root litter decomposition are known, their effects under field conditions remain

poorly understood (Cheng et al. 2014), particularly in the water-limited Mediterranean grassland ecosystem (Chou et al. 2008; St Clair et al. 2009).

Root litter in soil can be mineralized to CO₂, mobilized as dissolved organic carbon (DOC), and integrated into the bulk soil (i.e., broken down into smaller size particles or chemically transformed by physical and/or biotic actions) and microbial biomass. Numerous studies have used the litterbag method to quantify changes in litter mass, carbon, and nutrients during decomposition (e.g., Rovira and Vallejo 1997; Silver and Miya 2001; Zhang et al. 2008; Solly et al. 2015). However, this approach introduces artifacts, such as restricting the physical contact of litter with the soil matrix. Moreover, unless the litter material is isotopically labeled, this approach is limited because it does not provide information on the fate of litter that is not recovered in the litterbag (e.g., Bird and Torn 2006; Cotrufo et al. 2015; Hatton et al. 2015). Isotopic techniques provide a method to trace the fate of root-litter carbon into solid, dissolved, and gaseous forms (Bird and Torn 2006; Santos et al. 2016).

In this study, we traced the fate of root litter in a Mediterranean grassland. Our objectives were to assess the effects of soil warming and plant rhizosphere on the decomposition of ¹³C-labeled root litter buried at two different depths (A and B horizons). We measured root carbon lost as DOC, respired as CO₂, and retained in root versus bulk soil fractions after 6 and 15 months (i.e., at the end of one and two growing seasons) of in situ decomposition. We hypothesized that (1) root litter would break down into the bulk soil matrix and mineralize faster in the A horizon than in the B horizon; (2) heating would stimulate decomposition at depth, but exacerbate moisture limitation at the surface; and (3) the presence of rhizosphere would stimulate root litter decomposition in both horizons.

Materials and methods

Study site

The study took place at the Lysimeter Facility (Hawkes et al. 2005) of the Hopland Research and Extension Center, CA (39°00'N and 123°04'W). The climate is Mediterranean with hot, dry summers and mild, rainy winters. Lysimeters were first established in the 1970s and rebuilt in 2008. They are buried

within a sloped hillside to maintain the natural soil microclimate. Each lysimeter is contained in a steel drum (38 cm in diameter and 56 cm deep). The columns were packed with 33 cm of B-horizon Laughlin soil topped with 15 cm of A-horizon Laughlin soil from a nearby grassland. When we started the experiment in 2014, however, the total soil depth was 45 cm, indicating 3 cm of subsidence since the columns were packed in 2008. The lysimeters have a drain on the bottom, covered with filter fabric, 5 cm of clean sand and another layer of filter fabric. Each is plumbed to collect all water draining through the soil column in a 5 gallon glass carboy. The Laughlin series (fine-loamy, mixed, mesic Ultic Haploxeroll) is a typical grassland soil in the California North Coast region. SOC content decreases from 2.3% at the surface (0–8 cm) to 0.7% at depth (38–42 cm), while soil ¹³C = 28.0 to – 26.6‰ (Fig. S1). The lysimeters, or plots, were seeded in February 2014 and in October 2014 with an annual grassland mix of *Avena barbata* 40% (15 seeds, 16.0 g), *Bromus hordeaceus* 20% (25 seeds, 1.6 g), *Vulpia myuros* 20% (20 seeds, 0.3 g), and *Achillea millefolium* 20% (100 seeds, 0.1 g).

Experimental design

Treatments

Three environment factors were crossed with three substrate factors and replicated across four blocks. In total, 36 plots (3 × 3 × 4) were used in this study. The environment factors, designated as “treatments”, were control (ambient temperature, seeded), heated (ambient + 4 °C, seeded), and unplanted (ambient temperature, unseeded and weeded weekly). The three substrate factors, designated as “litter additions”, were root litter added to the A horizon (8–12 cm), root litter added to the B horizon (38–42 cm), and disturbance control (no root litter addition) (Fig. S2).

Plot design and soil microclimate monitoring

We divided each plot into quadrants (Fig. S2), one for temperature and moisture sensors, two for litter addition and respiration measurements, and the last for plant biomass measurement and soil nutrient assays. A single 50 cm long heating cable (SLMCAB10120B, BriskHeat Corp, Ohio) was placed

vertically in the center of each plot, inside a 1.9 cm steel conduit. Heating was controlled by a Campbell CR10X datalogger programmed to maintain a 4 °C difference in soil temperature between heated and control plots. Heating was initiated when litter was added in February 2014. Soil temperature was continuously monitored in the 12 disturbance-control plots—four each of control, heated, and unplanted treatments—using custom-made probes with thermistors (Omega 44005, USA) installed at 10 and 40 cm. Soil moisture was continuously monitored in the 12 disturbance-control plots—four each of control and heated treatments at 10 and 40 cm depth and four of the unplanted treatments at 10 cm depth only—using EC-5 moisture sensors (Decagon, USA). In December 2014 we installed dual temperature/moisture sensors (5TM, Decagon, USA) in 28 additional plots: Each of the four unplanted disturbance-control plots that previously had moisture sensors only at 10 cm depth received a 5TM sensor at 40 cm depth, and each of the 24 litter-addition plots that previously had no sensors received a 5TM sensor at 10 cm depth.

Litter additions

In February 2014 we added ¹³C-labeled *Avena fatua* roots (0.463 g C g root⁻¹, 5.6 atom%, grown for 12 weeks in a labeling chamber alternating ambient CO₂ with 10 atom% ¹³CO₂ every 3–4 days at the University of California (UC), Berkeley to either the A (8–12 cm) or B (38–42 cm) horizon in each of two quadrants per plot. Using a 3.8-cm diameter auger we removed soil from a sequence of depth intervals (0–8, 8–12, 12–20, 20–30, 30–38, and 38–42 cm). We cut root litter into ~ 1 cm long pieces and mixed it in soil that had been removed from either 8–12 or 38–42 cm depths. We subsequently repacked the soil from each depth interval into its original depth interval. We did not use litter-bags (e.g., Sanaullah et al. 2011) to maximize the contact between root litter and soil matrix. While all 36 plots experienced the same disturbance, only 24 plots received root litter addition—to either the A or the B horizon. The labeled litter was added to two quadrants of every plot, and one quadrat was harvested at the end of each of two growing seasons. Each litter addition consisted on average of 0.123 g of root material per soil core, corresponding to 109 g root m⁻² of the augered area.

Sampling and analyses

Soil CO₂ fluxes

Surface CO₂ fluxes were measured from 10-cm diameter PVC collars, which were centered over the litter addition, once prior to the addition of labeled root litter. Following root litter addition and plant germination, surface CO₂ and ¹³CO₂ fluxes were measured four times each during the first and second growing seasons (on 3/15/14, 4/5/14, 5/7/14, 5/20/14, 12/18/14, 2/3/15, 3/18/15, and 4/23/15). As plants were not clipped inside the collar, the measured CO₂ fluxes represent ecosystem respiration (including respiration from aboveground plants and belowground plants and microbes). In the first growing season, we used a Li-Cor 6400 analyzer and a Li-Cor 6400-09 soil respiration chamber to measure CO₂ flux and 1 M NaOH solution static trapping (24-hour) to measure the ¹³C value of CO₂ flux (Fig. S3). The trapped CO₂ was precipitated as SrCO₃ and measured for ¹³C (Harris et al. 1997). In the second growing season, we used a Cavity Ringdown Spectrometer (G2131-i, Picarro, USA, Fig. S3) to measure CO₂ and the Keeling-plot method to determine ¹³CO₂ fluxes (Torn et al. 2003). We conducted these measurements over a 5 min period on one collar per plot.

Dissolved C in leachate

Dissolved organic carbon (DOC) losses were quantified over the course of the study. After precipitation events (Fig. 1) we collected leachate from the bottom of each lysimeter (on 3/4/14, 3/15/14, 4/5/14, 5/7/14, 12/5/14, 12/11/14, 2/18/15, and 2/23/15). Leachate was weighed to determine volume and a subsample was frozen for analysis. In the lab, the subsamples were thawed and filtered through 0.45 μm acrodisc supor membrane filters (Pall Corporation, Switzerland) prior to analysis. The 0.45 μm pore size was chosen following a test that showed no difference (p = 0.85) in total organic carbon recovered after filtration through 0.2 μm, 0.45 μm, and control (no filter). Dissolved organic carbon (DOC) was calculated by taking the difference of total carbon and inorganic carbon (IC), which were measured directly with a TOC-V CSH Analyzer (Shimadzu, Japan). To obtain DO¹³C (3/4/14 and 3/15/14 samples only), subsamples were sent to the Stable Isotope Facility at

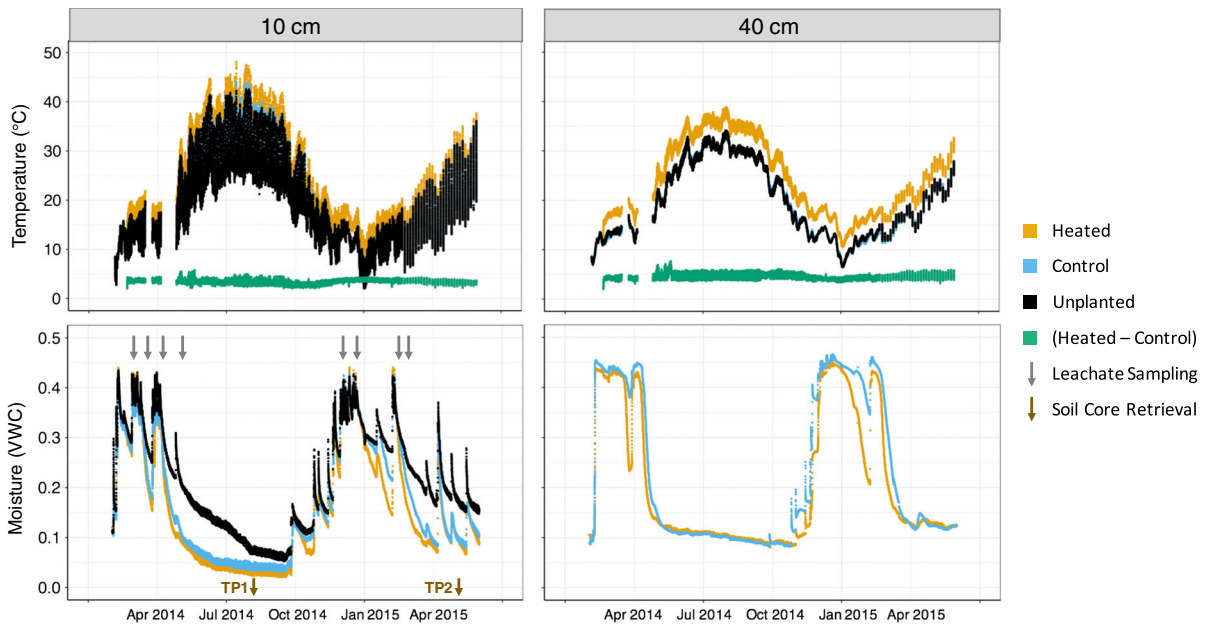


Fig. 1 Soil temperature and moisture at 10 and 40 cm for the period of the study in heated (yellow), control (blue), and unplanted (black) plots. The temperature difference between heated and control plots is shown in green. In the lower left

panel, gray arrows (top) indicate the rain events after which leachate was sampled and brown arrows (bottom) indicated soil core retrieval after Season 1 (August 2014, TP1) and after Season 2 (May 2015, TP2). (Color figure online)

UC Davis where they were acidified and purged prior to being run on a 1030 TOC Analyzer (OI Analytical, College Station, TX) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The contribution of labeled root carbon to DOC was determined to be a very small fraction of the applied litter ($< 0.2\%$, Fig. 5), and previous studies showed that leaching losses occurred mainly in the initial period after burial (Guelland et al. 2013; Soong et al. 2015), so samples from subsequent time points were only measured for DOC and not DO^{13}C .

Total C losses via DOC versus CO_2

Total DOC losses (g C m^{-2}) were determined by summing them over all rain events over the course of the experiment (from February 2014 to May 2015, 15 months). To extrapolate total CO_2 respired over the course of the experiment, we multiplied the mean rate per sampling campaign ($\text{g C m}^{-2} \text{ h}^{-1}$) by the hours in 12 months (excluding the three driest months—June to August 2014—when ecosystem respiration would have been negligible). We made each calculation separately for planted and unplanted plots.

Root litter ^{13}C recovery in soil

We recovered the soil to which the root litter had been applied after the first growing season in August 2014 and again at the end of the second growing season in May 2015 using a 7.6 cm diameter \times 45 cm long corer with a slide hammer attachment (AMS samplers Inc., USA). The intact cores were recovered in plastic liners, and capped. In 2014, we cut the cores into 0–5, 5–10, 10–15, 15–20, 20–35, 35–40, and 40–45 cm increments. We processed all depth increments of the disturbance control plots, the 5–20 cm sections of the plots with A horizon litter additions, and the 35–45 cm sections of the plots with B horizon litter additions. In 2015, we cut the cores into 0–7, 7–14, 14–20, 20–35, and 35–45 cm increments, and processed the 7–14, 14–20 and 35–45 cm sections of the disturbance control plots, the 7–14 and 14–20 sections of the plots with A horizon addition, and the 35–45 cm increment of the plots with B horizon addition. These sections spanned the depths to which root-litter was added (8–12 or 38–42 cm), and allow for potential downward movement of added root litter (Fig. S4).

In the lab, samples from each depth increment were weighed and then passed through a 2 mm sieve.

Gravimetric water content was determined on a subsample of the 2 mm soil. Rocks > 2 mm were separated and weighed. Coarse roots, including long roots that passed through the sieve, were separated by hand-picking for < 1 min. The 2 mm soils were subsampled using the cone-and-quarter technique to obtain a < 100 g aliquot for further processing. Sub-samples were weighed, and visible roots were hand picked (for 10 min per 50 g for shallow samples up to 14 cm depth and 5 min per 50 g for deeper samples) for heated and control plot samples. The root picking effort was halved for unplanted plot samples. Roots were washed and dried at 55 °C for 24 h, then weighed. The remaining “bulk” soil subsample was ground on a roller mill to pass a 250 µm mesh. Soil, coarse root, and fine root samples were analyzed for C and ¹³C at UC Berkeley (IsoPrime 100 IRMS in line with a Vario micro cube EA, Isoprime, UK).

We determined the proportion of carbon in our samples that was derived from the labeled litter (P_{litter}), in the leachate or solid samples, using a two end-member mixing model:

$$P_{litter} = (C_{sample} - C_{control}) / (C_{litter} - C_{control}),$$

where C_{sample} is the fractional abundance of ¹³C in the sample under consideration, $C_{control}$ is the average fractional abundance of ¹³C in the disturbance control (calculated separately for each sampling and treatment), and C_{litter} is the fractional abundance of ¹³C in the applied litter. For the solid samples, $C_{control}$ was calculated separately for the A and B horizons and P_{litter} was calculated for the root and bulk soil fractions. Litter recovery—in bulk soil, roots, leachate, or respired CO₂—was reported as the fraction of applied litter C recovered. Recovery as a fraction of applied litter was calculated by multiplying the proportion of carbon derived from the labeled litter (P_{litter}) by the mass of carbon recovered in those samples divided by the mass of carbon initially applied as litter.

Litter applied to the A horizon was found across various depth increments, namely the 5–10, 10–15, and 15–20 cm increments in 2014, and the 7–14 and 14–20 cm increments in 2015. And litter applied to the B horizon was found in the 35–40 and 40–45 cm increments in 2014, and the 35–45 cm increment in 2015. C_{sample} and $C_{control}$ were therefore calculated for the entire depth increment under consideration. Based on statistical differences across groups, bulk soil

$C_{control}$ was calculated separately by year and by horizon, and averaged across treatments. On the same basis, root $C_{control}$ was calculated separately by year, and averaged across horizons and treatments.

Soil moisture days

To investigate the cumulative differences in soil moisture between the A and B horizons over the course of the study, we created a simple metric called soil moisture days (SMD) akin to the commonly used growing degree days metric (based on Gilmore and Rogers 1958) in which the volumetric water content (VWC) of the soil was summed over the days of the field incubations. This metric considers both average daily water content and the number of days of the incubations, and is functionally relevant to litter decomposition rate.

Plant biomass and soil nutrient availability

Aboveground biomass (Fig. S5) was harvested for the full plot in July 2014, after the biomass had senesced, and for ¼ of the plot in April 2015, at peak biomass, avoiding the area above sensors and the collar used for soil flux measurements. Root biomass (Fig. S5) was calculated from roots separated from disturbance-control plot soil cores ($n = 4$) in 2014.

PRS probes (PRS probes, Western Ag Innovations, Canada; Johnson et al. 2007) were inserted to 7–12 cm (A horizon) to measure soil nutrient supply during the first growing season. Briefly, a pair of probes (one for cations and one for anions) was inserted in each of three locations per plot to measure nutrient (N and P) supply during both the early (2/20 to 4/5) and the late (4/5 to 7/11) growing season in 2014. Given that the litter addition to A or B horizons should not significantly affect soil nutrient mineralization, we pooled probes from the three litter-additions (A horizon, B horizon, and disturbance control) and measured nutrient supply rates for the three treatments (control, heated, unplanted, Fig. S6).

Statistical analyses

To determine how recovery of ¹³C-labeled root litter in bulk soil, root fraction, and leachate, and soil respiration were affected by sampling date, experimental treatment, or placement depth (Table 1), we fit

Table 1 Results (p values) of mixed model regression analyses with plot as a random effect

Response variable	¹³ C-labeled litter recovery in				Total C in		Soil moisture days
	Bulk soil	Root fraction	Leachate	Respiration	Leachate	Soil flux	
Source							
Observations	47 ^a	44 ^a	48 ^a	82 ^b	286 ^c	288 ^d	39 ^e
R ²	0.72	0.80	0.70	0.88	0.91	0.83	0.85
Date (D)	< 0.001	ns	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment (T)	ns	0.04	ns	ns	< 0.001	< 0.001	0.013
Horizon (H)	< 0.001	ns	< 0.01	NA	ns	ns	< 0.01
D × T	ns	ns	ns	< 0.01	< 0.001	< 0.001	NA
D × H	< 0.001	ns	< 0.01	NA	0.018	ns	NA
T × H	ns	ns	ns	NA	ns	ns	NA
D × T × H	ns	ns	ns	NA	ns	ns	NA

P values > 0.05 are not shown (ns). Recovery of litter in soil respiration was only analyzed for plots where litter was added to the A horizon

^aFor each of two time points targeted the 24 plots with litter additions ($n = 2 \times 24$)

^bFor each of 7 measurement campaigns targeted the 12 plots with litter added to the A horizon ($n = 12 \times 7$)

^cFollowing each of 8 rain events targeted all 36 plots ($n = 8 \times 36$)

^dFor each of 8 measurement campaigns targeted all 36 plots ($n = 8 \times 36$)

^eFor each of two time points targeted 12 disturbance control plots, where soil moisture sensors were installed. The 4 heated and 4 control plots each had sensors at 10 and 40 cm depth ($n = 2 \times 8 \times 2$). The 4 unplanted plots only had sensors at 10 cm ($n = 2 \times 4 \times 1$)

general linear models with fixed effects, including where appropriate their interactions, and added plot as a random effect using JMP statistical software (SAS). We used the Tukey test with $\alpha = 0.05$ to test for differences among means.

Results

Soil temperature and moisture

The warming treatment worked effectively. Throughout the experiment, the heated plots were ~ 4 °C warmer than the control plots while maintaining natural diurnal temperature variation, with more variation at 10 cm than at 40 cm depth in both treatments (Fig. 1). Integrating over each field incubation period, heating reduced soil moisture at both depths. Dry-down occurred faster and to a greater degree in the first compared to the second season and was less pronounced in unplanted compared to planted plots (Fig. 1).

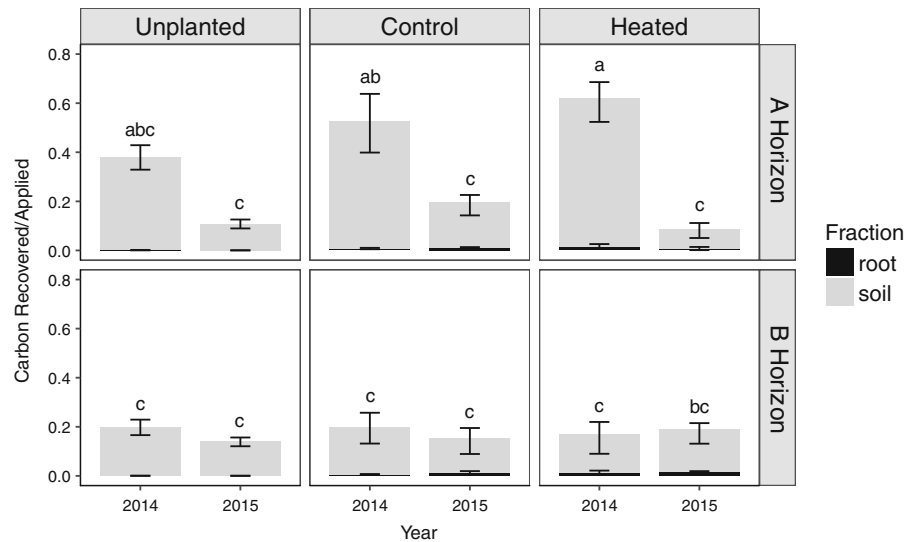
During the initial field incubation period (February to August 2014) cumulative Soil Moisture Days

(SMD) were 50% greater in the B compared to the A horizon. Over the entire incubation period (February 2014 to May 2015), which included a wetter year, cumulative SMD were 25% greater at depth. SMD differed significantly by treatment ($p < 0.01$, Unplanted > Heated), horizon ($p < 0.001$, B > A), and time-point ($p < 0.001$, Season 1 + 2 > Season 1). We did not test for interactions due to insufficient replication (Table 1).

Recovery of root litter C in the bulk soil and root fractions

On average, we recovered approximately one-third of the applied litter carbon after the first growing season and one-sixth after the second growing season (Fig. 2). Following Season 1 we recovered significantly more carbon in the A than in the B horizon ($\sim 50\%$ vs. 20%). In contrast, after Season 2 we recovered $\sim 20\%$ of applied carbon in both horizons. While heating and planting had no effect on recovery of litter ¹³C in the 2 mm bulk-soil fraction, there was a statistically significant effect of sampling date ($p < 0.001$), horizon ($p < 0.001$), and sampling

Fig. 2 Recovery (mean \pm SE) of applied ^{13}C -labeled root-litter carbon in root and soil fractions after 2014 and 2015 growing seasons by treatment (Unplanted, Control, Heated), horizon (A and B horizon), and year (2014 and 2015). Letters denote results of the Tukey test, conducted for the soil fraction. Means labeled with different letters are significantly different ($p < 0.05$)



date \times horizon ($p < 0.0001$; Table 1). Recovery of applied litter in the visible root fraction (fine roots plus coarse roots) was about an order of magnitude lower than in the bulk soil fraction, and was affected by treatment ($p = 0.04$), with lower recovery in the unplanted than in the heated plots (Fig. 2). The loss of applied root-litter carbon (i.e. one minus recovered root-litter carbon) was linearly correlated to

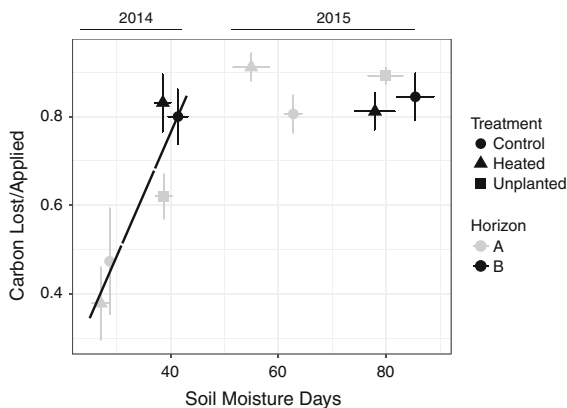


Fig. 3 Loss (mean \pm SE) of applied ^{13}C -labeled root-litter carbon as a function of cumulative Soil Moisture Days (soil VWC summed over the days of in situ incubation). Each point represents the average ($n = 4$) value for a given treatment (control = circles, heated = triangles, unplanted = squares), horizon (A horizon = open symbols, B horizon = closed symbols), and year (2014 = black, 2015 = gray). The parameters for the 2014 linear regression line are $y = -0.350 + 0.0279x$, $R^2 = 0.84$, $p = 0.028$, $n = 5$. We did not monitor soil moisture in the B horizons of unplanted plots

cumulative SMD for Season 1 ($R^2 = 0.84$, $p = 0.03$; Fig. 3), but not for Season 2.

DOC losses

We recovered leachate following four rain events in each water year, with three times more leachate recovered in 2015 than in 2014. Total DOC losses differed by environmental treatment ($p < 0.001$): they were greater in planted than in unplanted plots, but were not different between heated and unheated plots (Fig. 4, Table 1). Total DOC losses were also affected by leachate volume and thus varied by sampling date ($p < 0.001$). While there was no significant effect of litter addition ($p > 0.1$), there was a significant

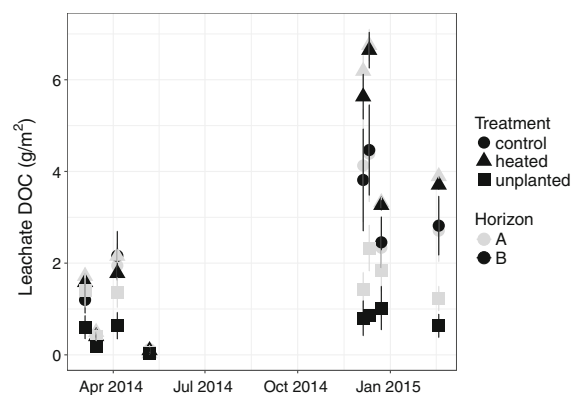


Fig. 4 Leachate DOC per m^2 area for all rain events by experimental treatment and depth of litter addition. Error bars represent one standard error of the mean ($n = 4$)

interaction of date \times litter addition (to A horizon, B horizon, or disturbance control), because after the 3/14/14, 5/6/14, and 12/10/14 precipitation events, DOC losses were larger for plots with litter added to A or to B horizons than for disturbance control. On average, any given rain event led to a DOC loss corresponding to approximately 0.2% of the carbon stock in the soil column. Similarly, roughly 0.15% of the labeled litter carbon in the column was lost as DOC in the first two rain events following litter burial. Losses of the labeled litter C were slightly higher for litter applied to the B than to the A horizon ($p < 0.01$), and higher in the first sampling date ($p < 0.001$), with no effect of experimental treatment (Fig. 5, Table 1).

CO₂ losses

Total ecosystem respiration varied by sampling date, experimental treatment (unplanted < control < heated), and their interaction ($p < 0.001$; Fig. 6), but not by depth of litter addition (Table 1) or between litter addition treatment and disturbance control. The respiration rate was higher in heated than in control plots early in the growing season each year, but the trend reversed late in the season when moisture was low and plants senesced earlier in heated plots. When soil was very dry (5/20/2014) and all plants had

senesced, the control, heated, and unplanted plots had the same total respiration rate. Neither the addition of root litter or placement horizon had a detectable effect on ecosystem respiration ($p > 0.7$). By extrapolating observed leachate and respiration losses while accounting for seasonal differences in these fluxes, we estimate that more than 98% of total carbon loss occurred via respiration with less than 2% lost as DOC. The ratio of these two loss pathways was similar for planted and unplanted plots.

Respiration in the plant-free plots decreased monotonically over time in Season 1, presumably as labile soil carbon stocks became depleted, and then leveled off in Season 2. We found no clear relationship between heterotrophic respiration (from unplanted plots) and soil microclimate.

The method we employed to sample soil ¹³CO₂ efflux, using 10 cm diameter collars inserted only 2–3 cm into soil, did not capture all of the respired root litter given that the enriched ¹³CO₂ did not only diffuse straight upward. This is apparent in the ¹³CO₂ flux results, which indicated faster loss of litter placed in the A horizon than in the B horizon in the first season, while the recovery data showed the opposite. We conclude that much of the ¹³CO₂ efflux from respired root litter diffused beyond the 10 cm diameter perimeter, especially for litter placed in the B horizon. Therefore, we interpret our results qualitatively and for litter placed in the A horizon only. Respiration of root litter ¹³C decreased very rapidly as soils dried during the first season and later, during the second season, as the amount of remaining litter decreased (Fig. 6), resulting in a significant effect of sampling date ($p < 0.001$). Comparing relative ¹³CO₂ losses (of litter applied to the A horizon) in heating and planting treatments, there was no detectable effect of heating or planting on respired root litter ($p = 0.19$; Table 1), consistent with the total recovery results. Extrapolating the detected ¹³CO₂ losses over the entire incubation period (minus 3 dry summer months) we obtain a flux rate that is consistent with the observed litter recovery in soil and roots.

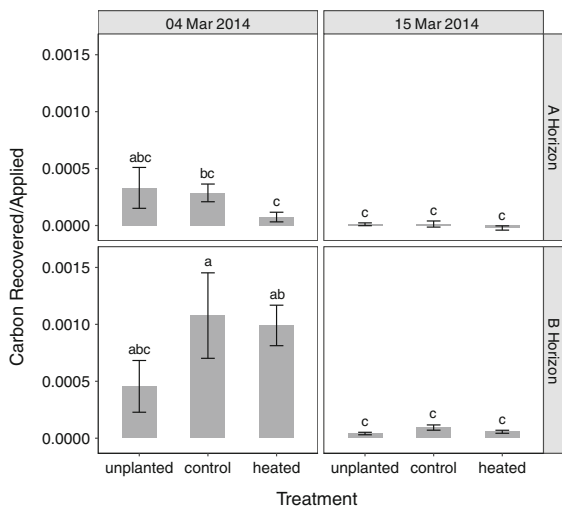


Fig. 5 Fraction of applied ¹³C-labeled root-litter carbon recovered in leachate DOC, by the horizon in which the litter was placed, for the first two samplings on March 4 and March 15, 2014. Letters denote results of the Tukey test—means labeled with different letters are significantly different ($p < 0.05$)

Plant biomass and soil nutrient availability

Aboveground biomass (AGB) was not significantly different between disturbance-control and litter-addition plots, and thus was pooled to compare across environmental treatments and years. AGB increased

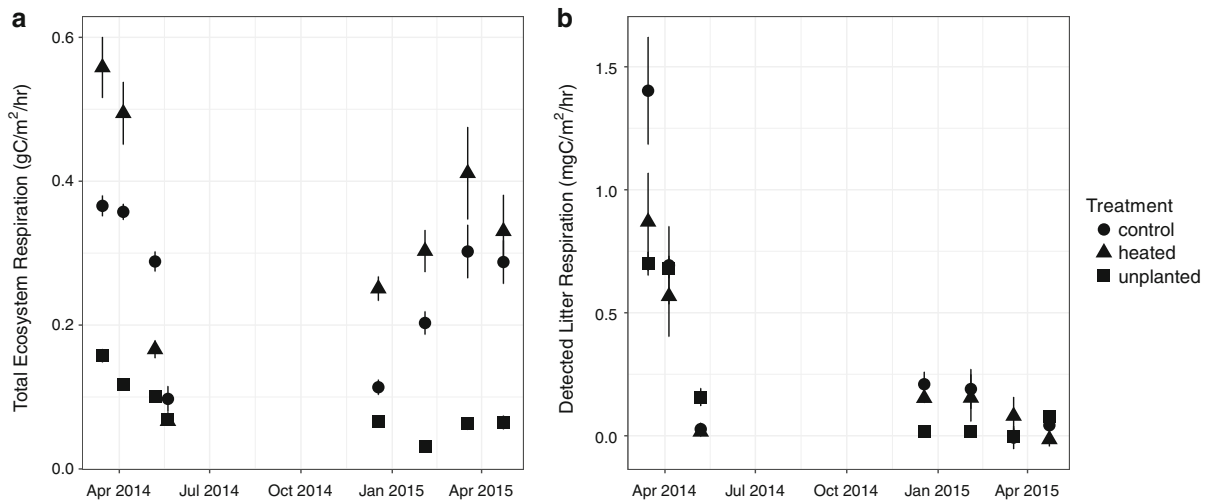


Fig. 6 **a** Total ecosystem respiration ($\text{g C m}^{-2} \text{ h}^{-1}$) and **b** detected root litter respiration ($\text{mg C m}^{-2} \text{ h}^{-1}$ from the A horizons) by sampling date for control, heated, and unplanted plots. Error bars represent one standard error of the mean (**a** $n = 12$, **b** $n = 4$)

significantly with heating, and was higher in the wet year of 2015, with no interaction between heating and year (Fig. S5). In contrast, belowground biomass (only measured in 2014) was not significantly affected by warming ($p > 0.05$).

We used PRS probes to measure soil nutrient availability during the first growing season (Fig. S6). Vegetation caused significant reductions in soil N availability (in both early and late season) and significant increases in soil P availability (only in the early season), while heating had no effect on soil N or P availability.

Discussion

Greater microbial biomass and more oxygen promote faster decomposition in topsoil relative to subsoil horizons (Rumpel and Kogel-Knabner 2011; Billings et al. 2015). We therefore hypothesized that root litter in the A horizon would break down into the bulk soil matrix and mineralize faster than in the B horizon. So, it was surprising that litter mineralized twice as fast in the B than in the A horizon during the first season. Yet this result generally agrees with Rovira and Vallejo (1997), who also measured litter decomposition in a water-limited Mediterranean system. California, including our site, experienced a severe drought in 2014, during the first growing season. Drying was less severe in the B horizon, which had greater cumulative

soil moisture compared to the A horizon (Fig. 1), consistent with faster initial root decomposition in the B horizon (Fig. 3).

We also hypothesized that (a) heating would stimulate decomposition at depth, but exacerbate moisture limitation at the surface, and (b) the presence of rhizosphere would stimulate root litter decomposition in both horizons, as observed by Bird et al. (2011). While heating stimulated ecosystem respiration early in the season (Fig. 6a), it did not affect detected litter respiration from the A horizon (Fig. 6b), even when soil moisture was not limiting (on 3/15/14, 4/5/14, and 12/20/14, when VWC at 10 cm depth was $> 25\%$ for all treatments). In addition, there was no effect of treatment on litter recovery (Table 1, Fig. 2). We surmise that heated and unplanted treatments had counteracting effects on soil moisture and thus on litter loss: the severe moisture limitation to decomposition in the A horizon during the summer was alleviated in the unplanted plots due to their lack of transpiration, and was somewhat exacerbated by increased transpiration in the heated plots (Fig. 1 lower panel). In the early season, when soil moisture was relatively high for both planted and unplanted plots, the presence of rhizosphere stimulated root litter decomposition ($^{13}\text{CO}_2$ loss, Fig. 6b), reduced N availability likely due to plant uptake, and elevated soil P availability (Fig. S6), likely due to the priming effect (Johnson et al. 2007; Bird et al. 2011; Zhu et al. 2014). However, this rhizosphere effect disappeared or even reversed in

the late season (Fig. 6b) when soil moisture was much lower in planted plots due to transpiration (Fig. 1). Overall, there was no significant effect of heating, rhizosphere, or depth on the rate of root litter decomposition after two seasons. However, all three factors do affect moisture regimes, and thus decomposition dynamics, albeit at smaller time scales.

In spite of greater soil moisture in the B horizon, increased root decomposition did not persist into Season 2, and we surmise that when only 20% of the applied litter remained, decomposition was controlled by factors other than climate, such as inherent recalcitrance of the remaining root material or energy/nutrient limitation of further microbial activity at depth (Schmidt et al. 2011). At depth, a lack of fresh labile carbon inputs limits the energy that microbes have to produce the enzymes they need to decompose recalcitrant carbon sources (Fontaine et al. 2007). Interestingly, while Solly et al. (2015) and Sanaullah et al. (2011) also report similar longer term decomposition rates across depths, the dominant factors that control decomposition (e.g. abiotic vs. abiotic limitations) differed across depths.

Decomposition is the sum of many processes: comminution or fragmentation, whereby litter is broken down into smaller pieces relatively quickly (weeks to months) by soil fauna and microorganisms (Baldock and Skjemstad 2000). Five months following root litter burial, we recovered an order of magnitude more labeled litter in the bulk soil than in the root fraction (including the smallest fragments recognizable to the naked eye), indicating a high degree of comminution. One explanation for the rapid break down of root litter into bulk soil could be that the applied *Avena* litter came from very fine roots, which were cut into 1 cm lengths before being thoroughly mixed with the soil. Moreover, while the rhizosphere had no statistically significant effect on total litter recovery, we recovered a greater fraction of the applied litter in the root fraction of planted compared to unplanted plots, which counter-intuitively indicates greater comminution of roots into bulk soil in the absence of the plant rhizosphere. This observation may be tied to greater soil moisture levels in unplanted plots leading to higher soil activity of the fauna responsible for comminution, or to differences in the microbial or macrofaunal communities in the planted and unplanted plots (e.g., Brandt et al. 2006; Bird et al. 2011; Shi et al. 2015). Populations of soil macrofauna,

which are key to litter comminution, can be reduced during dry conditions (Lindberg et al. 2002).

Another process that contributes to decomposition is leaching, whereby small molecular weight compounds are transported out of litter and through the soil profile via dissolution in water, primarily in the initial flushing events (Guelland et al. 2013; Soong et al. 2015). We recovered less than half a percent of the applied carbon in the leachate recovered from the two initial rain events following the burial of root litter (Fig. 5). As outlined above, we estimate that in total, < 2% of root-litter carbon losses were via DOC, thus we conclude that in this Mediterranean grassland ecosystem litter losses are overwhelmingly dominated by heterotrophic respiration. This contrasts sharply with a laboratory incubation study utilizing a variety of soil types in which DOC was found to account for at least 20% of the carbon losses (Soong et al. 2015), but agrees with (a) a similar field study in a coniferous forest with a Mediterranean climate (Bird and Torn 2006) that found minor movement of litter-derived DOC to deeper soil horizons, and (b) a recent field study in a temperate forest (Santos et al. 2016) that found less than 1% of applied root-litter carbon in DOC collected from zero-tension lysimeters below the litter burial depth.

Environmental treatment had no statistical effect on ^{13}C -labeled litter recovery, but did have effects on total carbon loss in respired CO_2 and in DOC. Total leachate DOC, as well as the differences between treatments (heated > control > unplanted plots), were magnified in Season 2, which had larger rain events. However, no effect of treatment on the recovery of applied carbon in the leachate was observed (following the two initial rain events), either because none existed, or because the error was large relative to the small amount of DO^{13}C recovered. We see clear differences in total ecosystem respiration rates across treatments, with generally higher values in heated than in unheated plots and much higher values in the planted compared to the unplanted plots. In contrast, recovery of $^{13}\text{CO}_2$, a product of heterotrophic respiration, differed less across treatments.

Finally, the system that we used to warm the soil (Hanson et al. 2011; Hicks Pries et al. 2017) was inexpensive, effective, and avoided many artifacts of laboratory incubations. It enabled the study of the soil profile in situ, under natural diel and seasonal

fluctuations of temperature and soil moisture with time and depth, and with minimal disturbance to soil structure.

In the coming years and decades, global warming is expected to increase the severity of both drought and precipitation events in California. We found that in this Mediterranean grassland ecosystem, soil moisture, which is affected by season, depth, heating, and plants, plays a dominant role in mediating the effect of those factors on root litter decomposition, which after two seasons did not differ by depth or by treatment. Therefore, it will be important to complement this study with others that manipulate soil moisture (e.g., Suttle et al. 2007; Khalili et al. 2016) to better understand how decomposition of plant litter and soil organic matter will respond and feedback to climate change.

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