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Environmental controls on extracellular polysaccharide accumulation in a California grassland soil

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A B S T R A C T

Areas with Mediterranean climate regimes, such as California, have cool wet winter growing seasons and hot dry summers. Summer is a time of stress for plants, yet soil microbes survive and biochemical processes continue. One mechanism soil microorganisms might use to survive drought is to produce extracellular polysaccharides (EPSac). We hypothesized that in dry soils, pools of microbial EPSac would therefore increase, but that this increase would depend on having carbon available from fresh plant inputs. We manipulated plant cover and dry season length and measured soil saccharides in a seasonally dry California grassland soil; we evaluated total sugars as well as the mix of sugars present in the soil. Soil cores were collected monthly from July 2014 to February 2015. Sugar residues were analyzed using Gas Chromatography—Mass Spectroscopy (GC-MS). Drier soils showed larger pools of sugar residues; these residues decreased as moisture increased across sample dates and treatments. Plant removal only slightly reduced soil saccharide levels. However, the pools of individual saccharides varied only modestly across all treatments and dates, and correlated with total microbial biomass, suggesting that extracellular polysaccharides may be a constitutive response to survival in soil, rather than an inducible response to dry conditions.

1. Introduction

The mechanisms by which microbes survive, metabolize, and reproduce in dry soils remain uncertain. It had been thought that soil microbes stay hydrated by producing compatible solutes (Kieft et al., 1987; Schimel et al., 2007; Warren, 2014), but recent work suggests this may be rare, at least in seasonally dry ecosystems, such as California grasslands (Boot et al., 2013; Kakumanu and Williams, 2014). Under dry conditions, rather, microbes may dehydrate or become disconnected from substrate, eventually leading to starvation (Parker and Schimel, 2011; Manzoni et al., 2012). These stressors are routine in seasonally dry ecosystems, such as those with a Mediterranean climate, yet microbes continue to function.

The Mediterranean climate has two dominant seasons—a cool wet winter growing season and a hot dry summer, which can go six months or more without rain (Bolle, 2003). Although many plants senesce or go dormant during summer, microbes survive and biochemical processes continue (Parker and Schimel, 2011); in California grasslands, microbial biomass may actually increase during these harsh times (Waldrop and Firestone, 2006; Parker and Schimel, 2011; Schaeffer et al., 2017).

Microbial physiology is regulated in soils by substrate and water availability (Skopp et al., 1990; Stark and Firestone, 1995). When soils wet up, pores fill and soils become hydrologically connected (Parker and Schimel, 2011; Manzoni et al., 2012), which allows substrates to diffuse through the soil (Parker and Schimel, 2011; Or et al., 2007). When soils dry, diffusion becomes limited and soil pores become physically disconnected, separating microbes from their substrates, leading to reduced activity that may drive microbes into dormancy (Fierer et al., 2005; Lennon and Jones, 2011; Manzoni et al., 2012). However, soil microbes don't shut down completely (Waldrop and Firestone, 2006; Parker and Schimel, 2011). To survive dry conditions, microbes appear able to shift resources from growth to survival (Schimel et al., 2007).

One mechanism soil organisms use to manage environmental stressors is to produce extracellular polymeric substances (EPS); these are predominantly polysaccharide, but also contain DNA, protein, and other constituents (More et al., 2014). Extracellular polysaccharides (EPSac) are produced by both plants and microorganisms (Oades, 1972); this material acts as a glue-like binding agent that adheres to soil particles, which promotes soil aggregate formation and stability (Blankinship et al., 2016; Martin, 1946; Whistler and Kirby, 1956). Plants produce polysaccharides in the rhizosphere (Gunina and

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Kuzyakov, 2015) and so may act as a substantial source of EPSac. Microbes, however, can also be a major source, as it is thought that they encapsulate themselves in EPS under stress, particularly starvation (Chen et al., 2014; Colica et al., 2014; Rossi et al., 2012; Steinberger and Holden, 2004; Wolfaardt et al., 1999) and desiccation (Chenu, 1993, 1995; Harris, 1981; Roberson and Firestone, 1992). During dry periods, resource pools shrink and concentrate in soil pore microsites. Substrates may become disconnected from soil microbes but EPS can create a matrix that physically connects microbes and substrates (Chenu and Roberson, 1996; Or et al., 2007). Further, as soils dry, EPS films may help maintain a beneficial microhabitat within soil aggregates by retaining moisture, increasing soil water-holding capacity, and delaying drying (Oades, 1984; Pointing and Belnap, 2012; Roberson and Firestone, 1992). However, producing EPS may be energetically expensive for microorganisms (Harder and Dijkhuizen, 1983; Wolfaardt et al., 1999). Thus, it remains uncertain how much microbial EPS production occurs in soil *in situ* and whether it is associated with specific environmental conditions or stresses (Schimel et al., 2007).

In this study, we asked: which environmental drivers in a natural grassland ecosystem control the production and accumulation of extracellular polysaccharides? Do EPSac concentrations increase with the extended length of the dry season and with greater plant C inputs? We hypothesized that with longer dry periods (i.e. extended drought), soils would have greater levels of EPSac due to the greater need for microbes to survive desiccation and starvation. We also hypothesized that in soils with more plants (i.e. higher C inputs) EPSac levels would be higher; not only would there be greater pools of plant-derived EPSac, but microbes would have more C to allocate to EPSac production; in soil without plants (i.e. lower C inputs), on the other hand, there would be less EPSac, due to reduced plant inputs and a lack of substrates for microbial EPSac production. To test these hypotheses, we manipulated plant cover and dry season length and regularly measured EPSac in a seasonally dry California grassland soil; we periodically evaluated pools of total EPSac as well as the mix of sugars making up EPSsac to try to separate plant from microbial constituents.

2. Materials and methods

2.1. Site description and field experiment

We implemented a plant cover and soil moisture field manipulation experiment in a seasonally dry grassland in Santa Barbara County (Blankinship et al., 2016; Homyak et al., 2016). The site is located at the University of California, Sedgwick Reserve in Los Olivos, California (34.712036°, −120.038797°). The reserve is approximately 28 km from the coast, and 370 m above sea level in the Santa Ynez Valley. The area has a Mediterranean climate, with long dry summers and cool wet winters. Average annual precipitation is 380 mm; however, during our 2-year study California was in the midst of a drought, where annual rainfall was approximately 50% below normal (175 mm in 2013 and 201 mm in 2014). Daily average air temperature is 16.8° C, with highs in the summer reaching into the 30's while winter lows below freezing are not uncommon. Meteorological data (air temperature, relative humidity, and rain/fog inputs) were obtained from a site on the Sedgwick reserve, approximately 2 km southwest of the field site (IDEAS, UCSB Geography Dept. http://www.geog.ucsb.edu/ideas/). Soil temp was measured from 15 cm depth. The sampling area is dominated by exotic annual grasses, primarily *Bromus diandrus*, *Bromus hordaceous*, and *Avena fatua*. These typically germinate with the first rains but senesce and die with the onset of the summer dry season (April).

Soils at the site are Pachic Haploxerolls. Texture in the A horizon is a silty clay loam with granular structure on nearly flat slopes (< 2%). The soil pH is 6.0, with 2.2% C, 0.21% N, and a bulk density of 1.2 g cm^{-3} in the upper 10 cm. The field experiment established field treatments that included manipulating plant inputs and the length of the summer growing season. All treatments were established in triplicate; there were three blocks of plots (1 × 2 m, separated from each other by 1 m) with each treatment replicated within each block; blocks were approximately 20 m apart.

2.1.1. Plant removal treatment

We created a gradient of fresh plant inputs into the surface soils by thinning live plants during the winter growing seasons. The treatments consisted of 0%, 33%, 66% and 100% removal and were done by hand throughout the growing season. Plant removal began in fall 2012. For this study, we sampled from plots with plants (0% removal) and without plants (100% removal) to focus on the extreme manipulations. During this study's summer focal period, plants were all dead, and there would be no inputs of fresh plant C. Hence, we hypothesized that the strongest effect of varying plant biomass during the winter/spring growing season would be to create a gradient of root litter C that would be established by the beginning of the summer dry season. The treatments might also, however, alter soil moisture during the early summer. Removing plants would reduce live root biomass during the growing season, reducing transpirational water loss and so increasing soil water content at the beginning of summer. However, removing plants would also reduce the amount of standing dead "thatch," which shades the soil surface; this would enhance evaporation off the soil surface. These effects might partially counter-balance each other early in the summer (more soil moisture but greater evaporation); by late summer, however, surface soils will inevitably dry fully.

2.1.2. Dry season manipulation

Dry season length was manipulated to alter the amount of time during which dry-season processes would continue (Fig. 1). Water manipulations included dry, control, short dry, and wet (i.e. no dry season). Water was added to the short dry plots (from May into July) and to the wet plots (from May into November) biweekly using backpack sprayers. Each scheduled watering consisted of two applications of 15 L each, roughly an hour apart, which equated to 1.5 cm of water. This maintained > 10% volumetric water content (VWC). Based on Fierer et al., 2005, we deemed 10% VWC to be an important threshold, as below this there was a noticeable decline in soil respiration, suggesting a loss of access to C substrate. In added-water treatments, we hand-weeded any plants that germinated to prevent summertime inputs of fresh plant-C. In dry treatment plots, we excluded precipitation from October through January using rain-out shelters made of clear corrugated polycarbonate roof panels (Suntuf, Palram Americas, Kutztown, Pennsylvania).

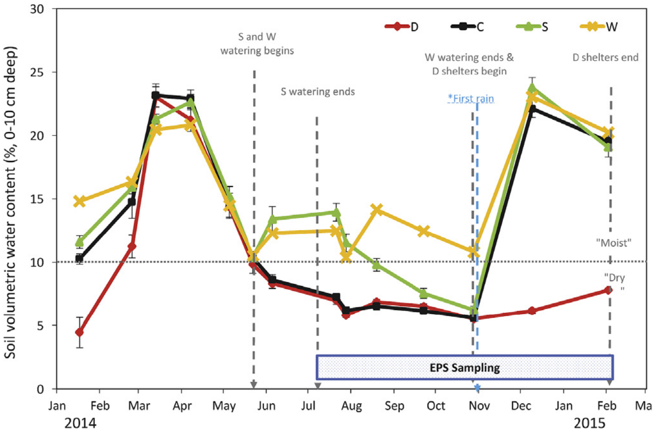

Fig. 1. Seasonal soil moisture patterns with ambient and manipulated conditions before and during EPS sampling. Treatments are Dry (D): red diamonds & line; Control (C): black squares & line; Short Dry (S) green triangles & line; Wet (W): purple X & line. Timing of treatments (grey dashed line) and rain events (blue dashed line) (± SE). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)