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Litter decomposition of three lignin-deficient mutants of *Sorghum bicolor* during spring thaw

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ABSTRACT

Decomposition of plant litter during the freeze-thaw season has recently gained attention as having a significant role in nutrient cycling in many cold ecosystems. However, few studies have examined decomposition of crop remnants during the freeze-thaw season in an agronomic setting when microbial activity is presumably low. We examined decomposition of four cultivars of sorghum (*Sorghum bicolor*) leaves in a field in Southern Minnesota, USA using the litterbag method. Three of the four cultivars we examined expressed the brown midrib (bmr) mutation which have altered/reduced levels of lignin in their secondary cell walls compared to the wild-type (WT). Litter was buried in the fall and harvested during the spring thaw. After 160 d the bmr mutants lost 57–62% of their initial mass, compared to 51% in the WT. Mass loss agreed with presumed initial litter quality, as the bmr litter had higher initial N, and holocellulose:lignin and lower lignin, C:N and lignin:N values compared to the WT. The increased decomposition of the bmr cultivars appears to be related to increased loss of hemicellulose and holocellulose (cellulose + hemicellulose) or higher initial N concentrations. Alterations in cell-wall deposition in the bmr cultivars may increase accessibility of microbial cell-wall degrading enzymes that accelerate mass loss. Our results demonstrate that alterations in initial lignin chemistry may influence decomposition of sorghum litter in an agronomic setting.

1. Introduction

Decomposition of plant litter is a key component in the global carbon cycle and contributes approximately 68 Pg C yr^{-1} to the total global annual carbon flux (Aerts, 1997). It was once assumed that decomposition rates of plant litter was regulated by a hierarchy of climate, litter chemistry and soil organisms. However, recent evidence suggests that this hierarchy may not always hold true, as the roles of litter quality and decomposers may overshadow climate in some systems (Currie et al., 2010; García-Palacios et al., 2013; Bradford et al., 2014). In addition, decomposition of litter was once thought to be negligible when soil temperatures drop below freezing; however, recent findings suggest that winter decomposition plays a major role in the release of carbon in many cold ecosystems (Uchida et al., 2005; Wu et al., 2010). Activity of cold-tolerant microbes appears to be partially responsible for litter mass loss and subsequent carbon efflux during winter months (Brooks et al., 1997; Uchida et al., 2005).

In agronomic systems, tillage of crop residues after autumn harvest results in increased nutrient release into the soil. This mechanical disturbance increases bacterial colonization of litter and decomposition in these systems can be much faster than in natural systems (Hendrix et al., 1986). Burial of crop residues in the fall during tillage increases availability of key nutrients such as N, P and K for crops planted in the next growing season (Freppaz et al., 2007; Zhu et al., 2012). Initial decomposition rates of these residues are often tied to the amount of soluble C in litter, but over longer time scales, rates are more often correlated with initial concentrations of cellulose, hemicellulose and lignin (Gregorich and Janzen, 2000; Trinsoutrot et al., 2000; Wilhelm et al., 2004). In a typical agronomic system, the half-lives of cellulose and hemicellulose is on the order of days, while the half-life of lignin, which is extremely recalcitrant, can often exceed one year (Kumar and Goh, 2000; Eiland et al., 2001; Wilhelm et al., 2004). Lignin is a complex polymer made from aromatic phenolic subunits that are products of the phenylpropanoid pathway. These phenolic subunits are covalently linked to polysaccharides in the secondary cell wall that increase structural rigidity and result in a polymer that is incredibly recalcitrant to microbial breakdown and decay (Meentemeyer, 1978).

Alterations in the phenylpropanoid pathway can lead to plants with a reduced or altered lignin content (Sattler et al., 2010; Baxter and Stewart, 2013). Reducing lignin content can be beneficial for feedstocks in cellulosic ethanol production, as lignin can hinder the conversion of polysaccharides in the cell wall to ethanol (Chapple et al., 2007). The

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brown midrib mutation (bmr) has recently been identified in sorghum (*Sorghum bicolor*), corn (*Zea mays*) and pearl millet (*Pennisetum glaucum*), which have impaired activity of cinnamyl alcohol dehydrogenase (CAD) and/or caffeate/5-hydroxyferulate-*O*-methyl transferase (COMT) enzymes (Palmer et al., 2008; Sattler et al., 2010), resulting in altered amounts of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) subunits of lignin. Phenotypic expression of these mutations typically include a reddish-brown leaf midrib and accumulation of reddish brown pigment in stalks and roots. In sorghum, the *bmr6* and the *bmr12* loci have impaired levels of CAD and COMT, respectively, and result in plants that typically contain 14–21% less lignin (Sattler et al., 2010). Therefore these bmr mutants would be ideal in examining the role of initial lignin content on litter decomposition.

In this study, we examined decomposition of four different sorghum cultivars (wild-type, *bmr6*, *bmr12*, and a stacked double mutant) in Southern Minnesota over the winter and early spring. We utilized the mesh litterbag technique (Pérez-Harguindeguy et al., 2013) in an agronomic field for 160 days. We suspected that litter with initially low lignin content should have increased mass loss rates and altered levels of cell-wall constituents at the end of the spring thaw. In addition, we characterized microbial communities between litter types and adjacent soil by examining differences in carbon-substrate catabolism.

2. Materials and methods

2.1. Study site and experimental design

The field experiment was conducted in a tilled agricultural field located near Janesville, MN, USA (44° 09′ N x 93° 45′W) from 16 November 2014 to 24 April 2015. The soil at this location is a Cordova Clay Loam and has a pH of 7.0. Phosphorus, potassium, zinc and sulfur concentrations averaged 115, 206, 4.3 and 15 ppm, respectively (data not shown). The average annual air temperature is 7.8 °C and the absolute minimum and maximum temperature ranges from −31 °C (January) to 36 °C (July). Average annual precipitation at the site averages 854 mm, and winter precipitation is relatively low, averaging 66 mm.

Seeds of wild type (WT), *bmr6* and *bmr12* cultivars of sorghum were obtained from the USDA-ARS at the University of Nebraska, Lincoln (Pedersen et al., 2006a). In addition, we created a *bmr6/bmr12* stacked hybrid (double mutant; DM) following the methods of Pedersen et al. (2006b). All four cultivars were grown in a greenhouse at Minnesota State University for two weeks then transplanted to a field on campus in early July 2014. These plants were harvested in mid-September when 75% of the flag leaves were fully expanded. Individual plants were placed in paper bags and oven dried at 60 °C for > 48 h. It is important to note that these leaves did not reach senescence and do not necessarily represent litter that would be representative of normal crop residues. However, a similar study using mutants of *Arabidopsis thaliana* with altered lignin levels demonstrated that this method could be utilized to examine the effects of lignin chemistry on decomposition (Talbot et al., 2012).

Dried plant material of each cultivar was individually separated into leaf and stem material and approximately 1.3 g of dried leaves was placed into fiberglass 2-mm mesh bags (12.5 × 12.5 cm) and sealed with nylon thread. Fifteen replicate samples were made for each cultivar for a total of 60 litter bags. Bags were then randomized and placed into the field on 16 November 2014 as soil started to freeze. Litterbags were placed in 1-m increments along transects and buried at a depth of 15 cm. We randomly collected five litterbag samples from each cultivar at three different harvest dates during the freeze-thaw season: 11 March, 6 April, and 24 April 2015. In addition, we sampled soil directly adjacent to the buried litterbags at each sampling date to examine the microbial community (see below).

2.2. Litter chemistry

On each collection date, five litterbags of each cultivar were gently removed from the soil and the remaining litter was lightly rinsed with a phosphate-buffered saline solution and using 100 µl of a 1:100 dilution for microbial community level phenotype profiling (below). Litter was then dried at 60 °C for > 48 h and weighed. Litter was then ground through a Wiley Mill (1 mm) and analyzed for litter chemistry. A fiber analyzer (model A200; ANKOM Technology, Macedon, NY, USA) was used to estimate concentrations of cellulose, hemicellulose and lignin of dried samples following Van Soest et al. (1991). Litter samples were sequentially digested in a neutral detergent fiber solution (NDF) to estimate the hemicellulose + cellulose + lignin fractions and then an acid detergent fiber solution (ADF) to determine cellulose + lignin fractions. Lastly, samples were agitated in 72% H_2SO_4 for 3 h and then placed in a muffle furnace (600 °C) to determine lignin and ash concentrations. A more detailed description of this procedure can be found in Warnke and Ruhland (2016). Additionally, we analyzed an extra set of initial samples for C and N concentrations with a flash combustion analyzer (TruSpec, Leco Corporation, St. Joseph, MO, USA).

2.3. Community-level phenotype profiling

Community-level phenotype profiling (CLPP) of the microbial community present on leaf litter and adjacent soil was performed by testing the ability of each community to use 95 different carbon sources present on BIOLOG GN2 microplates (Biolog, Hayward, CA, USA). In order to account for variance and to ensure that the microbes found on the litter were unique to each cultivar, samples from soil directly adjacent to each litterbag were also tested. Subsamples of litter were collected from three litterbags (n = 3) of WT and DM varieties (≈0.35 g) and adjacent soil (1 g) on the initial and final census dates. Subsamples were homogenized in 100 ml of phosphate-buffered saline (pH = 7.4) using a Waring blender. Samples were incubated for 10 min and then 10 ml was transferred from the top of the suspension into a pipette reservoir. A multichannel pipettor was then used to dispense 100 µl into individual wells of the microplate. Plates were then read every 24 h (λ = 490 nm) by a Multiskan Spectrum microplate reader (Thermo Electron Corporation, Waltham, MA, USA) for 7 d. A more detailed description of this procedure can be found in Garland and Mills (1991).

2.4. Statistical analyses

The general linear model procedure was used with a two-way analysis of variance (ANOVA) to examine differences in census dates and sorghum varieties. Initial chemical analyses were analyzed using a one-way ANOVA. The least significant difference test (LSD) was used to compare individual treatment means. A Welch's one-way ANOVA was used to examine differences in percent mass loss due to the small sample size and unequal variances on the last sample date. Unless specified, treatment means were considered significant at the $P < 0.05$ level.

Analysis of CLPP data has been discussed in detail elsewhere (Garland and Mills, 1991). The optical density of the control well (lacking a carbon source) of each microplate was subtracted from those of all other wells in the microplate, and the average well color development (AWCD) was then calculated for each reading by summing the optical densities of all wells of the microplate and dividing by 95. Replicates yielding AWCD values ranging from 0.5 to 0.7 were selected for detrended correspondence analysis (DCA) using PC-ORD software (MjM Software Design, Gleneden Beach, OR) following Garland (1997).