Ecology, 0(0), 2018, pp. 1–15

Effects of altered dry season length and plant inputs on soluble soil carbon

Peter M. Homyak [1,7] Joseph C. Blankinship,[2] Eric W. Slessarev,[3] Sean M. Schaeffer,[4] Stefano Manzoni [5,6] and Joshua P. Schimel[3]

[1]*Department of Environmental Sciences, University of California, Riverside, California 92521 USA*
[2]*Department of Soil, Water, and Environmental Science, University of Arizona, Tucson, Arizona 85721-0038 USA*
[3]*Department of Ecology, Evolution, and Marine Biology and Earth Research Institute, University of California, Santa Barbara, California 93106 USA*
[4]*Department of Biosystems Engineering and Soil Science, University of Tennessee, Knoxville, Tennessee 37996 USA*
[5]*Department of Physical Geography, Stockholm University, 106 91 Stockholm, Sweden*
[6]*Bolin Centre for Climate Research, Stockholm University, 106 91 Stockholm, Sweden*

Abstract. Soil moisture controls microbial activity and soil carbon cycling. Because microbial activity decreases as soils dry, decomposition of soil organic matter (SOM) is thought to decrease with increasing drought length. Yet, microbial biomass and a pool of water-extractable organic carbon (WEOC) can increase as soils dry, perhaps implying microbes may continue to break down SOM even if drought stressed. Here, we test the hypothesis that WEOC increases as soils dry because exoenzymes continue to break down litter, while their products accumulate because they cannot diffuse to microbes. To test this hypothesis, we manipulated field plots by cutting off litter inputs and by irrigating and excluding precipitation inputs to extend or shorten the length of the dry season. We expected that the longer the soils would remain dry, the more WEOC would accumulate in the presence of litter, whereas shortening the length of the dry season, or cutting off litter inputs, would reduce WEOC accumulation. Lastly, we incubated grass roots in the laboratory and measured the concentration of reducing sugars and potential hydrolytic enzyme activities, strictly to understand the mechanisms whereby exoenzymes break down litter over the dry season. As expected, extending dry season length increased WEOC concentrations by 30% above the 108 μg C/g measured in untreated plots, whereas keeping soils moist prevented WEOC from accumulating. Contrary to our hypothesis, excluding plant litter inputs actually increased WEOC concentrations by 40% above the 105 μg C/g measured in plots with plants. Reducing sugars did not accumulate in dry senesced roots in our laboratory incubation. Potential rates of reducing sugar production by hydrolytic enzymes ranged from 0.7 to 10 $\mu mol \cdot g^{-1} \cdot h^{-1}$ and far exceeded the rates of reducing sugar accumulation (~0.001 $\mu mol \cdot g^{-1} \cdot h^{-1}$). Our observations do not support the hypothesis that exoenzymes continue to break down litter to produce WEOC in dry soils. Instead, we develop the argument that physical processes are more likely to govern short-term WEOC dynamics via slaking of microaggregates that stabilize SOM and through WEOC redistribution when soils wet up, as well as through less understood effects of drought on the soil mineral matrix.

Key words: *Birch effect; carbon cycling; dissolved organic carbon; drought stress; exoenzymes; respiration.*

Introduction

Moisture is a key factor controlling soil carbon (C) cycling (Schimel et al. 2007, Borken and Matzner 2009); it controls microbial activity and, therefore, access to soil C and its fate (Schimel and Schaeffer 2012). Because microbial *activity* decreases as soils dry (Manzoni et al. 2012, Canarini et al. 2017), moisture is often conceptualized as a dominant control on microbial growth—moist conditions favor growth, whereas dry conditions constrain it. Yet, in contrast to these generalizations, microbial *biomass* can increase as soils dry (Matias et al. 2011, Parker and Schimel 2011, Boot et al. 2013, Homyak et al. 2014, Schaeffer et al. 2017). Disentangling how moisture regulates microbial access to C, and the fate of C is a necessary step toward validating assumptions built into models that predict C sequestration in soil organic matter (SOM) (Todd-Brown et al. 2014, Wieder et al. 2015, Crowther et al. 2016), especially because: (1) C models do not always capture dry season dynamics and (2) intensifying droughts and desertification are expected for many regions (Dai 2013).

Theory suggests that dry conditions constrain SOM decomposition because microbes become moisture stressed. However, as drought length increases, so does the concentration of water-extractable organic C (WEOC) (Zsolnay and Görlitz 1994, Xiang et al. 2008, Guo et al. 2014), suggesting that SOM decomposes even when soil is dry (Steinweg et al. 2013). In seasonally dry California grasslands, for example, a pool of WEOC increases linearly in step with microbial biomass C when soils are dry, peaking just before the onset of the wet season and then decreasing when rains return (Parker and Schimel 2011, Schaeffer et al. 2017), suggesting WEOC may contribute to CO_2 emission pulses when dry soils wet up (i.e., the Birch effect; Birch 1958). Though it remains unclear which processes cause WEOC to increase, the increase is thought to be controlled by both biological

Manuscript received 26 November 2017; revised 15 March 2018; accepted 13 July 2018. Corresponding Editor: Sasha C. Reed.
[7] E-mail: phomyak@ucr.edu

and physical processes, and their interactions (Miller et al. 2005, Lawrence et al. 2009, Schaeffer et al. 2017).

One perspective is that biological processes drive WEOC accumulation during the dry season. In particular, microbial C allocation is thought to control C availability (Kallenbach et al. 2016, Liang et al. 2017) and may help to explain WEOC accumulation. For example, some of the C acquired by microbes can be synthesized into cell walls, intracellular materials (e.g., proteins and nucleic acids), and extracellular materials that modify a cell's environment (e.g., extracellular polymeric substances; EPS) or help acquire resources (exoenzymes) (Schimel and Schaeffer 2012). Microbial C investment into synthesizing these relatively more complex molecules can stabilize C into forms that are not easily decomposed (Liang et al. 2017), which may contribute to the accumulation of WEOC. Exoenzymes, in particular, may continue to break down plant litter even under dry conditions (Schimel and Schaeffer 2012), producing plant-derived substrates that are not easily assimilated by microbes (Liang et al. 2017). Moreover, microbes can invest C into synthesizing osmolytes to adapt to dry conditions, which may contribute to the WEOC increase (Warren 2016). Microbial C allocation may, therefore, explain the rise in WEOC when soils dry, although identifying and disentangling the physiological processes that cause WEOC to accumulate under field conditions has proved challenging (Boot et al. 2013).

An alternate perspective is that physical processes primarily control WEOC accumulation under dry conditions. For example, drying–rewetting cycles can redistribute C as water moves into and out of pores and/or can slake aggregates exposing physically protected C to decomposers (Kemper et al. 1985, Denef et al. 2001, Kaiser et al. 2015). Destroying soil aggregates can mobilize substrates and increase CO_2 emissions on rewetting (Navarro-Garcia et al. 2012), while rewetting itself can release C that had been stabilized in soils for centuries (e.g., >600 yr old; Schimel et al. 2011). Furthermore, exposing soil to multiple drying–rewetting cycles can substantially increase microbial biomass, which suggests that C that is otherwise unavailable to microbes is mobilized from dry soil during wetting (Xiang et al. 2008). However, how the balance between, and interactions among, physical and biological processes regulate soil C dynamics in dry soil remains unclear.

The biological and physical processes for WEOC accumulation are not mutually exclusive. Rather, microbial C allocation and physical protection may interact to produce WEOC during the dry season. If exoenzymes remain active as soils dry, then plant detritus could still decompose during dry periods (Lawrence et al. 2009). Because diffusion is reduced in dry soil, the products of exoenzymes would accumulate, generating a pool of WEOC that increases with time as the dry season progresses (Steinweg et al. 2013, Manzoni et al. 2014, Schaeffer et al. 2017). In this way, drying can increasingly concentrate substrates as soils become hydrologically decoupled, generating pools of bioavailable materials that can diffuse to microbes when soils wet up (Manzoni and Katul 2014, Homyak et al. 2016, Canarini et al. 2017, Leitner et al. 2017).

The exoenzyme-based mechanism for WEOC accumulation, however, has not been experimentally demonstrated, limiting advances to conceptual frameworks that evaluate microbial C access and allocation (Dungait et al. 2012, Schimel and Schaeffer 2012). While models integrating these physiological and physical processes do well at capturing empirical observations (Lawrence et al. 2009, Manzoni et al. 2014, Zhang et al. 2014), it is essential to test their assumptions, otherwise preventing development of "real-world" processes on which soil C model structures should be built (Luo et al. 2016). Here, we focused on how soil moisture governs microbial access to C and asked: (1) Is exoenzyme-controlled decomposition of litter during the dry summer the source of WEOC? and (2) How does dry season length and access to plant litter drive WEOC production?

To address these questions, we manipulated a Mediterranean annual grassland to exclude plant C inputs and vary the length of the dry season. Plant C inputs (i.e., root exudates and litter) were excluded to test whether WEOC is derived from the breakdown of "fresh" plant C during the dry season (i.e., inputs from the antecedent winter's plant growing season). We also both shortened and extended the length of the dry season to: (1) determine whether WEOC concentrations varied according to the duration of drying and (2) assess microbial access to C by quantifying microbial biomass and CO_2 emissions in the field. Lastly, we coupled field observations with a laboratory incubation of grass roots, strictly to test mechanisms controlling litter breakdown. If exoenzymes break down litter over the summer dry season, we expected to observe increased concentrations of reducing sugars in litter over time. We hypothesized that: (1) WEOC increases because exoenzymes break down litter over the summer dry season—the longer soils remain dry, the more products of exoenzymes accumulate without diffusing to microbes—and (2) removing plants would lower WEOC concentrations because the enzymatic breakdown of fresh litter is the principal mechanism producing WEOC.

Materials and Methods

Site description

We studied a seasonally dry grassland at the University of California Sedgwick Reserve near Santa Barbara, California, USA (370 m ASL, 34.7120 N, 120.0388 W). The vegetation is dominated by nonnative Mediterranean annual grasses—primarily *Bromus diandrus*, *Bromus hordaceous*, and *Avena fatua*. The climate is Mediterranean with hot dry summers and cool wet winters (Appendix S1: Fig. S1A)—grasses grow during winter and die in summer. The mean annual precipitation is 380 mm with an average annual temperature of 16.8°C—daytime air temperatures average 33°C in summer and rarely drop below 0°C during winter nights. Roughly, 90% of annual precipitation falls between November and April. The Water Year (WY) officially begins on 1 October and ends on 30 September. During the 2 yr of the study, annual precipitation was roughly 50% below average (175 mm in WY 2013 and 201 mm in WY 2014).

The experimental sites are on nearly flat slopes (<2%) and are underlain by Pachic Argixerolls. The soils developed in alluvium derived from the Paso Robles Formation, which is a Plio-Pleistocene terrestrial deposit composed of clasts derived from Monterey Shale and Franciscan Formation mélange (mixtures of marine sediments and ultramafic ocean crust). The Paso Robles lithologies weather to produce 2:1 expansive clays as evidenced by noticeable cracks in

surface during the dry season. The upper 10 cm of the A horizon has silty clay loam texture and granular structure. It has pH of 6.0, with 2.2% C, 0.21% N, and a bulk density of 1.2 g/cm^3 in the upper 10 cm.

Vegetation and dry season length manipulations

To quantify the effect of soil moisture on WEOC accumulation and the magnitude of CO_2 produced, we maintained plots (2 m × 1 m) within ~0.1 ha for 2 yr with two levels of plant removal (0% and 90% plant thinning) and four levels of dry season length (Ambient, Extended Dry Season, Short Dry Season, and No Dry Season).

Our experimental plots were selected in December 2012 based on similar plant cover and composition; plots were segregated into three blocks, each block containing 8 plots (2 levels of vegetation × 4 levels of dry season length), for a total of 24 plots (n = 3 for each treatment). We oriented all plots with the longer 2 m side spanning from north to south and spaced at least 1 m apart to minimize edge effects. Thinning began in December 2012 and plots were maintained every 7–10 d during the growing season and as needed during the dry season. Edge effects of root growth from outside the plots were minimized by clearing a ~30 cm perimeter around every plot using a motorized weed trimmer.

The unthinned plots (0% thinning; denoted "with plants") were not altered. In the 90% thinning plots (denoted "without plants"), we removed all plants, but because of germination between site visits, we refrain from referring to the treatment as "100% thinning." Thinning occurred through the end of WY 2014. Averaged across 2 yr, 90% thinning increased soil moisture by 5% relative to Ambient plots.

The Ambient treatments represented field conditions—in California the dry season typically lasts 6 months (from May until October; Appendix S1: Fig. S1).

The Extended Dry Season delayed rainfall until January (i.e., ~9 months) using rainout shelters that kept soils dry. Rainout shelters (2.5 m × 1.2 m) used in Extended Dry Season plots were built from clear corrugated polycarbonate roof panels (Suntuf, Palram Americas, Kutztown, Pennsylvania) attached to a metal frame. We did not observe any consistent effect of the shelter on soil temperature (e.g., greenhouse effect) or soil moisture (e.g., dew accumulation). To extend the WY 2013 dry season, shelters were in place from 7 October 2013 until 30 January 2014 and from 24 October 2014 until 19 January 2015 for WY 2014 (Appendix S1: Fig. S1).

The No Dry Season treatment was designed to maintain soils consistently moist during the typical 6-month dry season (Parker and Schimel 2011). The Short Dry Season was similar to the No Dry Season, except that soils were irrigated and kept consistently moist for only about half of the dry season. Thereafter, soils were allowed to dry until rewet by rainfall. No Dry Season plots were irrigated 12 times in the 2013 dry season (May 14 through November 6; 180 mm total water added) and 10 times in the 2014 dry season (May 23 through October 21; 150 mm total water added; Appendix S1: Fig. S1). Short Dry Season plots were irrigated 5 times in the 2013 dry season (May 14 through July 2; 75 mm total water added) and 4 times in the 2014 dry season (May 23 through July 8; 60 mm total water added; Appendix S1: Fig. S1).

The predetermined threshold between "moist" and "dry" soil was 10% volumetric water content (VWC) or 18% water-filled pore space (WFPS). Soil WFPS was calculated as:

$$\mathrm{WFPS} = \frac{\mathrm{VWC}}{1 - \frac{\rho_b}{\rho_s}} \quad (1)$$

where VWC is soil volumetric water content, ρ_b is the bulk density (1.2 g/cm^3), ρ_s is the particle density (2.65 g/cm^3), and the denominator $1 - \rho_b/\rho_s$ is the soil porosity. In these soils, a marked decline in respiration occurs below 18% WFPS (Fierer et al. 2005), suggesting that microbes rapidly lose access to C substrates or reduce their activity as soils dry below this threshold. Therefore, we irrigated as soon as soils dried to 18% WFPS in the spring (when soils begin to dry) and continued irrigating until the first rain event in the fall (the onset of the wet season) for the No Dry Season or until July for the Short Dry Season.

Each irrigation event consisted of adding 30 L (equivalent to 1.5 cm rainfall) of local well water (1.1 mg DOC/L, 3 μg NH_4^+-N/L, and 1.6 mg NO_3^-N/L) to each plot every 2–3 weeks. Plots were irrigated using a backpack sprayer with a fine nozzle to minimize soil disturbance. Because of the sprayer's 15 L capacity and the desire to allow time for infiltration, the 30 L water was added in two 15 L "doses" spaced roughly 1 h apart. Based on field measurements of soil moisture using a portable MiniTrase Time Domain Reflectometer (Soil Moisture Equipment Corporation, Santa Barbara, California, USA), each irrigation event moistened soils in the top 10 cm to ~50% WFPS. Then, on average, soils dried by 2% WFPS per day, particularly during the middle of the summer. Thus, irrigation was required every 2 weeks to prevent drying below 18% WFPS.

Based on a soil porosity of 54% ($1 - \rho_b/\rho_s$), we expected the irrigation water to infiltrate at least 3 cm, wetting 66 kg of soil in the Short and No Dry Season plots. Under these conditions, and assuming steady state, irrigating soils for two dry seasons would have raised the C content of soils by only 11 μg DOC/g in the No Dry Season plots and to 4.5 μg DOC/g for the Short Dry Season plots.

CO_2 emission measurements

Three months prior to measuring CO_2 emissions, a polyvinylchloride collar (PVC; 30.5 cm diameter × 10 cm height) was inserted 6 cm into the ground at each of the 48 plots under vegetation and dry season length manipulations. The placement of collars did not impede the growth of plants.

Rates of soil CO_2 emissions were measured by soil chamber methodology (Davidson et al. 1991) using an infrared CO_2 analyzer (WMA-4; PP Systems, Amesbury, Massachusetts, USA). A PVC chamber (volume = 11 L), equipped with a small fan (4 cm diameter), was placed over the previously installed PVC collars. We measured the change in concentration of CO_2 inside the chamber headspace for approximately 3–6 min during which a linear increase in CO_2 concentrations was usually observed. During measurements, chamber air flowed into the analyzer and

makeup ambient air flowed into the chamber through a vent to prevent changes in pressure inside the chamber. Closing chambers for too long causes the rate of CO_2 accumulation within the chamber to decline because the CO_2 concentration gradient between the soil and the chamber decreases and because the difference between the CO_2 concentration inside the chamber and ambient makeup air increases. However, when the rate of CO_2 accumulation within the chamber is linear, these sources of error can be ignored. The flux of CO_2 was calculated based on the physical dimensions of the chamber, the linear portion of the rate of change in CO_2 concentration inside the chamber, and air temperature:

$$F = \frac{d\mathrm{C}}{dt} \times \frac{\mathrm{VC}}{\mathrm{ART}} \quad (2)$$

where F is the CO_2 flux rate ($\mu g\ CO_2\text{-}C \cdot m^{-2} \cdot s^{-1}$); $d\mathrm{C}/dt$ (ppmv CO_2-C/s) is the rate of CO_2 concentration increase inside the chamber computed by linear regression; V is the chamber volume (L); C is the atomic weight of carbon (12.01 g/mol); A is the area of the PVC collar (730 cm^2); R is the gas constant (0.0821 L atm $mol^{-1} \cdot K^{-1}$); and T is the chamber air temperature (K). The CO_2 analyzer was calibrated by mixing a CO_2 standard (1,000 ppmv CO_2-C; Scott Marrin, Riverside, California, USA) with zero-grade air. CO_2 emissions were measured approximately every 2–4 weeks from 8 May 2013 to 14 April 2015. During the dry season, CO_2 emissions were measured biweekly, within 3–5 h of irrigating soils. Therefore, CO_2 emission measurements made in Short and No Dry Season plots represent pulses of CO_2 generated after irrigating soils.

Soil sampling

We sampled surface soils (0–10 cm; A horizon) using a 10-cm corer (5 cm diameter). In the laboratory, soils were homogenized, sieved (4 mm), and analyzed for microbial biomass C, water-extractable organic C (WEOC), immediately respirable C (IRC), and total C. Microbial biomass C was measured by chloroform slurry extraction (Fierer and Schimel 2002). No correction for chloroform fumigation efficiency was made and we, therefore, report the "flush" of organic C after fumigation (MBCF). WEOC was measured by extracting each soil sample in deionized water by stirring 8 g of soil with 32 mL of water in a 50-mL Falcon tube on an orbital shaker (180 rpm) for 3 h followed by centrifugation (30,000 × g; 10 min). Both MBCF and WEOC extracts were analyzed for C concentration on a total organic carbon analyzer (TOC-V CSN, Shimadzu Scientific Instruments, Columbia, Maryland, USA). IRC was measured as the CO_2 released over a 3 h period after wetting soils in closed microcosms using a nondispersive infrared CO_2 analyzer (LI-820; Li-Cor, Inc., Lincoln, Nebraska, USA). Total C was measured by combustion in an elemental analyzer (NA 1500 Series 2; Fisons Instruments, Ipswich, UK).

Soil WFPS (upper 10 cm) was measured monthly at each of the 24 plots using a portable MiniTrase Time Domain Reflectometer and Eq. 1 (Appendix S1: Fig. S1B). A continuous record of soil moisture and temperature was obtained from the nearby Lisque weather station (2.6 km northwest of our site; 34.72449 N, 120.0635 W; 430 m ASL) operated by the Geography Department at UC Santa Barbara (Appendix S1: Fig. S1A).

Reducing sugar accumulation rates

We measured accumulation of reducing sugars in dry ripgut brome (*Bromus diandrus*, Roth) roots during a 4-week laboratory incubation to understand whether exoenzymes could break down litter over the dry season. Roots were collected in October 2014 from valley bottoms in five small drainages near the experimental plots. Roots were sampled by excavating individual senesced plants and hand-separating individual roots from mineral aggregates. The roots were then cut into 1-cm segments and incubated under field moisture (3% gravimetric water content) in glass mason jars. Because active microbes could consume the reducing sugars generated by exoenzymes, half of the roots collected from each drainage were incubated under continuous chloroform vapor to suppress microbial activity. Total reducing sugars were quantified in water extracts of subsampled root tissue after 1 d of incubation in sealed jars and then again after 4 weeks of incubation. Using this approach, any reducing sugars contained in microbial biomass and released by chloroform fumigation were quantified during the initial extraction, and subsequent accumulation of reducing sugars could be used to infer enzyme activity. Extractions were conducted using ultrapure water on an orbital shaker (180 rpm; 30 min), followed by filtration (0.2 μm pore size). Reducing sugars were quantified by the Prussian Blue method (Schinner and von Mersi 1990) using glucose as a standard.

We also quantified potential activities of the hydrolytic enzymes β-glucosidase, α-glucosidase, cellobiohydrolase, and xylosidase in the dead root tissue at the end of the incubation, as a way of assessing potential reducing sugar production rates. The products of these four enzymes are mono- and oligosaccharides with reducing groups that can be detected by the Prussian Blue assay. We thus assumed that the potential reducing sugar production rate for each enzyme was equal to the reaction rate under saturating substrate supply.

Root subsamples were prepared for enzyme assays by grinding under liquid nitrogen followed by suspension in 50 mmol/L sodium acetate buffer (pH 7). Potential enzyme activities were quantified by reacting methylumbelliferyl (MUB) tagged substrates with the root homogenates in 96-well microplates. To increase the precision of the assay, fluorescence generated by the reaction was measured hourly for 5 h without terminating the reaction by alkalization. Reaction rates were then obtained by fitting linear regressions to the change in MUB concentration over time, as inferred from measured increases in fluorescence. Scalar corrections were made to account for background quenching in the sample matrix. All reactions were conducted in eight replicate microplate wells and averaged.

Statistical analyses

We used a factorial randomized complete block design with repeated measures analysis of variance and Tukey post hoc tests to detect significant effects ($\alpha = 0.05$) of thinning and dry season length on soil CO_2 emissions, MBCF,

WEOC, IRC, and total C (SAS software; SAS Institute, Cary, North Carolina, USA). Our statistical analyses included all 24 plots, and although $n = 3$ for each treatment combination, the overall effect of thinning was assessed using 12 replicates (3 replicates across 4 levels of dry season length). To assess increases or decreases in MBCF, WEOC, and IRC over the dry season, we used linear regressions with time as the independent variable. For linear regressions, we focused on the 2014 dry season to maximize the likelihood of measuring an actual response to treatment manipulation. When necessary, data were log-transformed to meet the assumption of normality.

Results

CO_2 emissions

Across dry season length manipulations, thinning lowered CO_2 emissions by 32% below the 49.3 μg CO_2-C·m^{-2}·s^{-1} average measured in unthinned plots ($P < 0.0001$; Fig. 1). Increasing summer dry season length did not significantly affect CO_2 emissions ($P = 0.11$), though, on average, emissions declined compared to Ambient plots (Fig. 1A, B). Maintaining soils moist (both Short and No Dry Season treatments) increased CO_2 emissions above Ambient as a result of pulses generated upon rewetting soils ($P < 0.004$; Fig. 1A, C, D).

While thinning reduced CO_2 emissions, the magnitude of the reduction varied according to dry season length manipulation. Thinning lowered CO_2 emissions by 43% below the 39.5 μg CO_2-C·m^{-2}·s^{-1} average measured in Ambient plots ($P = 0.0064$; Fig. 1) but it had no effect in Extended Dry Season plots ($P = 0.67$; Fig. 1). In soils kept moist (Short and No Dry Season), the effect of thinning on CO_2 emissions decreased as irrigation intensity increased but the effects were marginally significant; thinning lowered CO_2 emissions by 33% below the 52 μg CO_2-C·m^{-2}·s^{-1} average in unthinned Short Dry Season plots ($P = 0.08$; Fig. 1C) and by 30% below the 107 μg CO_2-C·m^{-2}·s^{-1} average in unthinned No Dry Season plots ($P = 0.12$; Fig. 1D).

Microbial biomass C flush

Overall, thinning lowered MBCF by 20% below the 269 μg C/g average measured in unthinned plots across dry season length manipulations ($P < 0.0001$; Fig. 2). Extending or shortening the length of the dry season did not significantly affect MBCF ($P > 0.08$; Fig. 2B, C), whereas maintaining soils consistently moist (No Dry Season) lowered MBCF by 21% below the 271 μg C/g average measured in Ambient plots ($P = 0.003$; Fig. 2D). MBCF did not significantly change over the length of the 2014 dry season in response to altering dry season length or to thinning (Table 1).

Total WEOC

We did not detect an overall effect of thinning on WEOC across dry season length manipulations ($P = 0.18$; Fig. 3), but by exclusively considering dry season measurements (i.e., those outside hatched boxes in Fig. 3), thinning significantly increased WEOC by 40% above the 105 μg C/g average measured in unthinned plots ($P = 0.001$; Fig. 3).

Increasing summer dry season length increased WEOC concentrations; extending the length of the dry season increased WEOC by 30% above the 108 μg C/g average measured in Ambient plots ($P = 0.044$; Fig. 3A, B). In contrast, keeping soils moist had no effect on WEOC ($P > 0.4$ in Short and No Dry Season plots; Fig. 3A, C, D). Consistent with measuring more WEOC in dry soils (i.e., in Extended Dry Season plots), WEOC increased during the 2014 dry season across all experimental manipulations except for in plots with plants kept consistently moist (i.e., No Dry Season plots; Table 1).

Immediately respirable carbon (IRC)

Thinning did not significantly influence IRC concentrations across dry season length manipulations ($P = 0.25$; Fig. 4). However, in both Ambient and Short Dry Season plots with plants, IRC concentrations increased during the 2014 dry season, whereas in plots without plants, IRC did not change (Table 1).

Keeping soil moist lowered IRC concentrations; shortening the length of the dry season lowered IRC concentrations by 40% in Short Dry Season and by 70% in No Dry Season plots below the 10.6 μg C/g average measured in Ambient plots ($P < 0.0001$; Fig. 4A, C, D). In contrast, increasing summer dry season length had no effect ($P = 0.88$; Fig. 4B). At the onset of the wet season, in November 2014, IRC decreased in both Ambient and Short Dry Season plots, consistent with the IRC decline in consistently moist soils (No Dry Season plots; Fig. 4D).

Total soil C

Total soil C averaged 2% across treatment plots and did not vary significantly in response to plant thinning or moisture treatment ($P > 0.7$).

Reducing sugar accumulation rates

Reducing sugars did not accumulate in dry senesced roots over the 4-week incubation, regardless of whether chloroform was used to suppress microbial consumption of the sugars (Fig. 5). The mean change in reducing sugar concentration was 0.001 μmol·g^{-1}·h^{-1} in the chloroform-treated jars and −0.001 μmol·g^{-1}·h^{-1} in the untreated jars. Potential rates of reducing sugar production by hydrolytic enzymes ranged from 0.7 to 10 μmol·g^{-1}·h^{-1} and far exceeded the rates of reducing sugar accumulation during the incubation (Fig. 5).

Discussion

We excluded plant C inputs (i.e., root exudates and litter) and varied the length of the summer dry season to understand the mechanisms that control soil water-extractable organic C (WEOC) dynamics in drying soils, which is critical to validating assumptions built into C models. We tested the hypothesis that C derived from the enzymatic degradation of "fresh" litter (i.e., the antecedent winter's litter)

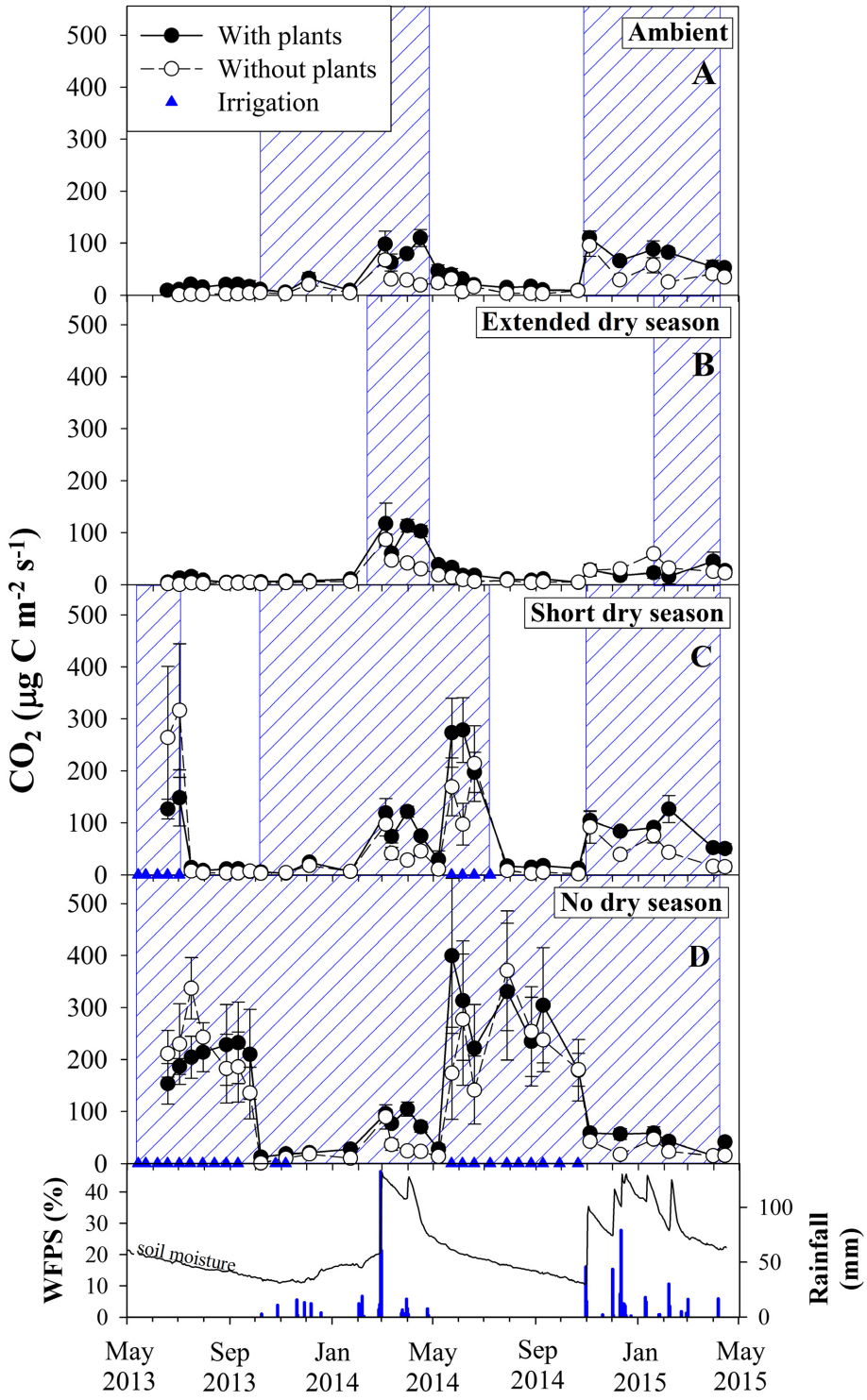

FIG. 1. Average (±SEM; $n = 3$) soil CO_2 fluxes from plots with and without plants under (A) Ambient conditions, (B) Extended Dry Season, (C) Short Dry Season, and (D) No Dry Season. Irrigation periods are represented by triangles on the *x*-axis of the Short Dry Season and No Dry Season treatments. Hashed boxes represent the wet season as controlled by natural rainfall (A), rainfall exclusion shelters (B), or combined irrigation with rainfall (C, D). The lower panel depicts soil water-filled pore space (WFPS) and rainfall measured at the nearby Lisque weather station.

produces a pool of WEOC that accumulates over the dry season because of limited microbial access to C (Miller et al. 2005, Lawrence et al. 2009, Blankinship and Schimel 2018). Contrary to our expectations, excluding plant C inputs did not prevent WEOC from accumulating over the summer dry season and roots did not break down to produce WEOC; there was no change in the concentration of reducing sugars in our root incubations.

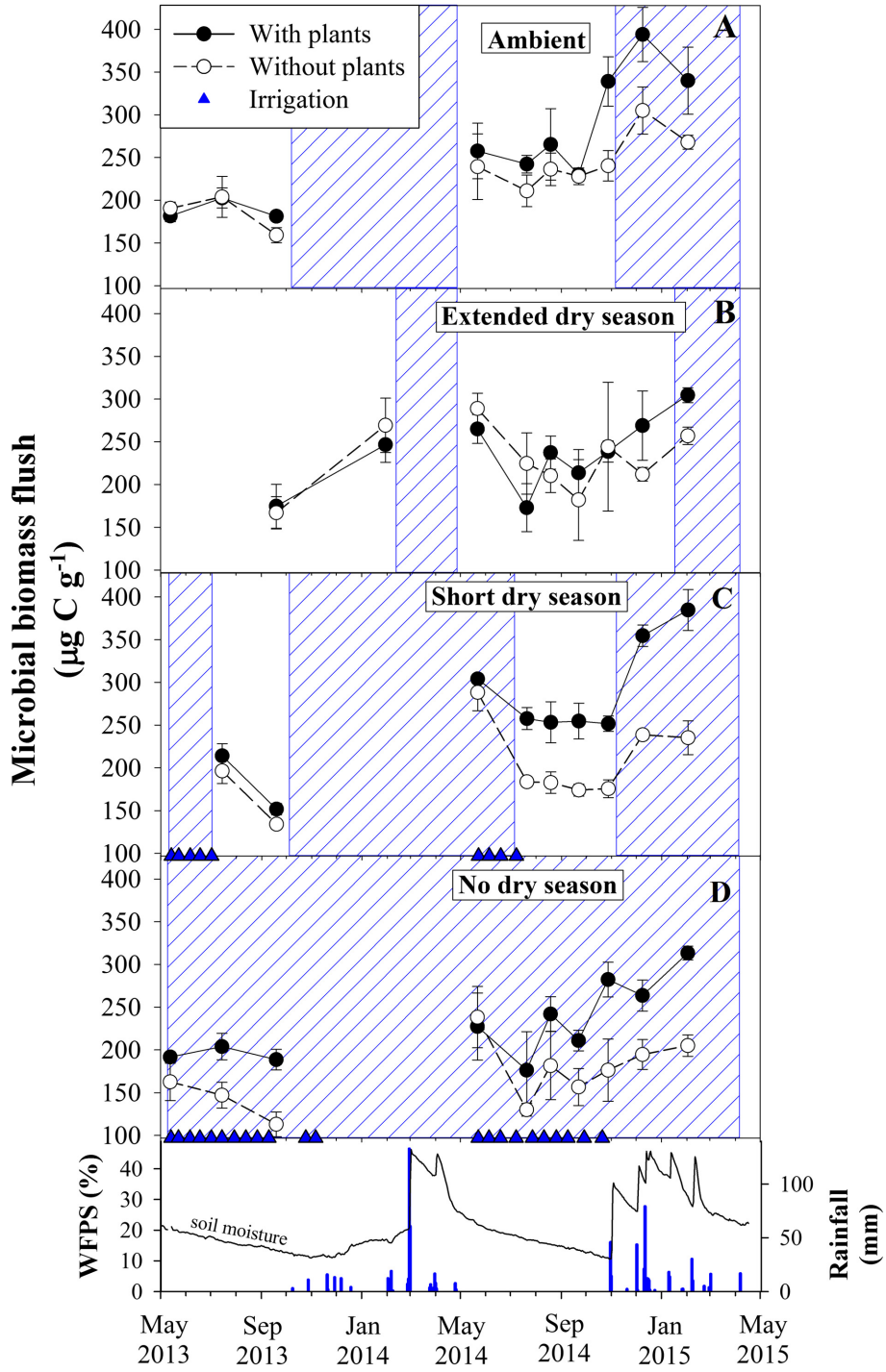

FIG. 2. Average (±SEM; $n = 3$) soil microbial biomass C flush from plots with and without plants under (A) Ambient conditions, (B) Extended Dry Season, (C) Short Dry Season, and (D) No Dry Season. Irrigation periods are represented by triangles on the x-axis of the Short Dry Season and No Dry Season treatments. Hashed boxes represent the wet season as controlled by natural rainfall (A), rainfall exclusion shelters (B), or combined irrigation with rainfall (C, D). The lower panel depicts soil water-filled pore space (WFPS) and rainfall measured at the nearby Lisque weather station.

Because mechanisms involving detrital breakdown over the dry season fail to capture our field observations, we instead develop the argument that physical interactions with the soil mineral matrix govern seasonal WEOC dynamics.

Water-extractable organic C dynamics

Excluding plants—surprisingly—increased WEOC, indicating that WEOC was not directly derived from live roots or decomposing fresh litter. If detrital breakdown governed

TABLE 1. Changes in microbial biomass flush, total water-extractable organic C (WEOC), and immediately respirable carbon (IRC) as a function of time (days) during the 2013 and 2014 dry seasons using linear regression.

Year, dry season length, and treatment	Microbial biomass flush (μg C·g^{-1}·d^{-1})		Total WEOC (μg C·g^{-1}·d^{-1})		IRC (μg C·g^{-1}·d^{-1})	
	Slope	*P*	Slope	*P*	Slope	*P*
2013						
Ambient						
With plants	−0.005 ± 0.11	>0.9	**0.43 ± 0.15**	**0.03**	0.013 ± 0.017	0.5
Without plants	−0.25 ± 0.18	0.2	**0.88 ± 0.12**	**0.0001**	0.033 ± 0.021	0.2
Extended						
With plants	0.54 ± 0.25	0.09	0.085 ± 0.17	0.6	−0.013 ± 0.018	0.5
Without plants	0.77 ± 0.28	0.05	0.17 ± 0.29	0.6	0.008 ± 0.023	0.8
Short						
With plants	**−0.94 ± 0.24**	**0.02**	**0.49 ± 0.14**	**0.02**	0.03	–
Without plants	**−0.94 ± 0.23**	**0.02**	**0.62 ± 0.22**	**0.05**	0.020 ± 0.020	0.4
No dry season						
With plants	−0.025 ± 0.13	0.9	−0.1 ± 0.065	0.2	**−0.12 ± 0.009**	**<0.0001**
Without plants	−0.39 ± 0.18	0.07	0.19 ± 0.081	0.06	**−0.075 ± 0.012**	**0.0005**
2014						
Ambient						
With plants	0.36 ± 0.26	0.2	**0.58 ± 0.12**	**0.0004**	**0.070 ± 0.022**	**0.0099**
Without plants	0.026 ± 0.17	0.9	**0.82 ± 0.17**	**0.0004**	−0.003 ± 0.034	0.9
Extended						
With plants	−0.1 ± 0.22	0.7	**0.38 ± 0.078**	**0.0003**	0.024 ± 0.046	0.6
Without plants	−0.39 ± 0.35	0.3	**1.09 ± 0.2**	**0.0001**	−0.001 ± 0.019	>0.9
Short						
With plants	−0.05 ± 0.22	0.8	**0.31 ± 0.10**	**0.0133**	**0.056 ± 0.022**	**0.03**
Without plants	−0.095 ± 0.11	0.4	**0.84 ± 0.38**	**0.05**	0.020 ± 0.019	0.3
No dry season						
With plants	0.32 ± 0.27	0.3	−0.092 ± 0.13	0.5	0.003 ± 0.024	0.9
Without plants	−0.33 ± 0.27	0.3	**0.39 ± 0.15**	**0.02**	−0.011 ± 0.019	0.6

Notes: Significant relationships ($P < 0.05$) are highlighted in bold.

WEOC accumulation as soils dried, then WEOC should have decreased across the gradient of plant removal, but it did not. The only data suggesting that detrital breakdown produced WEOC were the immediately respirable C (IRC); IRC increased as soils dried in plots with plants (in Ambient and Short Dry Season plots). Nevertheless, the increase in IRC was small, accounting for less than 6% of the WEOC accumulated during the dry season in Ambient plots. Moreover, extending the length of the dry season actually prevented IRC from accumulating, whereas WEOC increased through the summer dry season in all treatments. Because IRC was sensitive to drought and, unlike WEOC, increased when plants were present, it suggests that IRC is plant-associated, but it cannot explain why WEOC accumulates in dry soil.

That WEOC did not decrease after excluding the supply of fresh litter, suggests exoenzymes contribute little to the WEOC accumulating during the dry season. Because exoenzymes can maintain potential activities in dry environments (Stursova and Sinsabaugh 2008), or even accumulate during drought (Alster et al. 2013, Ren et al. 2017), we hypothesized exoenzymes produced WEOC. Indeed, enzyme activities can persist for months, with turnover times on the order of 24 d (Schimel et al. 2017), suggesting they might have the potential to break down SOM during the dry season. Nevertheless, while hydrolytic enzymes had measurable potential activities in dead roots at our field site, the concentration of reducing sugars in roots kept under field-moist conditions did not increase—even when microbial activity was suppressed with chloroform to minimize uptake of reducing sugars. Though exoenzymes remain present in dry soil, their efficiency in degrading substrates appeared to be effectively zero. Sustained exoenzyme-driven decomposition of litter during summer, therefore, is unlikely to explain the buildup of WEOC when soils are dry.

Although maintaining microbial access to fresh litter did not control the WEOC accumulation in dry soil, litter still indirectly influenced WEOC dynamics, with mechanisms working on both short (annual) and longer time scales (decades to centuries). In the short-term, the presence of live plants *reduced* WEOC levels. Soil microbial biomass and respiration were largest in plots with litter inputs, together suggesting that, in the presence of plants, microbes accessed more C. Indeed, the average difference in C stored in microbial biomass between Ambient plots with and without plants during the 2014 dry season (36 ± 16 μg C/g) were similar to the WEOC lost from plots with plants (38 ± 8 μg C/g), suggesting C may have been transferred from WEOC to microbial biomass in the presence of plants. Microbes accessing more WEOC in plots with plants may be consistent with the larger IRC measured in these plots, perhaps because microbial resource allocation to breaking down the more accessible IRC may have primed the decomposition of WEOC (Nottingham et al. 2009) as observed in another study where plant C inputs were excluded (Steinbeiss et al. 2008)—breakdown of labile C can increase decomposability of occluded C (Luo et al. 2017). It is also

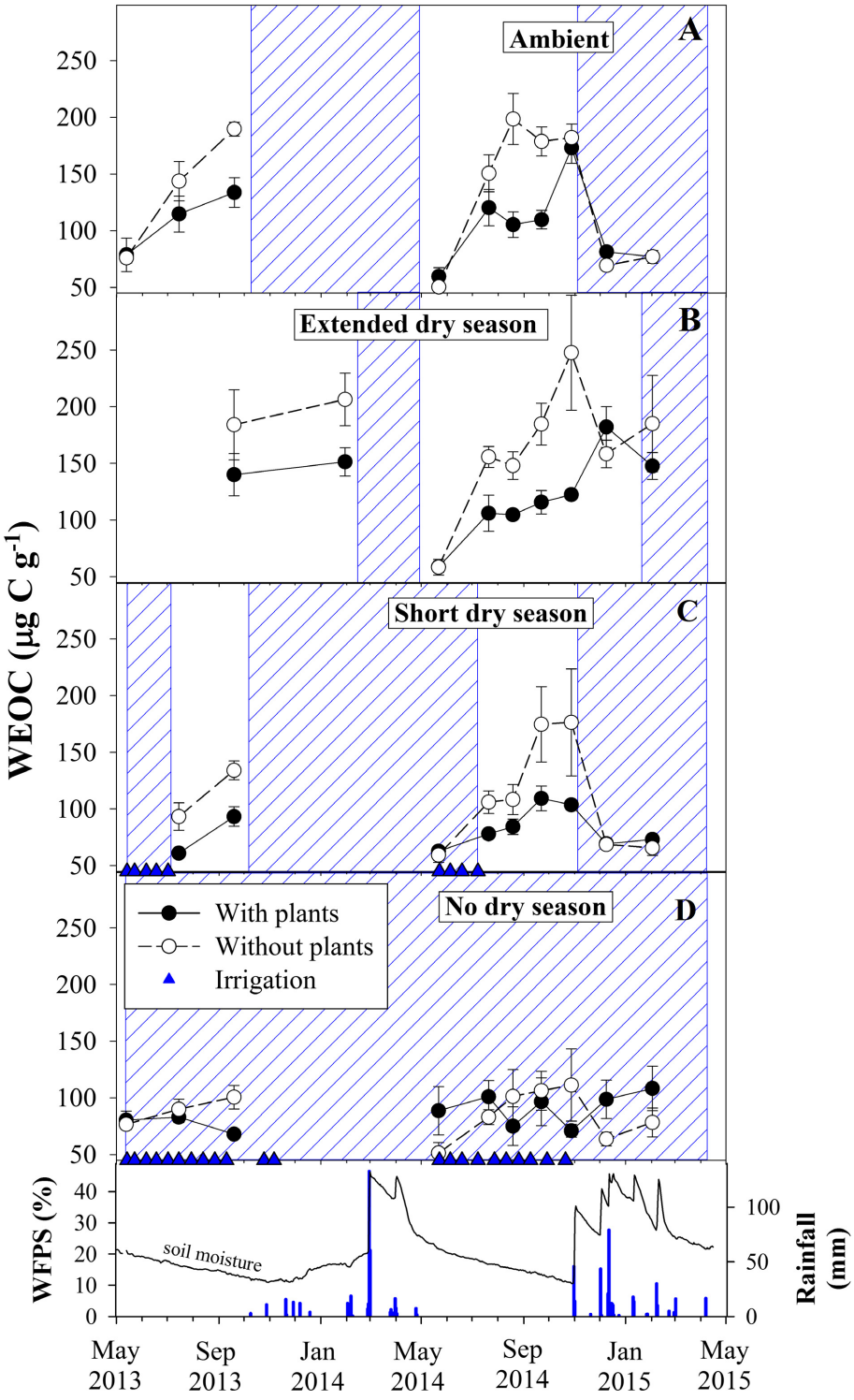

FIG. 3. Average (±SEM; $n = 3$) soil total water-extractable carbon (WEOC) from plots with and without plants under (A) Ambient conditions, (B) Extended Dry Season, (C) Short Dry Season, and (D) No Dry Season. Irrigation periods are represented by triangles on the *x*-axis of the Short Dry Season and No Dry Season treatments. Hashed boxes represent the wet season as controlled by natural rainfall (A), rainfall exclusion shelters (B), or combined irrigation with rainfall (C, D). The lower panel depicts soil water-filled pore space (WFPS) and rainfall measured at the nearby Lisque weather station.

possible microbes could have accessed more C in plots with plants because root exudates destabilize mineral-bound C through complexation and dissolution mechanisms (Keiluweit et al. 2015). Thus, the presence of plants may have lowered short-term WEOC pools by facilitating microbial access to that C.

Over the long-term (i.e., decades to centuries), litter influences WEOC by supplying C to soils (Hogberg et al. 2001,

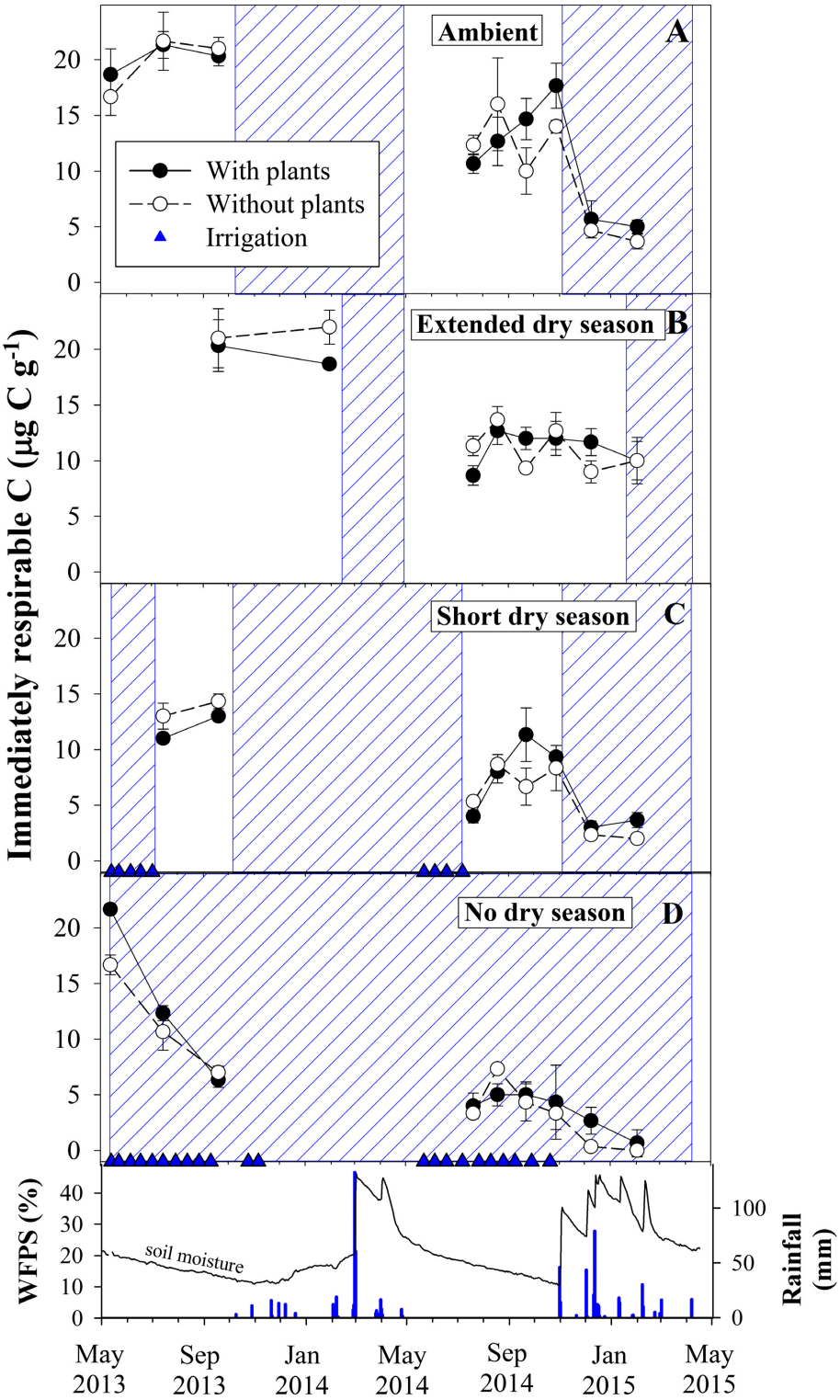

FIG. 4. Average (±SEM; $n = 3$) soil immediately respirable carbon (IRC) from plots with and without plants under (A) Ambient conditions, (B) Extended Dry Season, (C) Short Dry Season, and (D) No Dry Season. Irrigation periods are represented by triangles on the x-axis of the Short Dry Season and No Dry Season treatments. Hashed boxes represent the wet season as controlled by natural rainfall (A), rainfall exclusion shelters (B), or combined irrigation with rainfall (C, D). The lower panel depicts soil water-filled pore space (WFPS) and rainfall measured at the nearby Lisque weather station.

Cotrufo et al. 2013), which builds the SOM from which WEOC is derived. For example, the total C pool in the upper 10 cm of soil averaged 20 mg C/g, or about 80× the WEOC fraction, suggesting extant soil C stocks can generate WEOC for several decades before fresh litter C is required. In this sense, plant-derived C inputs operate as

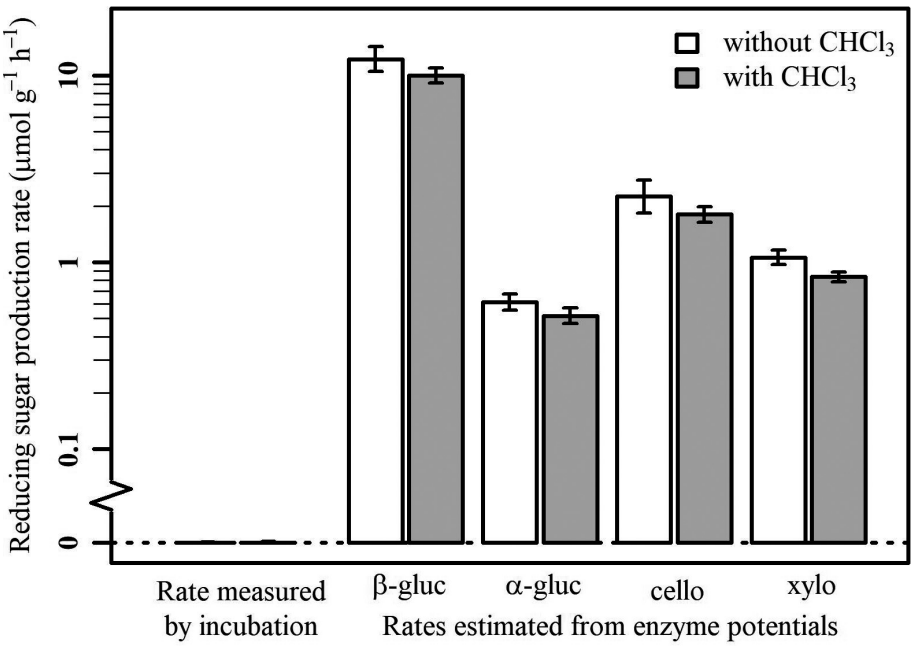

FIG. 5. Average (±SEM; $n = 5$) reducing sugar production rates in dry *Bromus diandrus* roots kept at 20°C and field moisture (3% gravimetric water content) with and without chloroform ($CHCl_3$). Chloroform was used to reduce microbial activity without affecting exoenzyme activity (Blankinship et al. 2014). "Rate measured by incubation" represents measured accumulation rates based on the change in total water-extractable reducing sugars, while "Rates estimated from enzyme potentials" represents potential production rates—what should have been measured—based on the activities of β-glucosidase, α-glucosidase, cellobiohydrolase, and xylosidase measured at the end of the incubation.

distal controls over long-term WEOC dynamics—building SOM from which WEOC is derived. Other proximate controls govern short-term microbial access to WEOC. The nature of these controls may be biological or physical.

Possible biological sources of water-extractable organic carbon

We identify three potential biological mechanisms that may explain WEOC accumulation: microbial death, osmolyte production, and production of extracellular polymeric substances (EPS). Drought-induced microbial death can produce a pool of bioavailable C (Blazewicz et al. 2014), which may explain why WEOC accumulates. However, microbial biomass did not decrease to support an increase in dead cells—on average, it increased slightly during the 2014 dry season in Ambient plots—consistent with studies measuring *increasing* microbial biomass as soils dry at our site (Parker and Schimel 2011, Boot et al. 2013, Schaeffer et al. 2017). If microbial death occurs by osmotic stress during rewetting, then WEOC should have been highest in plots with the highest microbial biomass, but it was not—microbial biomass was highest in plots with plants where WEOC was lowest. Moreover, microbial C does not influence the WEOC measured upon rewetting soils (Fierer and Schimel 2003) and, thus, cannot explain the WEOC accumulation. Similarly, while osmolytes may accumulate in some dry soils (Warren 2016), osmolyte production and release during rewetting does not appear to occur at our site (Boot et al. 2013) and cannot explain the WEOC accumulation. Alternatively, Schaeffer et al. (2017) hypothesized that microbes produce extracellular polymeric substances (EPS) or "glues" to withstand desiccation (Roberson and Firestone 1992) and facilitate nutrient supply in dry soils (Or et al. 2007); this may have caused WEOC to accumulate—EPS should be high in dry soils. Although EPS was highest at the end of summer in plots with plants (Marchus et al. 2018), it also cannot explain the WEOC accumulation because EPS did not accumulate in plots without plants, where WEOC was highest, and EPS did not increase in step with WEOC. Physiological mechanisms fail to capture the dry season WEOC accumulation, suggesting other proximal processes, likely physical, are more important.

Proposed physical controls on water-extractable organic carbon dynamics

Physical processes influence SOM turnover by influencing microbial access to C (Six et al. 2002*b*, Kleber et al. 2007, Sollins et al. 2009). For example, removing plants increased the abundance of microaggregates relative to macroaggregates at our site (Blankinship et al. 2016), influencing soil structure and potential access to C. In contrast to macroaggregates, microaggregates often have micropores too small to contain microbes (Chenu and Stotzky 2002, Keil and Mayer 2014), suggesting that WEOC may have increased in plots without plants because microbial access to C in microaggregates was constrained. Indeed, microbial biomass was lower in plots without plants than with plants. Furthermore, WEOC increased as soil moisture and C accessibility decreased, but WEOC did not accumulate when soils were kept moist, consistent with WEOC accumulating only if physical access to C is constrained (Blankinship and Schimel 2018). Correspondingly, IRC decreased in step with the

magnitude of CO_2 emission pulses generated upon successively irrigating soils, suggesting IRC decreased because microbial access to C was maintained. These patterns are consistent with the hypothesis that WEOC represents an extracellular C pool that accumulates when physical access to C is constrained under dry conditions. However, the physical processes that cause WEOC to increase during the dry period at our site remain unidentified.

Although we do not know which physical mechanisms increase WEOC over dry periods, the known physical effects of drying–rewetting on soil C offer some potential explanations. We operationally defined WEOC as the C extracted over a 3-h water extraction. However, consecutive drying–rewetting cycles can continuously expose previously unavailable C to microbes (Qualls and Bridgham 2005, Guo et al. 2012), albeit sometimes producing less WEOC than when soils first wet up (Xiang et al. 2008). That WEOC continues to be extracted over consecutive drying–rewetting cycles suggests that the disruptive energy caused by rewetting on soil structure and, the length of time of the extraction, influences how much WEOC is mobilized (Six et al. 2002*a*). Because wetting a dry soil can slake soil aggregates (e.g., via shrink–swell forces and compression of air bubbles; Denef et al. 2001) exposing occluded C to microbial decomposition (Borken and Matzner 2009, Navarro-Garcia et al. 2012, Guo et al. 2014), slaking may account for the initial, and frequently larger, WEOC pulse when soils first wet up. During subsequent rewetting, slaking should contribute less to WEOC—there are fewer aggregates to break down—but the movement of water into and out of pores can redistribute the C accumulating in thin water films as soils dry (Fig. 6A). For example, 2:1 clays, such as smectites, have both high shrink–swell and cation exchange capacity and can stabilize C on exchange sites and through cation bridges formed between clay surfaces and organic matter (Setia et al. 2013, Nguyen and Marschner 2016). The Pachic Argixerolls at our site are smectite-rich and are transitional to Vertisols—soils so clay-rich that they develop cracks as they dry—enabling soil mixing and the potential to destabilize occluded C during clay contraction and expansion. In this sense, slaking and C redistribution may explain why WEOC is high in dry soil, but other processes are required to explain why WEOC increases the longer soils stay dry.

The mechanisms responsible for WEOC accumulating over the length of the dry season are controlled by the balance of C fluxes into and out of the WEOC pool (Fig. 6B). C fluxes out of the WEOC pool are controlled by whether microbes can access that C (i.e., by whether soils are hydrologically coupled). In contrast, C fluxes into the WEOC pool may be controlled by the destabilization of complex C molecules in soil micropores (Smith et al. 2017). When soils dry, large pores drain first and micropores last because micropores retain water at more negative matric potentials (Papendick and Camprell 1981). Because organic matter is preferentially stored in micropores (Kaiser and Guggenberger 2007, Keil and Mayer 2014), increasing dry season length (i.e., time) can progressively drain pores of smaller diameter (Papendick and Camprell 1981), gradually destabilizing SOM-rich micropores when soils wet up (i.e., by slaking or redistribution). SOM stored in micropores contains more complex C, producing more CO_2 than the simpler and

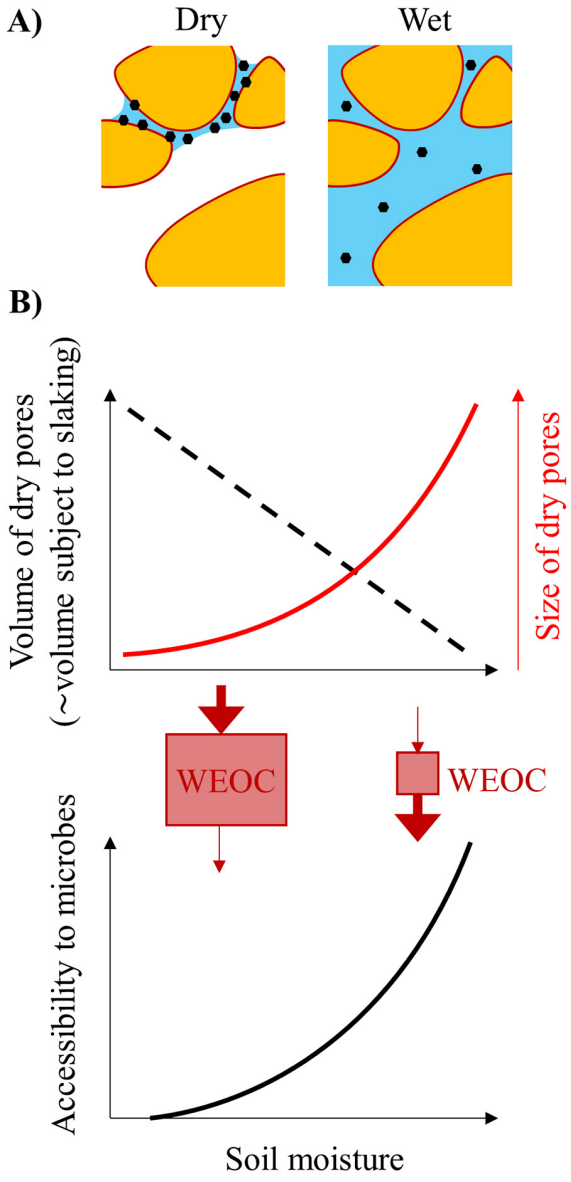

FIG. 6. (A) Conceptual diagram showing the distribution of C substrates (black hexagons) in a soil micropore under both wet and dry conditions. Carbon substrates diffuse through the soil solution when soils are wet. In contrast, C substrates accumulate on thin water films when soils are dry. (B) Soil water-extractable organic C (WEOC; red boxes) dynamics as controlled by the balance between C inputs and outputs (represented by the size of the red boxes and arrows). C inputs and outputs are governed by changes in soil moisture and its effect on the volume of dry pores that are subject to slaking, the size of dry pores, and WEOC accessibility to microbes. The volume of dry pores (dashed black line) decreases with increasing soil moisture, whereas the average size of the dry pores (red line) increases with increasing soil moisture—that is, large pores drain at relatively high matric potentials whereas smaller pores retain water at low matric potentials. WEOC accumulates in dry soil because C inputs are higher (the volume of dry pores subject to slaking is high with C derived from SOM-rich micropores (Bailey et al. 2017)) than C outputs (microbial access to C is low because soils are hydrologically disconnected). In contrast, WEOC is low in moist soil because C inputs are lower (the volume of dry pores subject to slaking is low with C derived from SOM-poor macropores (Bailey et al. 2017)) than C outputs (microbial access to C is high because soils are hydrologically connected).

less abundant C found in larger pores (Bailey et al. 2017), consistent with why WEOC increases with dry season length (Fig. 6; Miller et al. 2005, Guo et al. 2014, Manzoni et al. 2014, Schaeffer et al. 2017). That slaking of micropores releases previously inaccessible WEOC is also consistent with the rejuvenation of CO_2 pulses from one dry season to the next despite excluding plant C inputs (Fig. 1C, D). Furthermore, the larger WEOC pools measured in plots without plants are also consistent with the increase in microaggregate abundance in plots without plants (Blankinship et al. 2016). Still, WEOC increased with dry season length, as measured by bulk soil extractions that may have destabilized even physically protected C; this suggests complex physical processes govern WEOC dynamics.

Bulk soil extractions may have destroyed soil aggregates, suggesting WEOC stabilized in micropores should have been measured even if soils were not dry enough to slake. Nevertheless, because we extracted soils by stirring for 3 h on an orbital shaker—as opposed to by vigorous shaking—it is possible some microaggregates may have remained intact; high disruptive energies beyond stirring are required to break down microaggregates (Six et al. 2002*a*). Therefore, if microaggregates persist, except when dry enough to slake, then longer dry seasons should produce more WEOC—this is consistent with a greater abundance of microaggregates observed during the late dry season compared to the early dry season (Blankinship et al. 2016). Because soils gradually dried over the dry season (Appendix S1: Fig. S1), soils sampled in the late dry season should slake more often than soils sampled in the early dry season, possibly explaining our field observations.

Besides slaking, it is also possible that drying itself modifies the soil mineral phase to increase WEOC as soils dry. Conceptual models used to describe SOM binding onto clay surfaces suggest that drying shifts the orientation of amphiphilic molecules, weakening the strength of bonds between polysaccharides and clays (Kleber et al. 2007, Kaiser et al. 2015, Bailey et al. 2017). Specifically, the weakening of SOM–mineral bonds may be controlled by the ionic strength and pH of the soil solution—binding strength decreases at both high ionic strength and pH (Newcomb et al. 2017). Within the context of the pH 6 Pachic Argixerolls studied at our site, ionic strength may increase as solutes concentrate in thin water films during drying (Fig. 6A), lowering the binding strength between organic ligands and mineral surfaces at relatively favorable pH (Newcomb et al. 2017). Thus, prolonged drying should increase WEOC as SOM stabilized in the mineral surfaces of micropores is progressively exposed and decreasing water potentials drain smaller pores (Fig. 6). How these physical interactions may influence the accumulation of WEOC over the summer dry season is still a subject of active research (Bailey et al. 2017, Newcomb et al. 2017, Smith et al. 2017).

Implications for ecosystem C models

Microbial models of soil C cycling generally describe the coupled dynamics of organic C, WEOC, microbial biomass, and extracellular enzymes, using a discrete pool structure such as the one used by Schimel and Weintraub (2003). Here, we show that IRC (equivalent to the 3-h respiration pulse after rewetting) decouples from WEOC as soils dry—IRC stays generally flat except for in Ambient plots with plants, whereas WEOC consistently increases (Table 1)—suggesting that WEOC is not immediately accessible to microbes and that models miss the processes limiting microbial access to that C. Recent versions of these soil C models were extended to include a microbially unavailable WEOC pool, representing WEOC locked in hydrologically disconnected pores but made available upon reestablishing hydrological connections when soils wet up (Zhang et al. 2014, Manzoni et al. 2016). Because our empirical findings show that a fraction of the WEOC remains unavailable to microbes at rewetting, current models overemphasize the coupling of WEOC and respiration pulses.

Most C models do not describe plant–soil structure interactions and thus miss the mechanisms that we hypothesize prevent WEOC from accumulating during the dry season. Overcoming this structural limitation might require linking plant activity to a variable characterizing soil structure. Introducing additional parameters, however, is limited by uncertainties inherent to the new parameters and by generating potentially unstable dynamics when positive feedbacks occur between plants and soil structural properties (Caruso and Rillig 2011).

Conclusions

We hypothesized that the sustained exoenzyme-driven decomposition of plant litter produces a water-extractable organic C (WEOC) pool that accumulates as soils dry. Based upon field manipulations and laboratory incubations, we reject this hypothesis. Surprisingly, WEOC accumulated to a greater degree when fresh plant litter inputs were excluded, while hydrolytic enzymes were inefficient in breaking down litter under dry, field moisture, conditions. Microbial physiology does not appear to control the accumulation of WEOC as soils dry. Instead, physical processes possibly including (1) contraction of clays and slaking of SOM-rich micropores that redistribute C upon rewetting and (2) changes in how organic molecules bind to clays during drying may explain the WEOC patterns observed in these grassland soils. How physical mechanisms interact to control C dynamics under dry conditions is still not well understood and will require new approaches evaluating the nature of the mechanisms that mobilize WEOC when soils wet up. At a broader scale, our measurements imply that microbial access to soil C is the rate-limiting step governing whether soils behave as sinks or sources of atmospheric CO_2, and that in regions expected to see increased frequency of drying–rewetting cycles, there may be greater destabilization of soil C.

Acknowledgments

We are thankful to Oliver Chadwick for his suggestions on early versions of this manuscript, to Eddie Dominguez, Matt Kamiyama, and Krystal Vasquez for their assistance with CO_2 flux calculations and to members of the Schimel lab for their help in the field. Special thanks to Kate McCurdy and Sedgwick staff for maintaining and providing access to our research site. This research was funded by the National Science Foundation (postdoctoral fellowship to P.M.H [DBI-1202894] and DEB-1145875) and a Ford Postdoctoral Fellowship to P.M.H. The Swedish Research Council VR (2016-04146 and 2016-06313) partially funded S.M.

Literature Cited

Alster, C. J., D. P. German, Y. Lu, and S. D. Allison. 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biology & Biochemistry 64:68–79.

Bailey, V. L., A. P. Smith, M. Tfaily, S. J. Fansler, and B. Bond-Lamberty. 2017. Differences in soluble organic carbon chemistry in pore waters sampled from different pore size domains. Soil Biology and Biochemistry 107:133–143.

Birch, H. F. 1958. Patterns of humus decomposition in East African soils. Nature 181:788.

Blankinship, J. C., C. A. Becerra, S. M. Schaeffer, and J. P. Schimel. 2014. Separating cellular metabolism from exoenzyme activity in soil organic matter decomposition. Soil Biology & Biochemistry 71:68–75.

Blankinship, J. C., S. J. Fonte, J. Six, and J. P. Schimel. 2016. Plant versus microbial controls on soil aggregate stability in a seasonally dry ecosystem. Geoderma 272:39–50.

Blankinship, J., and J. Schimel. 2018. Biotic versus abiotic controls on bioavailable soil organic carbon. Soil Systems 2:10.

Blazewicz, S. J., E. Schwartz, and M. K. Firestone. 2014. Growth and death of bacteria and fungi underlie rainfall-induced carbon dioxide pulses from seasonally dried soil. Ecology 95:1162–1172.

Boot, C. M., S. M. Schaeffer, and J. P. Schimel. 2013. Static osmolyte concentrations in microbial biomass during seasonal drought in a California grassland. Soil Biology & Biochemistry 57:356–361.

Borken, W., and E. Matzner. 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology 15:808–824.

Canarini, A., L. P. Kiær, and F. A. Dijkstra. 2017. Soil carbon loss regulated by drought intensity and available substrate: a meta-analysis. Soil Biology and Biochemistry 112:90–99.

Caruso, T., and M. C. Rillig. 2011. Direct, positive feedbacks produce instability in models of interrelationships among soil structure, plants and arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 43:1198–1206.

Chenu, C., and G. Stotzky. 2002. Interactions between microorganisms and soil particles. Pages 3–39 *in* P. M. Huang, J.-M. Bollag, and N. Senesi, editors. Interactions between soil particles and microorganisms. Wiley, Weinheim, Germany.

Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef, and E. Paul. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology 19:988–995.

Crowther, T. W., et al. 2016. Quantifying global soil carbon losses in response to warming. Nature 540:104–108.

Dai, A. 2013. Increasing drought under global warming in observations and models. Nature Climate Change 3:52–58.

Davidson, E. A., P. M. Vitousek, P. A. Matson, R. Riley, G. Garcia-Mendez, and M. Maass. 1991. Soil emissions of nitric oxide in a seasonally dry tropical forest of Mexico. Journal of Geophysical Research 96:15439–15445.

Denef, K., J. Six, K. Paustian, and R. Merckx. 2001. Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry–wet cycles. Soil Biology and Biochemistry 33:2145–2153.

Dungait, J. A. J., D. W. Hopkins, A. S. Gregory, and A. P. Whitmore. 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18:1781–1796.

Fierer, N., O. A. Chadwick, and S. E. Trumbore. 2005. Production of CO_2 in soil profiles of a California annual grassland. Ecosystems 8:412–429.

Fierer, N., and J. P. Schimel. 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biology & Biochemistry 34:777–787.

Fierer, N., and J. P. Schimel. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67:798–805.

Guo, X. B., C. F. Drury, X. M. Yang, and W. D. Reynolds. 2014. Water-soluble carbon and the carbon dioxide pulse are regulated by the extent of soil drying and rewetting. Soil Science Society of America Journal 78:1267–1278.

Guo, X., C. F. Drury, X. Yang, W. D. Reynolds, and R. Zhang. 2012. Impacts of wet–dry cycles and a range of constant water contents on carbon mineralization in soils under three cropping treatments. Soil Science Society of America Journal 76:485–493.

Hogberg, P., A. Nordgren, N. Buchmann, A. F. S. Taylor, A. Ekblad, M. N. Hogberg, G. Nyberg, M. Ottosson-Lofvenius, and D. J. Read. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792.

Homyak, P. M., J. C. Blankinship, K. Marchus, D. M. Lucero, J. O. Sickman, and J. P. Schimel. 2016. Aridity and plant uptake interact to make dryland soils hotspots for nitric oxide (NO) emissions. Proceedings of the National Academy of Sciences USA 113:E2608–E2616.

Homyak, P. M., J. O. Sickman, A. E. Miller, J. M. Melack, T. Meixner, and J. P. Schimel. 2014. Assessing N saturation in a seasonally dry chaparral watershed: limitations of traditional indicators of N saturation. Ecosystems 17:1286–1305.

Kaiser, K., and G. Guggenberger. 2007. Sorptive stabilization of organic matter by microporous goethite: sorption into small pores vs. surface complexation. European Journal of Soil Science 58:45–59.

Kaiser, M., M. Kleber, and A. A. Berhe. 2015. How air-drying and rewetting modify soil organic matter characteristics: an assessment to improve data interpretation and inference. Soil Biology & Biochemistry 80:324–340.

Kallenbach, C. M., S. D. Frey, and A. S. Grandy. 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications 7:13630.

Keil, R., and L. Mayer. 2014. 12.12-Mineral matrices and organic matter. Pages 337–359 *in* H. D. Holland and K. K. Turekian, editors. Treatise on geochemistry. Second edition. Elsevier, Oxford, UK.

Keiluweit, M., J. J. Bougoure, P. S. Nico, J. Pett-Ridge, P. K. Weber, and M. Kleber. 2015. Mineral protection of soil carbon counteracted by root exudates. Nature Climate Change 5:588–595.

Kemper, W. D., R. Rosenau, and S. Nelson. 1985. Gas displacement and aggregate stability of soils. Soil Science Society of America Journal 49:25–28.

Kleber, M., P. Sollins, and R. Sutton. 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85:9–24.

Lawrence, C. R., J. C. Neff, and J. P. Schimel. 2009. Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. Soil Biology and Biochemistry 41:1923–1934.

Leitner, S., P. M. Homyak, J. C. Blankinship, J. Eberwein, G. D. Jenerette, S. Zechmeister-Boltenstern, and J. P. Schimel. 2017. Linking NO and N_2O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils. Soil Biology and Biochemistry 115:461–466.

Liang, C., J. P. Schimel, and J. D. Jastrow. 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2:17105.

Luo, Z., J. Baldock, and E. Wang. 2017. Modelling the dynamic physical protection of soil organic carbon: insights into carbon predictions and explanation of the priming effect. Global Change Biology 23:5273–5283.

Luo, Y., et al. 2016. Toward more realistic projections of soil carbon dynamics by Earth system models. Global Biogeochemical Cycles 30:40–56.

Manzoni, S., and G. Katul. 2014. Invariant soil water potential at zero microbial respiration explained by hydrological discontinuity in dry soils. Geophysical Research Letters 41:7151–7158.

Manzoni, S., F. Moyano, T. Kätterer, and J. Schimel. 2016. Modeling coupled enzymatic and solute transport controls on decomposition in drying soils. Soil Biology and Biochemistry 95:275–287.

Manzoni, S., S. M. Schaeffer, G. Katul, A. Porporato, and J. P. Schimel. 2014. A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils. Soil Biology & Biochemistry 73:69–83.

Manzoni, S., J. P. Schimel, and A. Porporato. 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93:930–938.

Marchus, K. A., J. C. Blankinship, and J. P. Schimel. 2018. Environmental controls on extracellular polysaccharide production in a Mediterranean grassland soil. Soil Biology and Biochemistry 125:86–92.

Matias, L., J. Castro, and R. Zamora. 2011. Soil-nutrient availability under a global-change scenario in a Mediterranean mountain ecosystem. Global Change Biology 17:1646–1657.

Miller, A. E., J. P. Schimel, T. Meixner, J. O. Sickman, and J. M. Melack. 2005. Episodic rewetting enhances carbon and nitrogen release from chaparral soils. Soil Biology & Biochemistry 37:2195–2204.

Navarro-Garcia, F., M. A. Casermeiro, and J. P. Schimel. 2012. When structure means conservation: effect of aggregate structure in controlling microbial responses to rewetting events. Soil Biology & Biochemistry 44:1–8.

Newcomb, C. J., N. P. Qafoku, J. W. Grate, V. L. Bailey, and J. J. De Yoreo. 2017. Developing a molecular picture of soil organic matter–mineral interactions by quantifying organo–mineral binding. Nature Communications 8:396.

Nguyen, T. T., and P. Marschner. 2016. Sorption of water-extractable organic carbon in various clay subsoils: effects of soil properties. Pedosphere 26:55–61.

Nottingham, A. T., H. Griffiths, P. M. Chamberlain, A. W. Stott, and E. V. J. Tanner. 2009. Soil priming by sugar and leaf-litter substrates: a link to microbial groups. Applied Soil Ecology 42:183–190.

Or, D., B. F. Smets, J. M. Wraith, A. Dechesne, and S. P. Friedman. 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media – a review. Advances in Water Resources 30:1505–1527.

Papendick, R. I., and G. S. Camprell. 1981. Theory and measurement of water potential. Pages 1–22 *in* J. F. Parr, W. R. Gardner, and L. F. Elliott, editors. Water potential relations in soil microbiology. Soil Science Society of America, Madison, Wisconsin, USA.

Parker, S. S., and J. P. Schimel. 2011. Soil nitrogen availability and transformations differ between the summer and the growing season in a California grassland. Applied Soil Ecology 48:185–192.

Qualls, R. G., and S. D. Bridgham. 2005. Mineralization rate of 14C-labelled dissolved organic matter from leaf litter in soils of a weathering chronosequence. Soil Biology and Biochemistry 37:905–916.

Ren, C., F. Zhao, Z. Shi, J. Chen, X. Han, G. Yang, Y. Feng, and G. Ren. 2017. Differential responses of soil microbial biomass and carbon-degrading enzyme activities to altered precipitation. Soil Biology and Biochemistry 115:1–10.

Roberson, E. B., and M. K. Firestone. 1992. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. Applied and Environmental Microbiology 58:1284–1291.

Schaeffer, S. M., P. M. Homyak, C. M. Boot, D. Roux-Michollet, and J. P. Schimel. 2017. Soil carbon and nitrogen dynamics throughout the summer drought in a California annual grassland. Soil Biology and Biochemistry 115:54–62.

Schimel, J. P., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386–1394.

Schimel, J., C. A. Becerra, and J. Blankinship. 2017. Estimating decay dynamics for enzyme activities in soils from different ecosystems. Soil Biology and Biochemistry 114:5–11.

Schimel, J. P., and S. M. Schaeffer. 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3:348.

Schimel, J. P., and M. N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology & Biochemistry 35:549–563.

Schimel, J. P., J. Å. M. Wetterstedt, P. A. Holden, and S. E. Trumbore. 2011. Drying/rewetting cycles mobilize old C from deep soils from a California annual grassland. Soil Biology and Biochemistry 43:1101–1103.

Schinner, F., and W. von Mersi. 1990. Xylanase-, CM-cellulase- and invertase activity in soil: an improved method. Soil Biology and Biochemistry 22:511–515.

Setia, R., P. Rengasamy, and P. Marschner. 2013. Effect of exchangeable cation concentration on sorption and desorption of dissolved organic carbon in saline soils. Science of the Total Environment 465:226–232.

Six, J., P. Callewaert, S. Lenders, S. De Gryze, S. J. Morris, E. G. Gregorich, E. A. Paul, and K. Paustian. 2002*a*. Measuring and understanding carbon storage in afforested soils by physical fractionation. Soil Science Society of America Journal 66:1981–1987.

Six, J., R. T. Conant, E. A. Paul, and K. Paustian. 2002*b*. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil 241:155–176.

Smith, A. P., B. Bond-Lamberty, B. W. Benscoter, M. M. Tfaily, C. R. Hinkle, C. Liu, and V. L. Bailey. 2017. Shifts in pore connectivity from precipitation versus groundwater rewetting increases soil carbon loss after drought. Nature Communications 8:1335.

Sollins, P., M. G. Kramer, C. Swanston, K. Lajtha, T. Filley, A. K. Aufdenkampe, R. Wagai, and R. D. Bowden. 2009. Sequential density fractionation across soils of contrasting mineralogy: evidence for both microbial- and mineral-controlled soil organic matter stabilization. Biogeochemistry 96:209–231.

Steinbeiss, S., V. M. Temperton, and G. Gleixner. 2008. Mechanisms of short-term soil carbon storage in experimental grasslands. Soil Biology & Biochemistry 40:2634–2642.

Steinweg, J. M., J. S. Dukes, E. A. Paul, and M. D. Wallenstein. 2013. Microbial responses to multi-factor climate change: effects on soil enzymes. Frontiers in Microbiology 4:146.

Stursova, M., and R. L. Sinsabaugh. 2008. Stabilization of oxidative enzymes in desert soil may limit organic matter accumulation. Soil Biology and Biochemistry 40:550–553.

Todd-Brown, K. E. O., et al. 2014. Changes in soil organic carbon storage predicted by Earth system models during the 21st century. Biogeosciences 11:2341–2356.

Warren, C. R. 2016. Do microbial osmolytes or extracellular depolymerisation products accumulate as soil dries? Soil Biology and Biochemistry 98:54–63.

Wieder, W. R., C. C. Cleveland, W. K. Smith, and K. Todd-Brown. 2015. Future productivity and carbon storage limited by terrestrial nutrient availability. Nature Geoscience 8:441–444.

Xiang, S. R., A. Doyle, P. A. Holden, and J. P. Schimel. 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biology & Biochemistry 40:2281–2289.

Zhang, X., G.-Y. Niu, A. S. Elshall, M. Ye, G. A. Barron-Gafford, and M. Pavao-Zuckerman. 2014. Assessing five evolving microbial enzyme models against field measurements from a semiarid savannah—What are the mechanisms of soil respiration pulses? Geophysical Research Letters 41:6428–6434.

Zsolnay, A., and H. Görlitz. 1994. Water extractable organic matter in arable soils: effects of drought and long-term fertilization. Soil Biology and Biochemistry 26:1257–1261.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2473/suppinfo

Supporting Information. Effects of altered dry-season length and plant inputs on soluble soil carbon. Peter M. Homyak, Joseph C. Blankinship, Eric W. Slessarev, Sean M. Schaeffer, Stefano Manzoni, and Joshua P. Schimel. *Ecology.* 2018.

Appendix S1

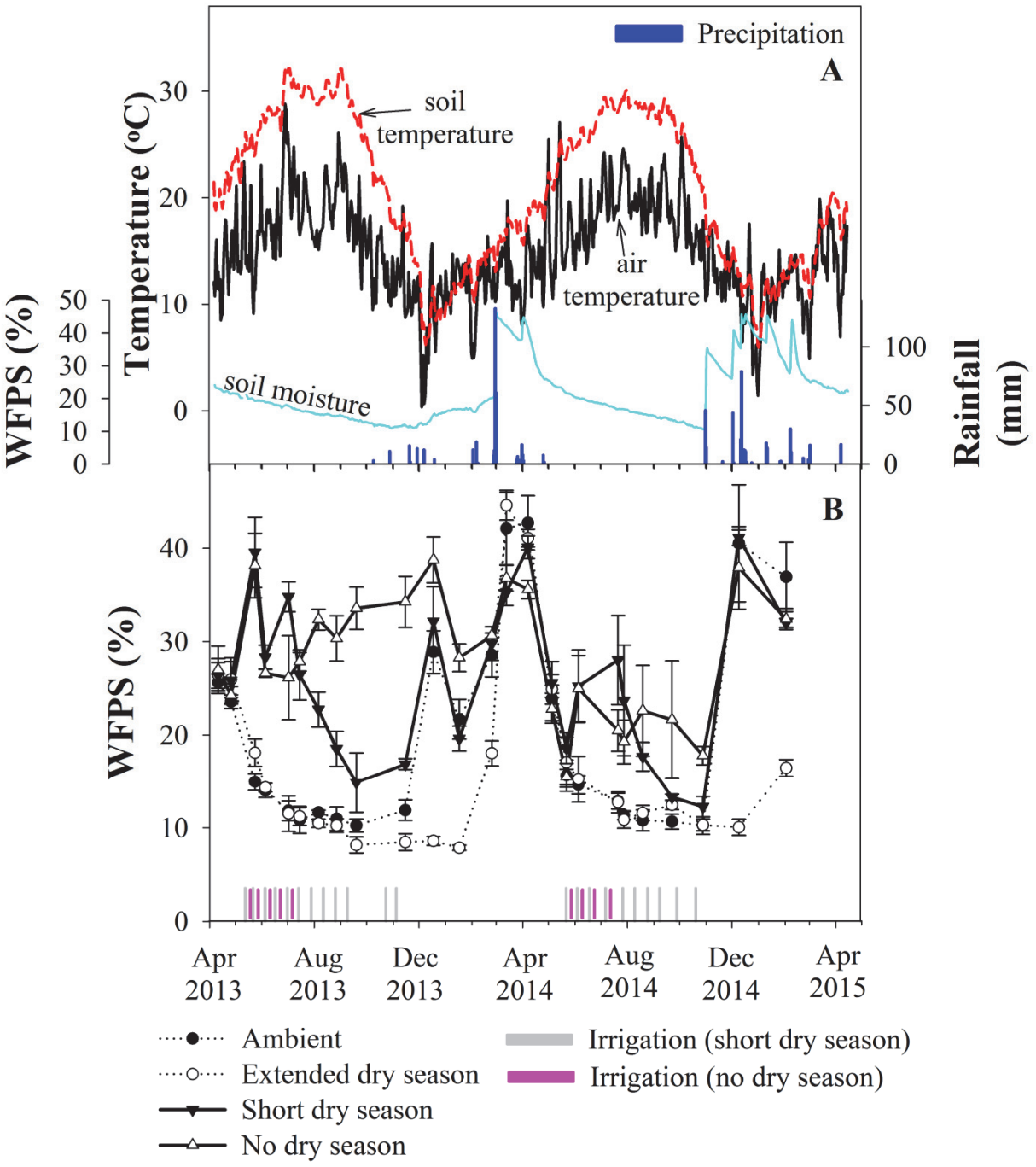

Figure S1. **A**) Climatic variables measured at the Lisque weather station operated by UC Santa Barbara and **B**) average (± SEM; n=3) soil water-filled pore space (WFPS) measured at a 10 cm depth in plots

with plants across the dry-season length manipulation treatments. Extended Dry Season rainout shelters were removed on 30 January 2014 for water year 2013 and 19 January 2015 for water year 2014. Irrigation periods are represented by bars across the x-axis, where duplicate bars represent watering of both the Short Dry Season and No Dry Season treatments. Reprinted with permission (Homyak, P.M., Blankinship, J.C., Marchus, K., Lucero, D.M., Sickman, J.O., Schimel, J.P., 2016. Aridity and plant uptake interact to make dryland soils hotspots for nitric oxide (NO) emissions. Proceedings of the National Academy of Sciences of the United States of America 113, E2608-E2616).