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Cell wall compositional changes during incubation of plant roots measured by mid-infrared diffuse reflectance spectroscopy and fiber analysis

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ABSTRACT

Plant roots, particularly the constituents of root cell walls (hemicellulose, cellulose and lignin) are important contributors to soil organic matter. Little is known about the cell wall composition of many important crop species or compositional changes as roots decay. The objectives of this study were to quantify changes in root cell wall composition during a four week laboratory incubation by forage fiber analysis and characterize those changes using diffuse reflectance infrared fourier transform spectroscopy (DRIFTS). The roots of six important crop, forage and native grass species were incubated at 25 °C and sampled weekly. Alfalfa lost 78% of initial mass over four weeks, while the remaining species lost between 19% and 38%. For all species the majority of this loss occurred during Week 1, and only alfalfa mass loss was significant ($P < 0.05$) each week. The trends observed for whole root decomposition were paralleled by the decomposability of root cell walls. Significant changes in hemicellulose, cellulose and lignin concentrations over time were only observed in alfalfa roots. Significant changes in decomposability of these constituents was likewise only observed in alfalfa, with cellulose the most decomposable fraction, followed by hemicellulose and lignin. Analysis by DRIFTS supported the fiber analysis results and revealed important changes in root cell wall composition. The disappearance of peaks due to starch in the perennial alfalfa and switchgrass roots following Week 1 helped to explain the greater initial mass loss in both of these species relative to the roots of the annuals. The spectral data also illustrated the resistance of alfalfa lignin to decomposition, the preservation of carbonyl compounds and the degradation of readily decomposed proteins. Finally, changes potentially indicative of wax compound preservation were found in the DRIFTS spectra of alfalfa even though the amount of wax was too small to quantify by fiber analysis. This research study reveals differences in the rate at which crop roots decompose and important changes that can occur in readily decomposable roots over relatively short time scales. These results provide valuable information contributing to the understanding and prediction of short term soil organic matter dynamics which will help to predict possible impact of management changes or soil disturbance on soil health and productivity as well as long term organic C stabilization and the potential for C sequestration.

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1. Introduction

Soil organic matter (SOM) represents the largest terrestrial carbon (C) pool, and there is indirect evidence that plant roots are a significant, though overlooked source of C for SOM formation (Gale and Cambardella, 2000; Lal, 2004; Puget and Drinkwater, 2001; Rasse et al., 2005). This is particularly true in subsoils which receive minimal C inputs from deposition of aboveground plant material and represent a large potential pool for soil C sequestration (Lorenz et al., 2007). Globally, 59% of the soil organic C (SOC) in the top meter of cropped soils is found below the 20 cm depth (Jobbágy and Jackson, 2000). The radiocarbon age of soil organic C increases with depth and can exceed 1000 years, but information is lacking regarding the processes governing the formation of subsoil SOM including inputs and stabilization mechanisms (Rumpel and Kögel-Knabner, 2011). In agricultural systems, changes in crop species and management practices alter root inputs to SOM throughout the soil profile, but the impact of these changes in root inputs on soil C storage is unknown. Increasing SOM in agricultural soils would yield numerous additional ecosystem services, including reductions in water and nutrient losses, improved soil aggregation and increased productivity on degraded soils (Lal, 2009).

Abbreviations: ADF, Acid Detergent Fiber; DRIFTS, diffuse reflectance mid-infrared fourier transform spectroscopy; NDF, Neutral Detergent Fiber.

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Root-derived macromolecules (i.e., lignin and plant waxes such as suberin) are likely to be important contributors to SOM (Kögel-Knabner, 2002; Winkler et al., 2005; Otto and Simpson, 2006). Labile plant derived compounds from root biomass are an important input to subsoil SOM and play an important role in agricultural soil quality and nutrient cycling (Vancampenhout et al., 2012). Differences in root cell wall composition among plant species and the relative resistance of cell wall constituents to decomposition and mineralization are important factors for understanding the role of root-derived C in SOM cycling, as well as for organic C stabilization. Growing scientific consensus suggests that stabilization of these molecules in recalcitrant SOM is likely due to physico-chemical processes rather than inherent biochemical recalcitrance of the root compounds themselves (Kleber and Johnson, 2010; Marschner et al., 2008). Nevertheless, biochemical recalcitrance and physico-chemical protection occur together during root tissue mineralization (Garcia-Pausas et al., 2012). Knowledge of the nature of root-derived compounds stabilized in SOM and their relative recalcitrance is necessary to guage the potential rate and magnitude of SOM loss following soil disturbance such a change in tillage practice, logging or fire or to predict quantitative gains in C storage in SOM resulting from growing different plant species.

Soluble intracellular root components consist of proteins and free sugars as well as the storage polysaccharides starch and, in grasses, fructan (Kögel-Knabner, 2002; Gunnarsson et al., 2008). Non-cell wall components are rapidly decomposed in the initial days of root decomposition (Abiven et al., 2005; Machinet et al., 2011). Root cell walls are primarily composed of hemicellulose, cellulose, lignin and wax in varying proportions depending upon species (Aulen et al., 2012; Machinet et al., 2009, 2011; Picon-Cochard et al., 2012; Redin et al., 2014; White et al., 2011). The cell walls of legumes such as alfalfa also contain relatively larger amounts of pectins compared to grasses (Åman, 1993; Gunnarsson and Marstorp, 2002). Alfalfa pectin has been demonstrated to be readily degradable and increasing pectin concentrations leads to greater alfalfa digestibility in forage studies (Hatfield, 1993; Jung and Engels, 2002). Cell wall structural molecules exist in a complex interrelationship which influences biological degradation (Amin et al., 2014; Bertrand et al., 2006). Interactions among cell wall components, particularly lignin and cellulose are important factors controlling decomposition (Melillo et al., 1982; Talbot and Treseder, 2012). Lignin protects cellulose and hemicellulose from decay, while cellulose decomposition helps to facilitate lignin degradation (Talbot and Treseder, 2012). Lignin is a complex polyphenolic molecule composed of *p*-coumaryl, coniferyl and synapyl alcohol monomers with varying degrees of methoxylation of the aromatic ring. Non-methoxylated monomers are designated *p*-hydroxyphenyl, while monomethoxylated and dimethoxylated units are designated syringyl and guaicyl, respectively. In aboveground plant tissues lignins with a higher proportion of guaicyl exhibit greater resistance to decomposition, while conversely those predominated by *p*-hydroxyphenyl units are most susceptible to degradation (Bertrand et al., 2006; Jung and Vogel, 1992; Talbot et al., 2012).

Hemicellulose structure and the extent of crosslinking between hemicellulose and lignin by ferulic acid also influences root decomposition (Amin et al., 2014; Machinet et al., 2009). Hemicellulose is composed of a complex mixture of polysaccharides consisting of β-1-4 linked xylan chains with varying degrees of substitution with arabinose, glucouronic acid, 4-*O*-methyl glucouronic acid, galactose and acetyl groups (Hatfield, 1989). In grasses, the principle substituent to the xylose backbone is arabinose, while in legumes rhamnose is a characteristic constituent (Dehority, 1993; Machinet et al., 2009). More highly substituted arabinoxylans exhibit greater resistance to degradation (Amin et al., 2014; Machinet et al., 2009). Ferulic acid forms esterified linkages between lignin and hemicelluloses (Ralph and Helm, 1993). Cross linking between lignin and hemicellulose limits enzyme access to cellulose and hemicellulose resulting in lower rates of root decomposition (Amin et al., 2014; Talbot et al., 2012).

Different plant species with varying proportions of lignin, cellulose and hemicellulose in their roots have been shown to influence both the rate and extent of root C mineralization in soil (Abiven et al., 2005; Bertrand et al., 2006; Machinet et al., 2009, 2011; Redin et al., 2014). Among a wide variety of crop plants, large differences in the amount of C mineralized and the rate of C mineralization during incubation of aerial plant components have been documented (Redin et al., 2014). In contrast to aboveground plant tissues, studies of corn root tissue decomposition have found poor correlations between root lignin, including the ratio of syringyl to guaiacyl subunits and long-term C mineralization rates (Abiven et al., 2005; Machinet et al., 2009). In corn root tissue, C mineralization rates were negatively correlated with the degree of arabinose substitution in hemicellose as well as to the ratio of arabinose to lignin (Machinet et al., 2009, 2011). Total C mineralization was also negatively correlated with the ratio of lignin:cellulose, the amount of esterified *p*-coumaric acid and the amount of hemicellulose in the root (Machinet et al., 2009). Moreover, corn root lignin and highly substituted hemicellulose persist relative to cellulose and less substituted hemicelluloses during decomposition (Machinet et al., 2009).

Given the evidence that the rate of C mineralization differs by plant species and that the C mineralization rate arises from differences in the decomposability of root cell wall constituents, it is necessary to investigate root cell wall composition prior to decomposition and compositional changes that occur during root degradation. This information is vital to understanding the cycling of root C in soil and the potential for root-derived compounds to contribute to SOM. However, as noted in White et al. (2011) there is little information on root cell wall composition for most species or knowledge of root compositional changes during decomposition. The objectives of this study were to i) quantify changes in root cell wall composition during short-term incubations of six important grain, forage and native grass species representative of widely grown crops in the US. utilizing fiber analysis techniques, and ii) characterize changes in root cell wall composition and molecular structure using diffuse reflectance infrared fourier transform spectroscopy (DRIFTS.)

2. Methods

2.1. Root samples

Alfalfa (*Medicago sativa*), soybean (*Glycine max*), corn (*Zea mays*), tall fescue (*Festuca arundinacea*), orchardgrass (*Dactylis glomerata*) and switchgrass (*Panicum virgatum*) root samples were collected during the months of September and October from research plots and production fields at the Beltsville Agricultural Research Center (BARC) in Beltsville, Maryland, USA (39.0° N, 76.9° W). At least three root samples of each species were collected from randomly selected locations within a single production field or research plot. Entire plants were collected, including roots and soil to a depth of 15 to 20 cm. Soil was gently washed from the roots with water. Root samples were dried at 60 °C and ground in a cyclone grinder to pass a 20 mesh (0.841 mm) screen. Characterization data including hemicellulose, cellulose, lignin and wax concentrations for each species are shown in Table 1 as reported by White et al. (2011).

2.2. Incubation

Triplicate ground root samples were sealed in ANKOM Technology (Macedon, NY, USA) 5.0 cm × 5.5 cm polyester/polyethylene fiber bags with a pore size of 25 μm (Adesogan, 2005). Ground roots were used to eliminate species differences in root size, root thickness or effects of root tissue architecture in order to solely assess the biochemical recalcitrance of root cell wall constituents (Lindedam et al., 2009). The root bags were buried in acid-washed coarse quartz silica sand in individual 7.6 cm × 7.9 cm vented incubation containers. Deionized water was added to each container to simulate gravimetric water content at field capacity (0.137 g g^{-1} moisture). Nitrogen was also added with the water as KNO_3 to simulate N concentrations found in subsoils (2 mg N kg^{-1}). Each incubation container was then inoculated with 2 μL of a 2:1 deionized water:soil extract using soil collected from