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Extracellular enzyme activity response to defoliation and water addition in two ecosites of the mixed grass prairie

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ABSTRACT

Grassland composition is affected by livestock grazing and moisture availability, yet little is known about how defoliation and soil moisture interact to affect belowground processes. In particular, microbial activity, the proximate driver of decomposition, may be affected by plant responses and environmental variability. We hypothesize that grassland soils with different defoliation and moisture treatments will differ in soil biogeochemical cycling in response to both physical environment (i.e. soil moisture) and biotic (i.e. plant community) shifts. To understand how microbial function is affected by defoliation and moisture, we measured extracellular enzyme activity (EEA) at two mixed grass prairie ecosites (mesic lowland and xeric upland) in Alberta, Canada using a fully factorial experiment that manipulated growing season defoliation and water addition over 4 years. The defoliation treatments were: high intensity – high frequency (HIHF), high intensity – low frequency (HILF), or low intensity – high frequency (LIHF) from May through August each year, and a treatment where defoliation was deferred until the end of each summer. The watering treatments were: ambient and water addition (150 mm month^{-1} above ambient), which was intended to eliminate moisture limitations. In the fourth year of treatment, we measured the activities of 5 hydrolytic extracellular enzymes responsible for carbon (C), nitrogen (N) and phosphorous (P) release. We observed that water addition reduced activity of most EEAs across both sites, although these effects were distinctly mitigated by defoliation at the lowland site. Within the lowland, water addition reduced C cycling enzyme activities under deferred and HILF defoliation, with a similar pattern in the activity of the P cycling enzyme in response to HILF and LIHF defoliation. Defoliation effects on EEA in the lowland were limited largely to ambient moisture conditions, where severe (HIHF) defoliation reduced C cycling EEA, with a similar pattern in the upland, though only for β-D-cellobiosidase activity. Independent of moisture treatment, deferred defoliation reduced activity of phosphatase in the lowland, as well as the activity of one enzyme responsible for C cycling in the upland site. There was no effect of defoliation or water addition on *N*-acetyl-β glucosaminidase activity at either site. Overall, we demonstrate that while EEAs are strongly affected by environmental conditions, defoliation during the growing season may interact with the physical environment and regulate biogeochemical cycling.

1. Introduction

Grasslands cover more than 40% of the earth's terrestrial surface and contain nearly 30% of the soil organic carbon (C) pool (Lal, 2002; Derner and Schuman, 2007). This carbon enters grasslands above ground via photosynthesis, but along with large nutrient pools (e.g. nitrogen and phosphorous; hereafter 'N' and 'P') the majority is stored in belowground roots and enter soil biogeochemical pools via plant litter decomposition. The chemical complexity and structure of soil organic matter (SOM) is determined by plant community composition (e.g. cool vs warm season grasses) (De Deyn et al., 2008), litter abundance and its associated chemical composition and quality (i.e. C:N or lignin:N ratios) (Wickings et al., 2012).

When senesced plant material and root exudates enter the litter pool, the primary mechanism of decomposition and associated cycling of C and nutrients is microbial activity, which liberates nutrients from organic material via extracellular enzyme activity (EEA) (Saiya-Cork et al., 2002; Allison et al., 2007). Enzymes are

Abbreviations: C, carbon; N, nitrogen; ANPP, aboveground net primary production; GWC, gravimetric water content; OM, organic matter; SOM, soil organic matter; BG, β-1,4-glucosidase; Cello, β-D-cellobiosidase; Xylo, β-1,4-xylosidase; Phos, acid phosphatase; NAG, *N*-acetyl-β glucosaminidase.

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released by microbes into the litter and soil layers, where these compounds catalyze the decomposition of target molecules. Similarly, organically bound nutrients, which are often limited in grasslands (Huenneke et al., 1990; Vitousek and Howarth 1990), can be released from organic matter by microbial enzymes making them available in the soil. Factors regulating the production and release of EEAs are typically related to the metabolic needs of the microbial community and the availability of substrate within litter and soil layers (Sinsabaugh et al., 2008). As a result, the plant community and associated chemical composition of root exudates and litter has the potential to influence SOM chemistry (Allison and Vitousek, 2005; De Deyn et al., 2008; Wickings et al., 2012). At present we lack a clear link between how changes in vegetation composition in response to disturbance affect these biologically mediated ecosystem processes.

The composition of grassland communities is broadly controlled by regional climate (e.g. temperature and moisture) over long time scales (Chapin et al., 2011). However, heterogeneous disturbances such as fire and grazing by livestock can lead to shifts in plant species dominance and co-dominance (Milchunas et al., 1989; Milchunas and Lauenroth, 1993; Koerner and Collins, 2014). These changes in turn, can enhance the growth and vigor of adapted plants and microbes (Zogg et al., 1997; Bardgett et al., 2001; Grayston et al., 2004; Hart et al., 2005). Moreover, the cascading effect of these disturbances can impact competitive interactions among plants (Yates et al., 2000), further affecting community structure.

There is great debate about how different grazing systems affect rangeland ecosystems (Briske et al., 2008, 2011; Teague et al., 2013). Grazing systems can range from continuous (i.e. season-long) grazing to management intensive grazing (MIG) with varying rest intervals between intermittent defoliation periods (i.e. rotational). Studies aimed at understanding the effect of grazing systems on plant community composition and productivity have been carried out across a broad range of grasslands in North America (Holechek et al., 2000; Briske et al., 2008). Additionally, several studies have assessed the influence of grazing on grassland C stores (Derner et al., 1997; Derner et al., 2006; Derner and Schuman 2007; Ingram et al., 2008) and microbes (Bardgett et al., 2001; Grayston et al., 2004; Patra et al., 2005; Teague et al., 2011; Yang et al., 2013). However, the results of these studies have been contradictory in many cases. For example, grazing and its associated effects have been shown to enhance, decrease, or have no effect on the quantity of C stored in grassland soils (Derner and Schuman, 2007).

To develop mechanistic links between land-use and associated changes in plant community structure and production, as well as the fate of C and organic nutrients, studies that directly measure the cycling of C and nutrients immobilized in litter and SOM are necessary. We measured the extracellular enzyme activity (EEA) of microbial enzymes responsible for decomposing plant based organic compounds within the soil of two contrasting grassland types that had received 4-years of experimental defoliation and water addition. We hypothesized that water addition would affect the physical environment and subsequent measures of EEA. We also hypothesized that EEA would be affected by both defoliation intensity and frequency during the growing season due to changes in substrate (e.g. quantity and quality) associated with unique plant communities, and plant physiological responses to defoliation.

2. Methods

2.1. Study sites and experimental design

We used two existing long-term study sites at the University of Alberta Mattheis Research Ranch first established in 2010. Both sites are located in the Brooks Plain of SE Alberta, Canada, within the Mixedgrass Prairie natural sub-region (Adams et al., 2005). Mean annual precipitation and temperature are 354 mm and 4.2° C, respectively. The first site was established on a relatively mesic loamy lowland site (50° 53′ 40.2″ N, 111° 52′ 26.3″ W; hereafter 'lowland site'), while the second was established on a more xeric sandy upland site (50° 52′ 23.8″ N, 111° 52′ 26.2″ W; hereafter 'upland site'). Both sites were internally uniform in topography and initial plant composition. The lowland site was comprised of a Gleyed Eluviated Brown Chernozemic soil of sandy loam texture, while the upland site had a Rego Brown Chernozemic soil with a loamy sand texture. Prior to establishment, both sites were grazed by cattle for several decades at moderate stocking rates, and were considered to be in good range health (>80% range health score; Adams et al., 2009).

At each site, a fully randomized factorial design was used to manipulate water and defoliation, beginning in 2010 and continuing through 2013. We implemented four growing season defoliation treatments and two water treatments, with each combination replicated 6 times at the lowland (n = 48 plots) site and 7 times at the upland site (n = 56 plots). Treatments were applied to 1 × 1 m permanently marked plots with a minimum of 0.5 m buffer between plots. Defoliation treatments were applied via manual clipping in the following intensity by frequency combinations: high intensity – high frequency (HIHF), high intensity – low frequency (HILF), low intensity – high frequency (LIHF) or a single annual deferred defoliation. Deferred treatments were clipped once to a 2 cm stubble height in August, while the HIHF and HILF plots were clipped to 2 cm every 3 or 6 weeks, respectively, from mid-May through the end of August each year. In contrast, the LIHF treatment was clipped to 5 cm every 3 weeks. All plots were defoliated to 2 cm at year-end. Moisture treatments were either no addition (i.e. ambient rainfall) or water addition via spray irrigation, which augmented ambient conditions to maintain a monthly average of 150 mm during the summer months. Watering was done every 10 days, commencing in early June and ceasing at the end of August. All treatments were initiated in 2010 and continued throughout each subsequent growing season. Plant community composition was documented by identifying all species present within each plot during the third and fourth years of treatment.

2.2. Sample collection and processing

On July 28, 2013 we removed three mineral soil cores (1.25 cm diameter, 10 cm deep) from each experimental plot. Cores were combined for each plot, bagged and promptly frozen. Samples were kept cold during transport to the University of Alberta where they were stored at −20 °C until prepared for analysis. While freezing samples may alter EEA (DeForest 2009; Peoples and Koide, 2012), the extended travel period associated with collecting samples over such a large area necessitated that samples be frozen to ensure preservation. All samples were run through a 2 mm sieve to remove coarse material (e.g. rocks and roots). Duplicate subsamples were taken to measure soil pH, gravimetric water content and organic matter content at the plot level. Soil pH was measured using a Fisher Accumet probe using a 40 g: 80 ml mix of soil to deionized water. Gravimetric water content was measured by drying 25 g of soil at 105 °C for 48 h. Soil organic matter was measured using loss-on-ignition by igniting soil at 350 °C for 4 h. Another subsample was dried at 60 °C and ground to a fine powder in a ball mill (Retsch MM400 Mixer Mill, Retsch, Haan, Germany), and then fumigated with HCl to remove carbonates. Subsamples were analyzed in duplicate to quantify total organic C and total N concentration using a LECO TruSpec CN elemental analyzer (LECO Corporation, St. Joseph, MI, USA). After every tenth sample, a LECO soil standard (502-308; LECO