ABSTRACT

Title of Dissertation: CHARACTERIZATION OF PLANT ROOT CELL WALL STRUCTURAL CHANGES DURING DECOMPOSITION

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Plant roots are important contributors of organic carbon compounds for soil organic matter (SOM) formation, particularly hemicellulose, cellulose and lignin, but little is known about the composition of many species. Knowledge of compositional changes as plant roots decompose is also limited. This information is essential to understand the role of root-derived macromolecules in SOM dynamics and carbon sequestration. The paucity of available data necessitates analytical techniques to assess root composition and changes during decomposition. The objectives of this research were to evaluate diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) for assessment of root cell wall composition and to quantify and characterize changes in the cell wall composition of important crop and forage species during decomposition in 30 and 270 day incubations. Results indicate that the roots of the same species are similar despite differences in climate, soil and fertilization, while important differences were noted between roots of different species. Spectral analysis was consistent with chemical fiber analysis composition

data and revealed features that may be indicative of root suberin content. Between Day 0 and Day 30 significant ($P<0.05$) changes in alfalfa root hemicellulose, cellulose and lignin were observed as roots became enriched with lignin relative to hemicellulose and cellulose. No changes were observed in the other studied roots over this interval. In the 270 day incubation large species dependent variations were observed in the extent of root tissue decomposition. In contrast to the short term results, lignin, cellulose and hemicellulose in the roots of all studied species degraded proportionally over time. Analysis by DRIFTS supported the fiber analysis results and revealed important changes as roots decompose. Spectra illustrated changes in hemicellulose structure and potential suberin preservation in decomposing roots. These results help to increase understanding and prediction of 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 carbon stabilization and the potential for C sequestration. Variability in root composition and degradation suggest that characterization of a range of individual species is necessary to predict the soil carbon contribution from roots.

CHARACTERIZATION OF PLANT ROOT CELL WALL STRUCTURAL CHANGES DURING DECOMPOSITION

by

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Chapter 1: Introduction

Globally, the soil organic carbon (SOC) contained in soil organic matter (SOM) to the 1 m depth totals about 1550 Gt, making it the largest terrestrial C pool (Jobbágy and Jackson, 2000; Lal, 2004). Agricultural soils have lost an average of 50 to 75% of their pre-cultivation SOM with losses ranging from 30 to 60 Mg C/ha (Lal, 2007). The amount of C lost between 1850 and 2000 is estimated to be 78 Gt (Lal, 2009). Increasing SOM also leads to numerous ecosystem benefits including improved soil structure and aggregation, increased water holding capacity, increased nutrient cycling and greater soil health (Lal et al., 2009). Clearly, restoring SOM in agricultural soils would also aid in the sequestration of atmospheric C.

Subsoils contain an important and overlooked pool of SOM and represent an important potential sink for atmospheric C sequestration (Lorenz et al., 2007). The majority of SOC in agricultural soils is found below the 20 cm depth, and plant production and the proportion of above ground to below ground biomass exert a strong influence on the distribution of C (Jobbágy and Jackson, 2000). Subsoil SOM is affected by land use and management changes (Wright et al., 2007; Follet et al., 2009). The radiocarbon age of SOC increases with depth, as does the size of the stable SOM pool, suggesting that increasing this pool may be an effective means of increasing long term C sequestration (Lorenz and Lal, 2005, Lorenz and Lal, 2006).

Preservation of SOM is dependent on soil factors such as associations between organic compounds and soil minerals and protection of SOM within stable soil aggregates (Kögel-Knabner et al., 2008; Rumpel and Kögel-Knabner, 2011). Examination of soil humic substances by nuclear magnetic resonance spectroscopy

has revealed that humus is composed of a complex mixture of biopolymers (proteins, carbohydrates, lignin, and aliphatic compounds from fatty acids and waxes) of plant, microbial and faunal origin (Kelleher and Simpson, 2006). The composition of SOM is controlled by a combination of the nature and chemical composition of vegetative inputs and factors such as climate which affects microbial decomposition (Vancampenhout et al., 2009). In addition, climate change may alter the patterns of SOM retention versus mineralization. Recent research has found increased sequestration of aliphatic waxes, coupled with greater oxidation of lignin with rising soil temperatures (Feng et al., 2010). Therefore, knowledge of the chemical composition of plant residue inputs is crucial to understanding and predicting C dynamics and the composition of SOM for a given location under a specific cropping or land management system.

Plant roots are likely a significant source of C for SOM formation as root C is more likely to be stabilized relative to aboveground C sources leading to an average 2.4 times longer soil residence time (Rasse et al., 2005). Given their inherently close relationship with the soil matrix, roots are ideally positioned to contribute C for stabilization by physico-chemical protection by association with soil minerals and physical protection with soil aggregates (Rasse et al., 2005). Crop roots are an important source of SOM in agricultural soils. For example, in simulated no till soils 42% of root-derived ^{14}C remained in soil compared to 16% of above-ground residue-derived ^{14}C one year after oat harvest (Gale and Cambardella, 2000). Puget and Drinkwater (2001) found that 49% of root C from a spring vetch cover crop remained following corn harvest, compared to 13% of incorporated shoot-derived C. Though

thirteen years of stover removal resulted in a decline in total SOC, corn crop-derived ^{13}C abundance in the soil still increased (Clapp et al 2000), indicating the contributions of root-derived C. Root lignin and hemicellulose have been demonstrated to be the primary influences on long-term C accrual in managed tropical forest ecosystems (Russell et al., 2004). Root biomass is likely the major contributor to subsoil SOM but direct evidence of this connection is lacking. Root chemistry is likely the principle factor affecting root decomposition in soil (Silver and Miya, 2001). However, there is scant research characterizing the chemical structure of roots and root decomposition (Lindedam et al., 2009). Knowledge of crop root composition and conversion to SOM is essential to determine the role of agricultural systems in the terrestrial C cycle and to develop management practices that promote soil health and C sequestration.

Root cell walls are primarily composed of lignin, cellulose, hemicellulose and wax, in species-dependent proportions (Aulen et al., 2012; Machinet et al., 2009, 2011; Picon-Cochard et al., 2012; Redin et al., 2014; White et al., 2011). Macromolecules from root cell walls such as lignin and plant waxes such as suberin are likely important sources of C for SOM formation (Kögel-Knabner, 2002; Winkler et al., 2005; Otto and Simpson, 2006).

Lignin provides structural rigidity to the cell wall. Plant roots are more lignified than shoots. Root biomass averages 11% lignin compared to 5% in above ground biomass (Rasse et al., 2005). In addition, the lignin to nitrogen (N) ratio was three times greater in the roots compared to the shoots. Lignin is a complex, high molecular weight macromolecule composed of *p*-coumaryl, coniferyl and synapyl

alcohol monomers with varying degrees of methoxylation of the aromatic ring. Differences in methoxylation influence lignin susceptibility to degradation. Non-methoxylated monomers (*p*-hydroxyphenyl) are more degradable relative to dimethoxylated (guaiacyl) monomers (Bertrand et al., 2006; Jung and Vogel. 1992; Talbot et al., 2012). Numerous studies have found that lignin chemistry determines the rate of decay through its inherent structural stability and through varying degrees of cross-linking with hemicellulose (Bertrand et al., 2006; Talbot et al., 2012).

Cellulose is a polymeric polysaccharide composed of long chains of β(1→4) linked glucose monomers. It is the major structural component of the plant cell wall. Hydrogen bridges form between hydroxyl groups on adjacent cellulose molecules leading to a fibril structure (Kögel-Knabner, 2002). Cellulose is decomposed slowly by many bacteria and fungi and only persists as a small proportion of SOM (Gunnarsson and Marstorp, 2002; Kögel-Knabner, I., 2002).

Hemicellulose is a polysaccharide composed of polymerized β(1→4) linked backbone sugars (e.g. xylose) with varying degrees of branching and substitution with other sugars (e.g. arabinose, glucouronic acid, 4-*O*-methyl glucouronic acid, galactose and acetyl groups) (Hatfield, 1989; Kögel-Knabner, 2002; Scheller and Ulvskov, 2010). A greater degree of branching and substitution leads to increased resistance to degradation (Gunnarsson and Marstorp, 2002, Machinet et al., 2009). Hemicelluloses are a heterogeneous group and vary structurally by species (Scheller and Ulvskov, 2010). In grasses, the principle hemicellulose is arabinoxylose, while in legumes the polysaccharide is rhamnoxylose (Dehority, 1993; Machinet et al., 2009). The persistence of hemicellulose soil is intermediate between soluble cellular components

such as sugars and fructans and other insoluble components such as cellulose (Gunnarsson and Marstorp, 2002).

Suberin is a polyester molecule with both an aliphatic and aromatic moiety (Bernards, 2002). The suberin molecule acts as a protective barrier and serves within root tissues to regulate water and gas exchanges (Kollattukudy, 1984). The suberin macromolecule is composed of an ester linked polymer of hydroxycinnamic acids and lignin monomers embedded within the cell wall which is covalently linked to the glycerol-based polyester aliphatic domain comprised of long chain fatty acids and alcohols extending outward from the cell wall (Bernards, 2002). Though suberin should be readily decomposed, suberin-derived aliphatic compounds are preserved in soil without significant alteration (Nierop et al., 2003, Allard, 2006, Mendez-Millan et al., 2010). However, little is known about suberin preservation and degradation in decomposing roots.

Soluble non-cell wall proteins, free sugars, starch and fructans (in grasses) are rapidly decomposed in the initial days of root decomposition (Kögel-Knabner, 2002; Abiven et al., 2005; Gunnarsson et al., 2008; Machinet et al., 2011). Following this initial period, differences in the proportions of root cell wall lignin, cellulose and hemicellulose among roots has 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). For example, Redin et al. (2014) found that grass roots with high hemicellulose concentrations and low cellulose and total nitrogen concentrations degraded at a slow rate and exhibited low C mineralization over 120 days incubation.

Structural macromolecules form a complex network within the cell wall that limits access by extracellular enzymes during tissue degradation (Amin et al., 2014; Bertrand et al., 2006). The three dimensional structure of the root cell wall consists of cellulose fibrils in close association with lignin and hemicellulose (Kögel-Knabner, 2002; Scheller and Ulvskov, 2010; Amin et al., 2014). Hemicellulose links the cellulose fibrils both by being physically trapped within the fibril network during cell wall formation and by hydrogen bonding (Scheller and Ulvskov, 2010). Lignin is bound to cellulose via hydrogen bonds (Kögel-Knabner, 2002). In addition, ferulic acid forms esterified linkages between lignin and hemicellulose (Ralph and Helm, 1993; Amin et al., 2014).

The interrelationship between root cell wall constituents are important factors controlling decomposition. For instance, lignin protects cellulose and hemicellulose from decay while cellulose decomposition in turn facilitates lignin degradation (Melillo et al., 1982; Bertrand et al., 2006; Talbot and Treseder, 2012). In addition, the degree of hemicellulose substitution and extent of crosslinking to lignin has been shown to be an important factor controlling root decomposition. More highly substituted arabinoxylans exhibit greater resistance to degradation and the structure of hemicellulose as do those with greater degrees of ferulic acid crosslinking with lignin (Amin et al., 2014; Machinet et al., 2009; Talbot et al., 2012). For example, C mineralization rate in corn root tissue were negatively correlated with increases in hemicellulose concentration, arabinose substitution in hemicellulose as well as the ratios of arabinose:lignin, lignin:cellulose (Machinet et al., 2009; 2011). Root lignin and highly substituted hemicellulose persists relative to cellulose and less substituted

hemicellulose during decomposition (Machinet et al., 2009). These factors influence enzyme activity and make cellulose and hemicellulose more resistant to enzymatic hydrolysis and oxidation (Amin et al., 2014).

Given the importance of differences in cell wall composition on the rate and extent of root decomposition characterizing root composition requires analytical tools capable of accurately assessing the composition of these sources of SOM. These techniques will facilitate the large scale assessments of root composition and the formation of SOM that will be necessary for validation of C sequestration in soils. Infrared spectroscopy has been well established as a method for the characterization of forages, SOM, humic acids, root anatomy and differences in fatty acid composition related to mycorrhizal colonization (Reeves, 1993; McCarty et al., 2002; Rumpel et al., 2001; Zeier and Schreiber, 1999; Dokken and Davis, 2007; Quénéa et al., 2005; Sarkhot et al., 2007; Calderon et al., 2009). Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) is an ideal method for the analysis of biomass samples which would otherwise require time-consuming laboratory assays. A single spectrum can reveal multiple details about the structural characteristics of a sample and can be used to reveal compositional data once appropriate calibrations have been developed. However, DRIFTS has not yet been used to characterize the cell wall composition of whole root samples.

The objectives of this study are to i) compare the DRIFTS spectra of roots of different species using whole root samples and root fiber fractions and to identify spectral features indicative of important root macromolecules ii) quantify changes in root cell wall composition of important grain, forage and native grass species

representative of widely grown crops during both the initial and longer term stages of root degradation, iii) characterize changes in root cell wall composition and molecular structure using DRIFTS.

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Chapter 2: Mid-infrared diffuse reflectance spectroscopy for the rapid analysis of plant root composition

ABSTRACT

Roots are an important contributor of recalcitrant organic carbon compounds for soil organic matter formation, but little is known about the composition of many species. There is a need for additional techniques capable of assessing significant, but often overlooked, carbon sinks such as roots. Diffuse reflectance infrared spectroscopy (DRIFTS) has great potential for the analysis and characterization of plant root composition. The objectives of this research were to compare the DRIFTS spectra of roots of different species using whole root samples and root fiber fractions and to identify spectral features indicative of important root macromolecules in order to evaluate the potential of DRIFTS to determine root composition. A wide variety of roots from agronomic and horticultural crops, ornamental plants, and native plants were collected and analyzed by DRIFTS. Samples were ground in a cyclone grinder to pass a 20 mesh screen and scanned without KBr dilution from 400 to 4000 cm^{-1} using KBr as the background. In addition, traditional fiber analysis of a subset of roots and DRIFTS analysis of the resulting fiber fractions (neutral detergent fiber, acid detergent fiber, hemicellulose, cellulose, lignin and wax) were utilized to identify spectral features associated with those fractions. Results indicate that the roots of the same species are similar despite differences in climate, soil and fertilization, while important differences were noted between roots of different species. Tree root lignins appeared to be similar to their above ground counterparts based on comparison with published data. Root lignins for all studied species varied by species. Spectral analysis was consistent with chemical fiber analysis composition data and revealed

features that may be indicative of root suberin content. Further research is necessary to confirm that these features are related to suberin. Overall, the results of this research demonstrate the potential of DRIFTS for the characterization of plant root composition and as a tool to screen large numbers of samples for more effective utilization of more time-consuming analytical procedures.

INTRODUCTION

The need to make swift progress in advancing our understanding of the terrestrial carbon (C) cycle and soil C sequestration requires analytical tools capable of accurately assessing the composition of the sources of soil organic matter (SOM). Roots are an important source of recalcitrant macromolecules for SOM formation. For example, in simulated no till soils 42% of root-derived ^{14}C remained in soil compared to 16% of residue-derived ^{14}C, with only 11% residue-derived ^{14}C remaining on the soil surface 1 year after oat harvest (Gale and Cambardella, 2000). In field experiments 49% of root C from an *in situ* ^{13}C-labeled spring vetch cover crop remained following corn harvest at the end of the season, compared to 13% of incorporated shoot-derived C which the authors attributed in part to greater biochemical recalcitrance of root litter (Puget and Drinkwater, 2001). Chemical recalcitrance of root compounds may be a significant contributor to slower mineralization, leading to an average 2.4 times longer soil residence of root C as compared to shoot C (Rasse et al., 2005). Root lignin and hemicellulose were the primary influences on long-term C accrual in managed tropical forest ecosystems and was related to species effects on C sequestration (Russell et al., 2004). Yet there is virtually no information available about the composition of many species. Knowledge

of the properties of root biomass is crucial to understanding the role of roots in SOM formation and is essential for developing management practices, new crops and crop plants that maximize inputs to stable SOM. Chemolysis and analytical pyrolysis are the primary methods currently used to assess the composition of plant materials and SOM. Chemolysis involves the chemical decomposition of complex organic substances to yield simpler component molecular structures for analysis. Similarly, pyrolysis utilizes heat in the absence of oxygen to thermally decompose organic substances prior to analysis. Unfortunately, these methods are laborious which severely limits the number of samples that can be analyzed. More rapid techniques capable of characterizing thousands of samples will facilitate larger scale assessments of SOM and are essential for validation of C sequestration in soils. Infrared spectroscopy has been used for decades in the analysis of biomass and soils including the characterization of forages, SOM and humic acids, and root anatomy (Reeves, 1993; McCarty et al., 2002; Rumpel et al., 2001; Zeier and Schreiber, 1999; Dokken and Davis, 2007; Quénéa et al., 2005; Sarkhot et al., 2007). In particular, diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) is perfect for biomass and soil samples which may require time consuming, expensive and laborious assays, as the need for further conventional analysis is potentially reduced by 90% or more. The method has been successfully applied to assess differences in root fatty acid composition related to mycorrhizal colonization (Calderon et al., 2009). Furthermore, a single spectra can reveal multiple parameters for which appropriate calibrations have been developed. However, DRIFTS has not been used to characterize the lignin, cellulose, hemicellulose and suberin composition of whole root samples and research

is needed to assess its capability to distinguish the root components most likely to contribute to stable SOM and C sequestration.

Recalcitrant root-derived macromolecules (i.e., lignin and suberin) are likely to be important contributors to stable SOM (Kögel-Knabner, 2002, Winkler et al., 2005, Otto and Simpson, 2006). Recalcitrant compounds are concentrated during biodegradation and may constitute as much as 50% of the stable, long-term SOM pool (Krull et al., 2003; Falloon and Smith, 2000). Recent nuclear magnetic resonance (NMR) research suggests that soil humic substances may be a complex macromolecular mixture of plant, microbial and faunal origin and their degradation products, rather than a distinct chemical category (Kelleher and Simpson, 2006). The authors examined an International Humic Substances Society Florida peat humic acid (HA) standard as well as HA extracted from a grassland and forest soil in comparison to protein, lignin, carbohydrate, and cutin standards. The predominant contributors to the studied humic acids were proteins, carbohydrates, lignin, and aliphatic biopolymers from fatty acids and waxes; a large percentage of which still had structures derived from their parent compounds.

The poly-phenolic structure of lignin likely makes it resistant to microbial attack. Rasse et al. (2005) report more lignin in roots than in above ground biomass, with root lignin contents averaging 11% compared to 5% in above ground biomass. The lignin to N ratio of the roots was three times greater than that of the shoots, suggesting greater resistance to degradation. It should be noted that standard methods for lignin analysis may overestimate lignin content due to the presence of other compounds within the lignin residue, such as waxes (Preston et al., 2006). For

example, in an analysis of leaf and fine root litter of *Prestoea montana* and *Dacryodes excelsa*, two subtropical tree species, wet chemical fiber analysis results indicated root lignin concentrations averaging 28% compared to 14% in the leaves (Galletti et al., 1993). However, analytical pyrolysis and DRIFTS data revealed a lower total lignin content and a high proportion of material that was determined to be lignin but could not be ascribed to lignin phenolics. The high root lignin concentrations reported in the literature may be due in part to the presence of suberin in root tissues.

Suberin, a waxy component of endodermal cell walls, is composed of both aliphatic and phenolic domains, with differing proportions of aliphatic components among plant species (Bernards, 2002). The aliphatic moiety is generally described as a glycerol-based polyester compound composed of long chain saturated alkanoic acids, α,ω-alkanedioic acids, ω-hydroxy acids, alkanols (chain lengths ≥C20) and also includes some esterified hydroxycinnamic acids (Bernards, 2002). The phenolic moiety is likely similar to lignin with a polymeric network derived from the p-coumaryl, coniferyl, and sinapyl alcohols but also from hydroxycinnamic acids and their derivatives (Bernards, 2002). Suberin is preserved in soil environments and has been found to be a significant contributor to SOM (Bull et al., 2000; Nierop et al., 2003, Nierop and Verstraten, 2003), for example, comprising between 17 to 47% of SOM in grassland subsoils (Feng and Simpson, 2007).

Clearly in order to accurately assess root contributions to SOM it is necessary to differentiate the lignin and suberin components of root biomass, as well as other important constituents such as cellulose and hemicellulose. The objectives of this

research were to compare the DRIFTS spectra of roots of different species using whole root samples and root fiber fractions and to identify spectral features indicative of important root macromolecules in order to evaluate the potential of DRIFTS to determine root composition.

METHODS

Root samples

Root samples were collected in September and October from a variety of sources. This timing was chosen to be close to onset of annual plant senescence or perennial plant dormancy. Agronomic grain (corn, soybean) and forage (alfalfa, orchardgrass, fescue, switchgrass) crop roots were collected from production fields at the Beltsville Agricultural Research Center (BARC) in Beltsville, MD. Three root samples of each species used for spectral and fiber analysis were collected from three randomly selected locations within a single field or research plot. The switchgrass root samples were collected from research plots at BARC which had received two different rates of nitrogen fertilization at 35 kg N ha^{-1} and 235 kg N ha^{-1}. Horticultural crop (tomato, lettuce) roots were collected from home gardens representing different soil and climatic conditions in central and western Maryland, USA. Tree roots (white pine, white oak) were collected from small trees (30 – 50 cm height) growing in a single brushy, overgrown location. For most samples entire plants were dug, removing roots and soil to a depth of 15 to 20 cm. However, some tomato plants were collected by simply pulling the plant from the soil by its stem. This allowed for some assessment of the impact of sample collection methods on the resulting spectra. All root samples were composed of at least three plants. For some species additional

plants were collected to ensure sufficient root material for analysis. Soil was gently washed from the roots and subsamples were divided into fine (<1 mm) and coarse (>1 mm) fractions. Samples were dried at 60˚ C and ground in a cyclone grinder to pass a 20 mesh screen prior to analysis.

Spectroscopy

All mid-IR spectra were obtained using DRIFTS on a Digilab FTS-7000 Fourier Transform Spectrometer (Digilab/Varian/Agilent, Santa Clara, CA, USA) equipped with a glow bar source, KBr beam splitter and Peltier cooled DTGS detector. Spectra were obtained from 4000 to 400 cm^{-1} at 4 cm^{-1} resolution using a Pike Autodiff autosampler (Pike Technologies, Watertown, WI) with KBr as the background and each sample scanned as ground non-KBr diluted material. Each spectrum consists of 64 co-added, background corrected scans.

Spectral analysis

Spectra were compared and analyzed using irAnalyze ver. 3.0.19.0 (LabCognition, Analytical Software GmbH & Co, KG) or GRAMS/AI ver. 8.0 (Thermo Electron Corp., Waltham, MA, USA).

Fiber Analysis

Fiber analysis was carried out in triplicate in sealed bags using an Ankom Technology A200 Fiber Unit (Macedon, NY, USA). Samples were analyzed sequentially for neutral detergent fiber (NDF) and acid detergent fiber (ADF) using the A200. The NDF was determined gravimetrically following extraction under pressure at 100 ˚C for 75 min. in a solution containing 0.1M sodium lauryl sulfate +

0.05M Disodium EDTA + 0.02M sodium borate decahydrate + 0.03M Na_2HPO_4 + 0.07M triethylene glycol, then the ADF was similarly determined following extraction under pressure at 100 ˚C for 60 min. in 0.5M H_2SO_4 + 0.05M cetyl trimethyl ammonium bromide. Further information on the NDF and ADF methods used may be found at http://www.ankom.com/analytical-procedures.aspx. The ADF was then analyzed for permanganate lignin. In addition, samples were analyzed for cellulose by treatment of the permanganate lignin residue with 72% H_2SO_4. Both permanganate lignin and cellulose was determined in beakers using the permanganate method outlined by Goering and Van Soest (1970) and Van Soest (1994), but using the fiber bags as opposed to crucibles as outlined in the original method. Briefly, following NDF and ADF extraction with the Ankom unit, the bags were placed in a large beaker and covered with a permanganate solution [0.21M $KMnO_4$ + 1.1 x 10^{-4}M Ag_2SO_4 + 0.005M $Fe(NO_3)_3 \cdot 9\ H_2O$ + 3.0 x 10^{-4}M $AgNO_3$ + 2.9M $C_2H_4O_2$ + 0.017M CH_3CO_2K + 1.4M $(CH_3)_3COH$]. A smaller beaker was placed inside the large beaker to keep the bags submerged. The samples were then placed on an orbital shaker and allowed to react for 90 min. Samples were also stirred by hand to turn and mix the bags every 30 min. The H_2SO_4 extraction was conducted similarly using a 72% by weight solution for 180 min, but the beakers were not placed on the orbital shaker. The residue remaining following 72% H_2SO_4 extraction is composed of waxes and minerals. In roots this wax may be suberin, though additional research is necessary to definitively confirm its composition; which was quantified by subtracting the weight of the ash remaining following sample combustion at 550 ˚C.

Differences in fiber fractions were tested using ANOVA and means separations were performed by LSD at the P<0.05 level of significance.

RESULTS and DISCUSSION

Comparison of spectra

As shown in Figure 1, the DRIFTS spectra of switchgrass taken from two plots are virtually identical, indicating that the composition of the roots of different plants of the same species may well be characteristic of that species. This agrees with

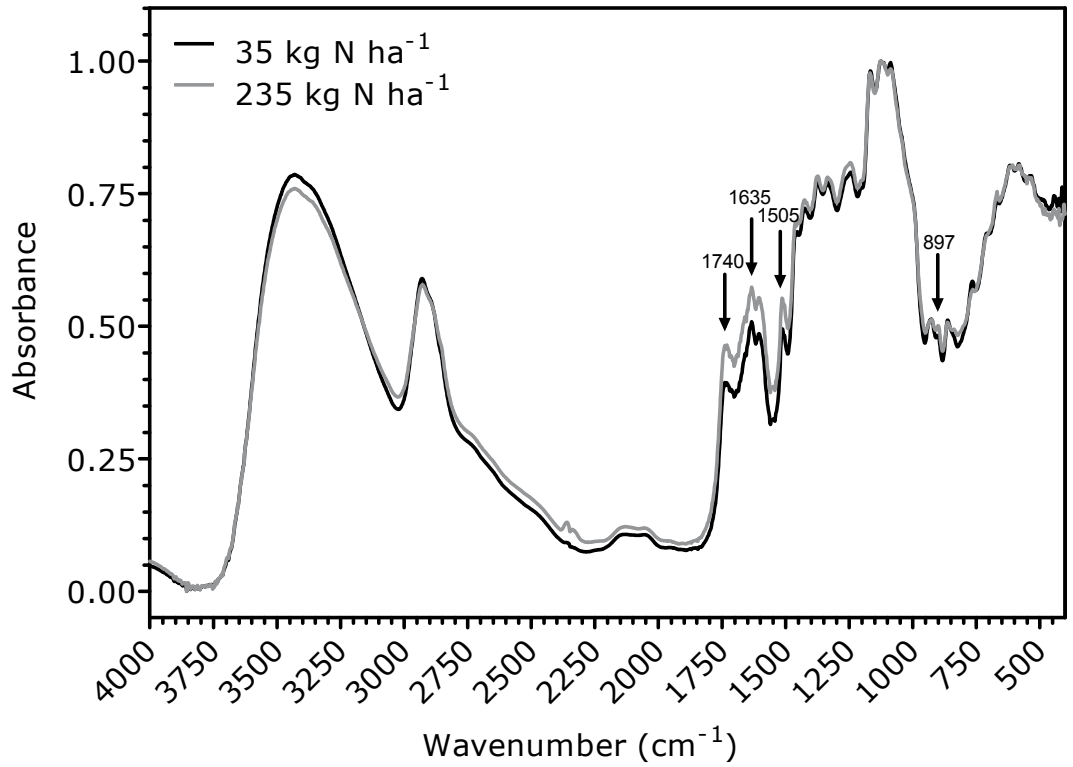

Figure 1: Mid-infrared spectra of switchgrass roots collected from two field research plots which received nitrogen fertilizer at a rate of either 35 kg N ha^{-1} or 235 kg N ha^{-1} (Spectra normalized from 0 to 1).

decades of research into the fiber composition of above ground biomass. Further, as these plots received very different rates of N fertilizer (35 kg ha^{-1} vs. 235 kg ha^{-1}) it

appears that differences in crop management do not influence switchgrass root composition. The differences absorption seen in the peaks between 1750 cm^{-1} and 1500 cm^{-1} do not represent differences in qualitative composition but rather are likely due to increased concentrations of protein and lignin (Reeves, 1993; Pandey, 1999; Dokken and Davis, 2007) with increased fertilization. Similarity in root composition within a species grown under differing conditions is also evident in tomato root samples obtained from two different gardens by two very different means (Figure 2). For one sample ("Pulled"), the roots were obtained from a drought

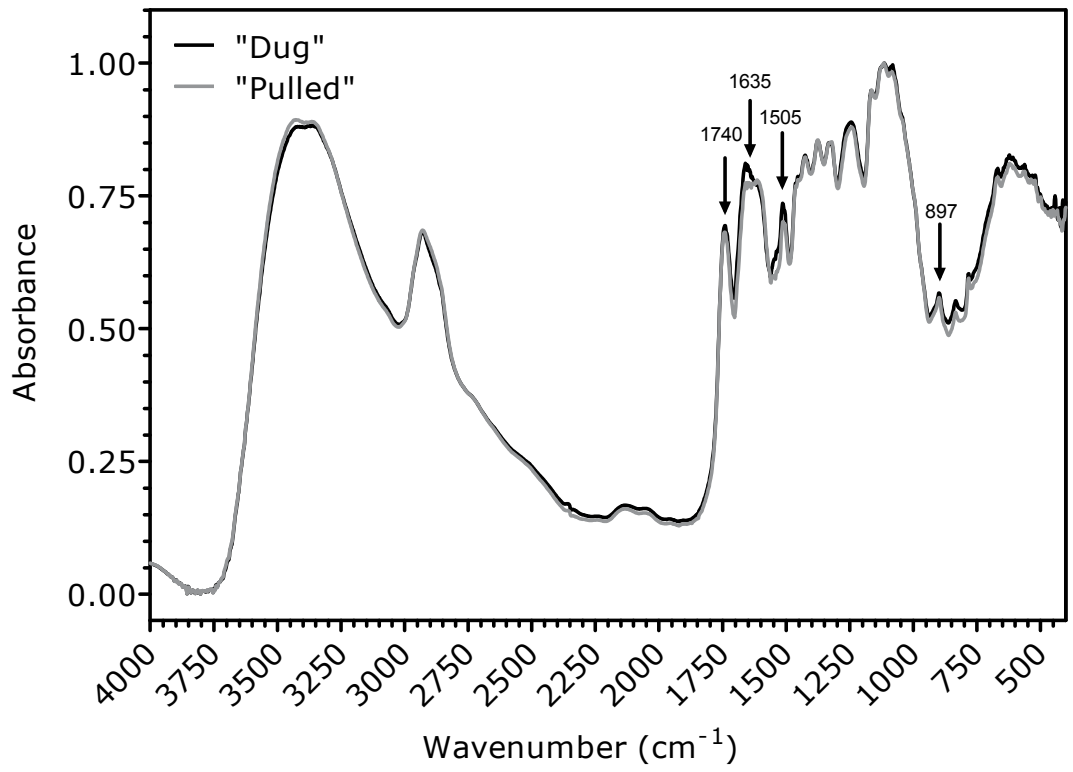

Figure 2: Mid-infrared spectra of tomato roots harvested either by careful digging ("Dug") or hand pulling ("Pulled") (Spectra normalized from 0 to 1).

stricken plant simply pulled from the soil and retaining few, if any, fine roots. The other sample ("Dug") was obtained from a healthy plant by carefully digging up the

plant and washing the soil from the roots. Comparison of Figure 1 and Figure 2 reveals that the roots from these two species are very different in many important areas of the spectra. Differences at 1740 cm^{-1} (carbonyls) (Reeves, 1993; Séné et al., 1994; Pandey, 1999; Dokken and Davis, 2007) as well as 1635 cm^{-1} and 1505 cm^{-1} (lignin) (Reeves, 1993; Pandey, 1999; Dokken and Davis, 2007) indicate that the lignin in tomato roots is of a very different composition and probably present at a much lower concentration than in the switchgrass roots. In contrast, the peak at 897 cm^{-1} (Reeves, 1993; Pandey, 1999; Séné et al., 1994) is known to come from the α1-4 linkage in cellulose indicating that tomato roots contain more cellulose relative to switchgrass roots. The obvious question this data raises is, "What is the macromolecular composition of plant roots?" For example, in the tomato root spectra the similar bands at 1100 to 1200 cm^{-1} indicate the presence of carbohydrates (Séné et al., 1994; Dokken and Davis, 2007), but the spectra do not provide clear evidence of their structure. For instance, pectins have high carbonyl content, however, further support for its presence, such as peaks in the region of 1445 cm^{-1} to 1460 cm^{-1} and 854 cm^{-1} (Séné et al., 1994, Dokken and Davis, 2007), is not apparent. Hemicelluloses come in many forms and may be the source for the spectrum, but further work will be needed to identify the structural characteristics of root macromolecules. The objective of this effort was not to delineate all the differences in roots or to determine absolute composition, but to evaluate the capability of DRIFTS as a tool to identify important root components. Even these few spectral plots indicate that DRIFTS has tremendous potential for analysis of root samples and greatly increase our knowledge of root composition. Using DRIFTS as the starting point,

samples can be screened and selected for further detailed chemical analysis, increasing the information gained from time consuming standard analytical methods.

Lignin type

As can be easily seen in Figure 3, softwood lignins in the spectrum of white pine (guaiacyl lignin) produce a peak at 1510 cm^{-1} while the lignin peak is shifted to

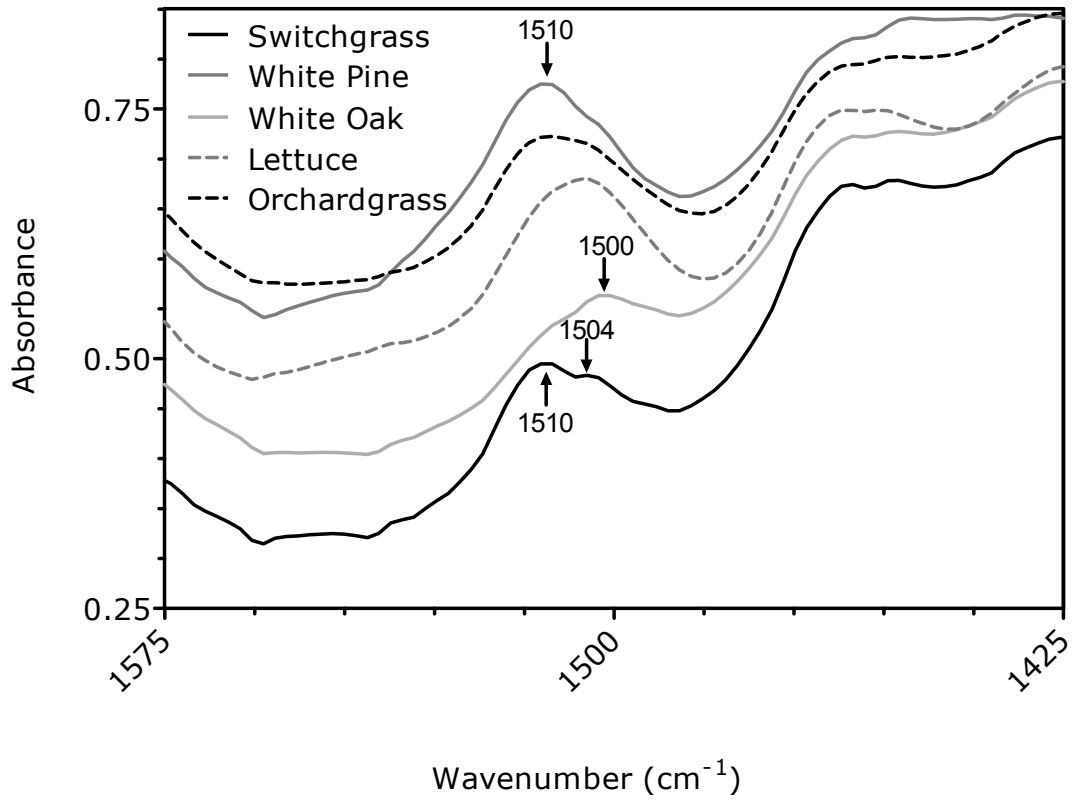

Figure 3: Partial mid-infrared spectra of switchgrass, lettuce, orchardgrass, white pine, and white oak roots (Spectra normalized from 0 to 1).

1500 cm^{-1} in the white oak sample indicating hardwood lignins (guaiacyl-syringyl lignin) consistent with the lignins in their respective above ground biomass (Pandey, 1999). Examination of a variety of grasses (corn, fescue, switchgrass), alfalfa, horticultural plants (lettuce, tomato, pumpkin) and other roots (cactus) did not find any which seem to be predominantly hardwood type in nature, although those from

orchardgrass appear to be close (Figure 3). As illustrated in Figure 3 most of the roots examined appear to contain a combination of the two lignin types. For example, the lettuce root spectrum features one large peak between 1500 cm^{-1} and 1510 cm^{-1} indicating no predominance of one type of lignin over the other. In comparison, the switchgrass root spectrum indicates a shift in the ratio between lignin types with a peak at both 1510 cm^{-1} and a shoulder peak at 1504 cm^{-1}. Although future analysis using pyrolysis-gas-chromatography mass spectrometry is planned to further elucidate the differences in lignin composition of roots, the potential of DRIFTS to reveal important differences in root composition is clear.

Fine versus coarse roots

Roots vary in size from fine (<2 mm) roots which are responsible for taking up nutrients from the soil (Jackson et al., 1997, Zobel et al., 2007) to coarse roots which provide the framework for root system architecture and transport water and nutrients to the above ground plant (McCully, 1999). In Figure 4A, the spectra of fine and coarse roots for non-BT hybrid corn are shown. While there is a difference apparent at 1655 cm^{-1} (amides)(Séné et al., 1994; Dokken and Davis, 2007), overall the two spectra are remarkably similar. This would seem to indicate that plant roots have largely the same composition regardless of size. Given that roots uniformly contain the same cellular components and structures (Raven, 1999; Raven and Edwards, 2001) it seems reasonable that root composition is similar regardless of size. If substantiated by further efforts, this would simplify root compositional analysis because coarse roots are much easier to collect than fine roots, which are

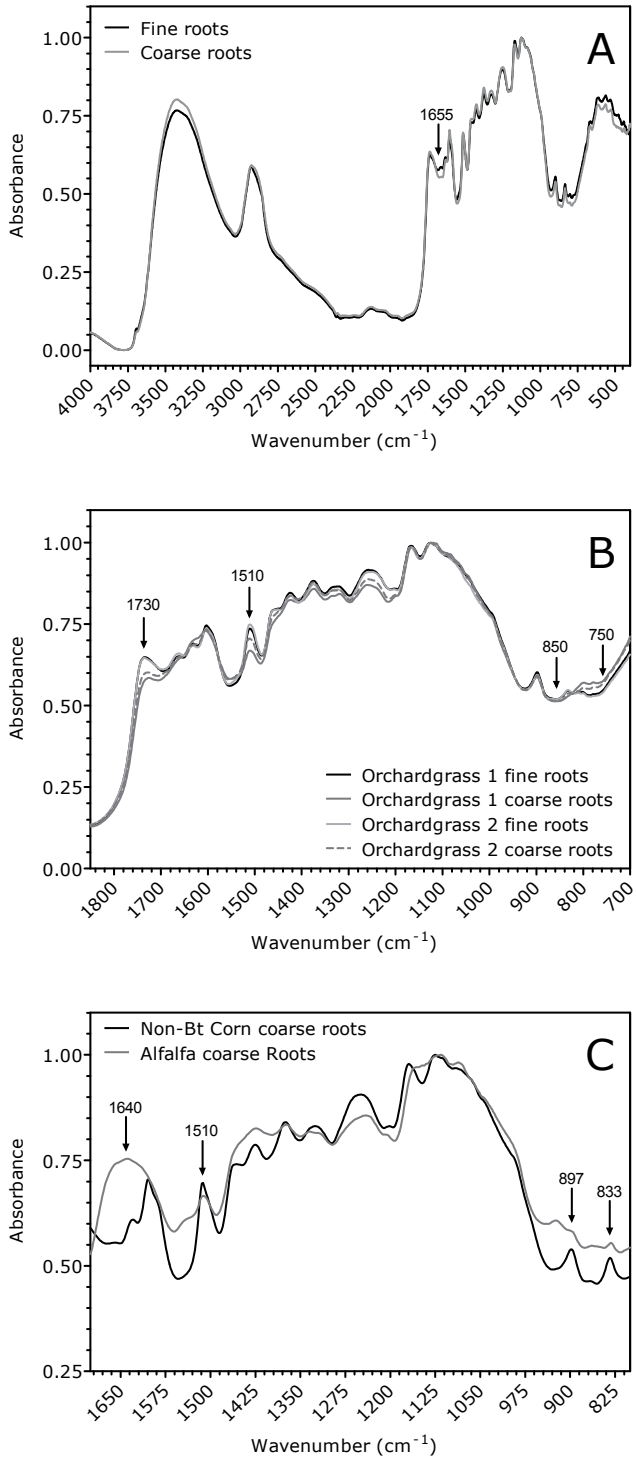

Figure 4: Mid-infrared spectra of fine and coarse non-Bt corn roots (A), partial mid-infrared spectra of two samples of fine and coarse orchardgrass roots (B), and partial mid-infrared spectra of coarse alfalfa and non-Bt corn roots (C) (Spectra normalized from 0 to 1).

difficult to separate from the soil. Extremely fine roots may be as small as 25 μm in diameter (McCully, 1999) requiring magnification for truly accurate sampling.

On the other hand, as demonstrated in Figure 4B the fine and coarse roots collected from two different samples of orchardgrass roots appear to differ in composition with more carbonyls (1730 cm^{-1}) (Reeves, 1993; Séné et al., 1994; Pandey, 1999; Dokken and Davis, 2007) and more or different lignin composition (1510 cm^{-1}) in the fine roots than in the coarse. Distinctive differences were also found in the spectral region between 850 cm^{-1} and 750 cm^{-1}. Similar features have been observed in lignin and biochars indicating multi-substituted aromatics (unpublished data). This supports the conclusion that orchardgrass fine and coarse roots have different lignin composition. This in turn could affect root decomposition and subsequent contributions to soil C pools. Examination of roots from tall fescue, another forage grass, revealed no clear trends in the spectra of coarse and fine roots, while for alfalfa roots, a legume, changes at 1730 cm^{-1} and 1510 cm^{-1} mimic the results seen in orchardgrass (data not shown). For some species differences in fine and coarse root composition may necessitate size separation to assess the total contribution of its roots to SOM. These results further demonstrate the potential for DRIFTS as a screening tool to identify species requiring more extensive sampling and analysis to determine important root compositional differences.

Above ground biomass versus root composition

While above ground biomass was not collected along with the roots, some comparisons can be made based on previous compositional analysis of above ground plant biomass of the same species. For example, alfalfa is known to produce high

quality, high protein hay, while the biomass of corn is basically a highly lignified cellulosic material (Reeves, 1987). Figure 4C illustrates the spectra for coarse alfalfa and corn roots. As can be clearly seen, the alfalfa spectrum has a large band at 1640 cm^{-1} (Séné et al., 1994; Dokken and Davis, 2007) due to protein amides which is not seen in corn roots. Also, differences in the bands at 1510 cm^{-1} and 833 cm^{-1} indicate more lignin (Pandey, 1999; Dokken and Davis, 2007) in corn roots relative to alfalfa roots. Further, the band at 897 cm^{-1} reveals relatively more cellulose in corn compared to alfalfa roots. These features are all consistent with known compositional data for the above ground biomass for the two species. Further research is needed comparing the composition of above ground and below ground biomass. If similarities are found in the composition of the roots and above ground biomass for agricultural crops, then data from decades of work on crop biomass and the ratio of above ground biomass to below ground biomass could be used to estimate the potential for root lignin contributions to soil, improving efforts to model C cycling in agricultural systems.

Spectroscopy versus fiber analysis

The results of root fiber analysis are consistent with the composition indicated by spectral analysis (Table 1). For example, corn roots contain 55%, 47%, and 45% more NDF, ADF, and cellulose, respectively than alfalfa roots. The lower overall fiber (NDF) for alfalfa indicate the presence of soluble proteins and other materials consistent with the data shown in Figure 4C.

Further agreement between spectral and chemical analysis can be seen in comparison of suberin content. Soybean roots contain 50.5 mg g^{-1} suberin, the highest concentration,

Table 1: Results of preliminary sequential fiber extraction and total carbon (C) and nitrogen (N) analysis of roots from different species. Numbers in the same column with the same letter are not significantly different by LSD (0.05). NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber.

Species	NDF	ADF	Hemi-cellulose	Lignin	Cellulose	Wax	Total N	Total C	C:N
	----------	----------	----------	mg g^{-1}	----------	----------	----------	----------	
Corn	679^{b}	399^{b}	279^{b}	42.1^{c}	301^{b}	23.3^{bc}	6.53^{de}	389^{c}	59.5
Switchgrass	468^{c}	272^{c}	197^{c}	59.1^{b}	198^{c}	12.8^{bc}	4.23^{e}	434^{a}	103
Soybean	732^{a}	561^{a}	170^{c}	82.5^{a}	406^{a}	50.5^{a}	14.2^{b}	430^{a}	30.2
Orchardgrass	729^{a}	400^{b}	329^{a}	62.3^{b}	291^{b}	36.5^{ab}	12.9^{b}	427^{a}	33.1
Fescue	651^{b}	389^{b}	262^{b}	55.5^{bc}	285^{b}	22.4^{bc}	8.21^{d}	412^{b}	50.1
Alfalfa	308^{d}	213^{d}	94.9^{d}	42.7^{c}	165^{d}	6.47^{c}	23.1^{a}	432^{a}	18.7

while alfalfa roots contain the least at only 6.47 mg g^{-1} (Table 1). As can be seen in Figure 5, the peak due to C-H between 2800 cm^{-1} and 3020 cm^{-1} (Pandey, 1999; Dokken and Davis, 2007) in the soybean root spectrum is larger relative to the valley at 3030 cm^{-1} and is broader than the alfalfa root spectrum possibly indicating the aliphatic component of suberin. In addition, the shoulder seen in the alfalfa root spectrum at 2890 cm^{-1} is absent in the soybean root. The spectra of switchgrass (Figure 1) (another root with relatively low suberin content) and orchardgrass (which has an intermediate suberin content) further support the conclusion that changes in the shape and absorbance of the peak between 2800 cm^{-1} and 3020 cm^{-1} may be due to the C-H groups of suberin. However, one should note that lipids and other root components can also contribute to this peak. Examination of this region reveals that switchgrass roots resemble alfalfa roots while the orchardgrass root spectrum resembles that of soybean roots. However, alkyl C-H groups come from many sources, including lignin. Further work, including the isolation and characterization of suberins for spectral identification, is needed before this feature can be positively linked to the molecule. Further spectral evidence for the difference in suberin content between roots of different species can be seen in the peak at 1740 cm^{-1} which is much larger for soybean roots relative to alfalfa roots (Figure 5). Numerous authors have attributed this peak to carbonyl stretching in esters

such as those found in suberin (Séné et al., 1994; Zeier and Schreiber., 1999; Schreiber et al., 2005; Dokken and Davis, 2007).

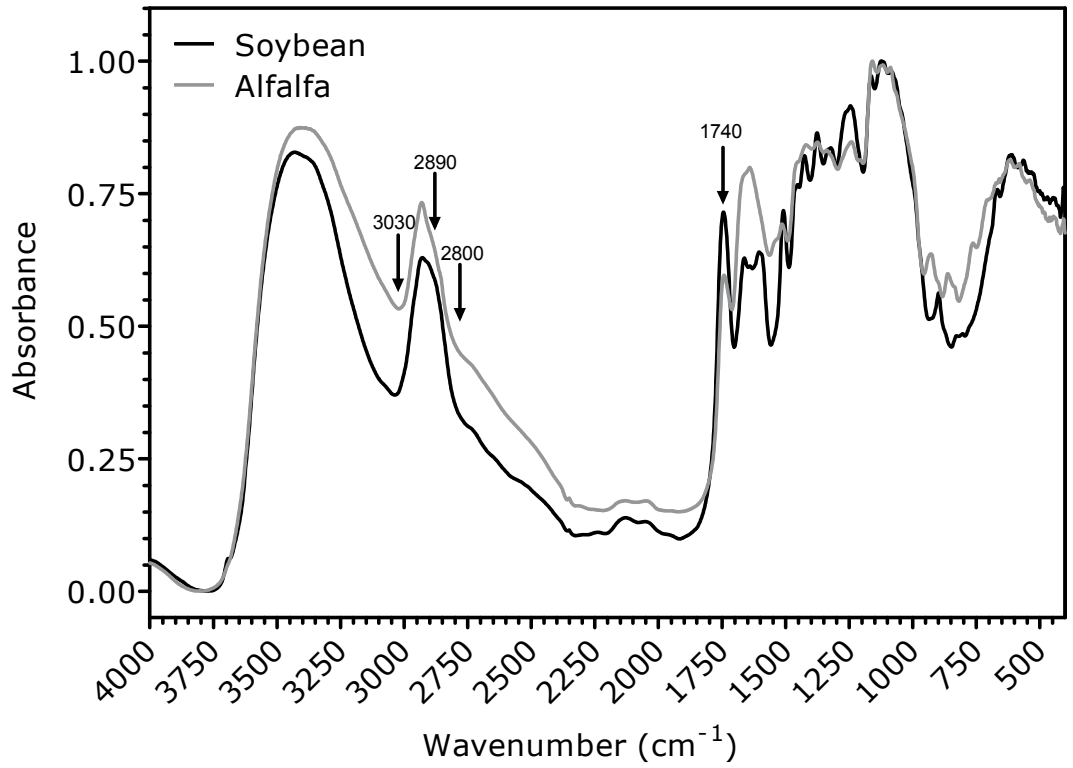

Figure 5: Mid-infrared spectra of alfalfa and soybean roots (Spectra normalized to between 0 and 1).

Spectral analysis of isolated fiber fractions

The primary change in soybean roots due to NDF extraction to remove soluble cell compounds was a reduction in the peak at 1660 cm^{-1} due to removal of protein amides (Figure 6) (Pandey, 1999; Dokken and Davis, 2007). Following ADF extraction further reduction in the peaks at 1660 cm^{-1} and the peak at 1740 cm^{-1} is due to a reduction in the acid carbonyls of hemicelluloses. The fiber remaining following this extraction consists primarily of cellulose, lignin and waxes (suberin in below ground biomass). Permanganate extraction to remove lignin resulted in a large

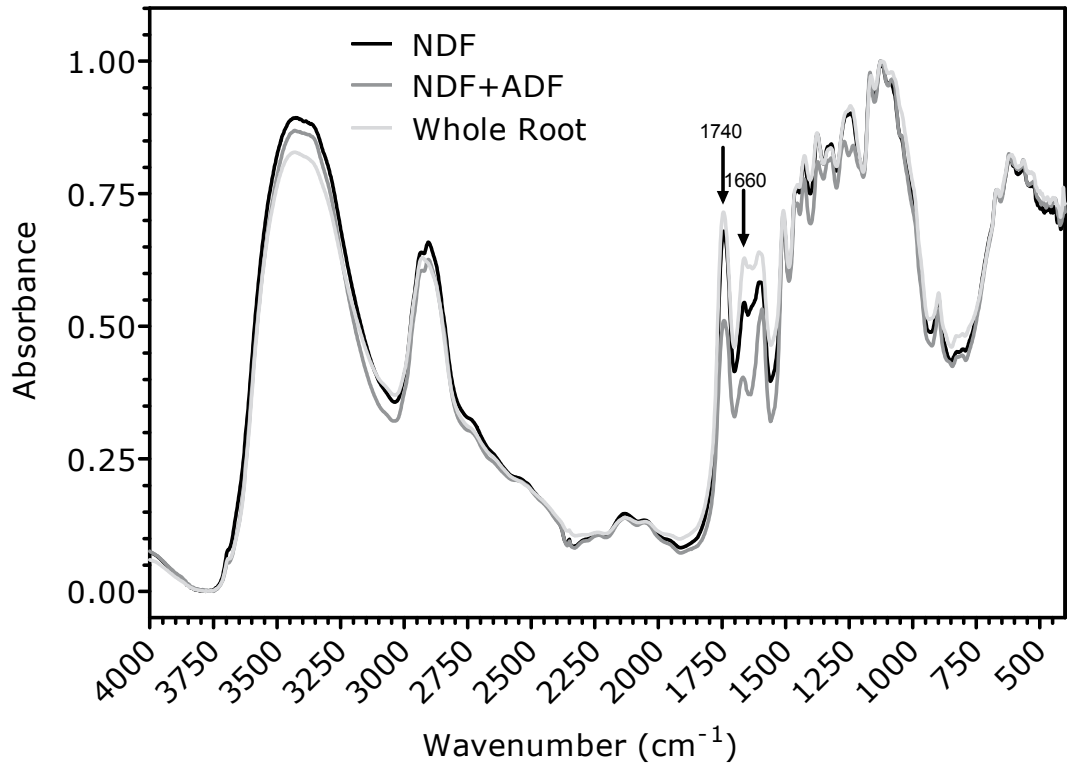

Figure 6: Mid-infrared spectra of whole soybean roots and the neutral detergent fiber (NDF) and acid detergent fiber (NDF+ADF) derived by sequential fiber extraction of soybean roots (Spectra normalized to between 0 and 1).

relative decrease in the diagnostic lignin peak at 1506 cm^{-1} (Figure 7). Finally, extraction with 72% H_2SO_4 removed the cellulose presumably revealing the spectra of the waxy materials (Figure 7). As in the whole root spectrum, in the spectra of soybean root ADF before and after extraction with permanganate and 72% H_2SO_4 (Figure 7) the C-H peak between 3000 cm^{-1} and 2800 cm^{-1} is consistent with the hydrocarbons of waxes. In addition, the small peak remaining at 1506 cm^{-1} and the peak at 1740 cm^{-1} may indicate the aromatic moieties and carbonyl groups, respectively, known to be present in suberin (Zeier and Schreiber, 1999; Bernards, 2002).

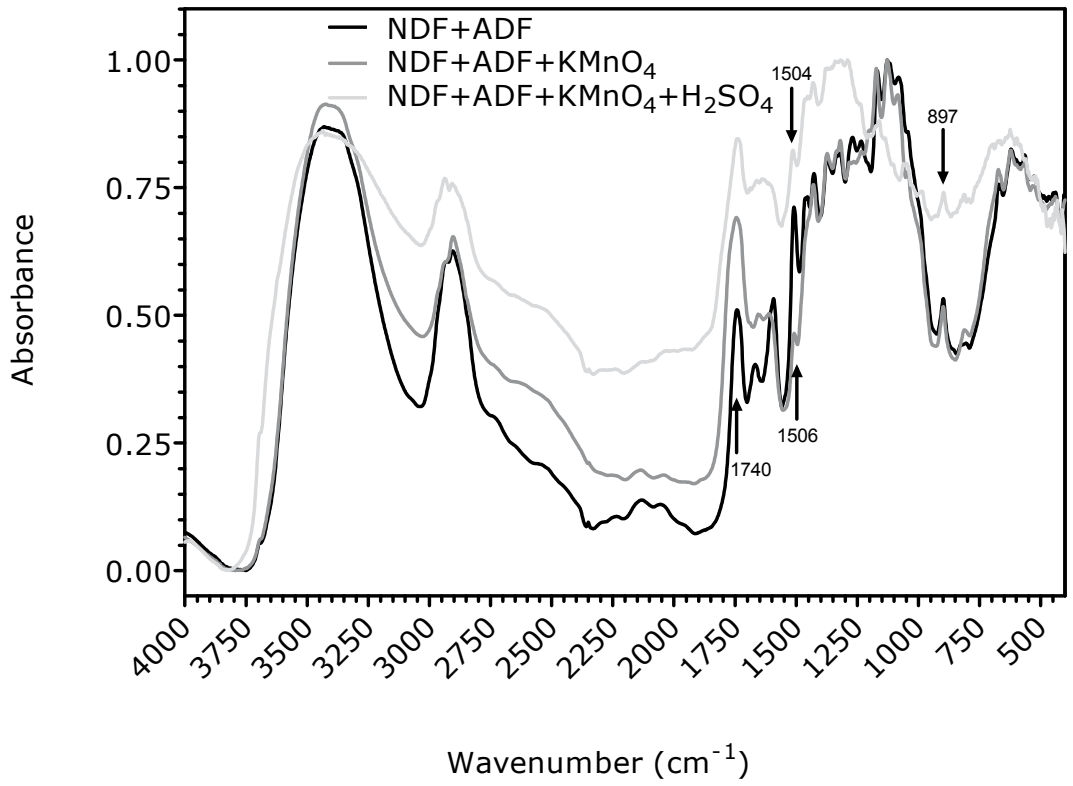

Figure 7: Mid-infrared spectra of soybean acid detergent fiber (NDF+ADF) before and after extraction with permanganate (NDF+ADF+$KMnO_4$) followed by 72% sulfuric acid (NDF+ADF+$KMnO_4$+H_2SO_4) (Spectra normalized to between 0 and 1).

Further examination of the collected spectra suggests that the fiber extraction procedure failed to remove all of the cellulose from some samples. While the extraction procedure utilized methods well established for forage analysis, when applied to roots incomplete extractions were observed. This is revealed by the presence of a small peak at 897 cm^{-1} in the spectra of the soybean fiber fraction remaining following 72% H_2SO_4 extraction, indicating the presence of residual cellulose (Figure 7). Incomplete cellulose extraction was readily apparent in one orchardgrass root replicate during fiber extraction (Figure 8). The final fiber fraction appeared predominately white and resembled thick paper, clearly indicating incomplete cellulose extraction by the 72% H_2SO_4 reagent. Further, many similarities

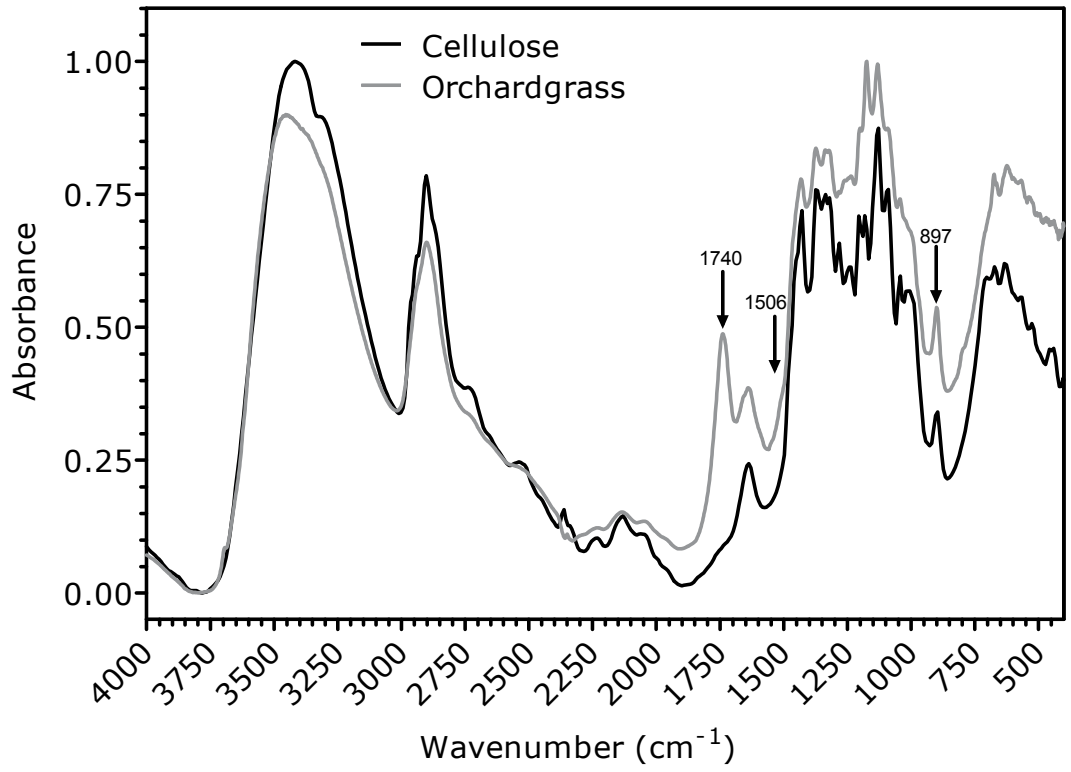

Figure 8: Mid-infrared spectra of an incompletely extracted orchardgrass root sample following all four sequential extraction steps (NDF+ADF+$KMnO_4$+H_2SO_4) and that of cellulose (Spectra normalized to between 0 and 1).

are evident between the sample spectra and one for cellulose (Figure 8). This factor may present difficulties for spectral interpretation as even small amounts of cellulose remaining in the sample may exceed the suberin content and conceal parts of its spectrum. This would prove especially problematic with species possessing a high ratio of cellulose to suberin such as alfalfa and switchgrass (Table 1). However, in this sample the reduction in the peak at 1506 cm^{-1} and the presence of a large peak at 1740 cm^{-1} support the presence of suberin despite the influence of residual cellulose which produces no peak at these locations. The spectra of the final fiber extraction fraction of soybean, fescue and alfalfa roots also feature a large peak at 1740 cm^{-1}

indicating suberin (Figure 9), but again, further work with extracted/purified suberin will be needed to confirm these conclusions.

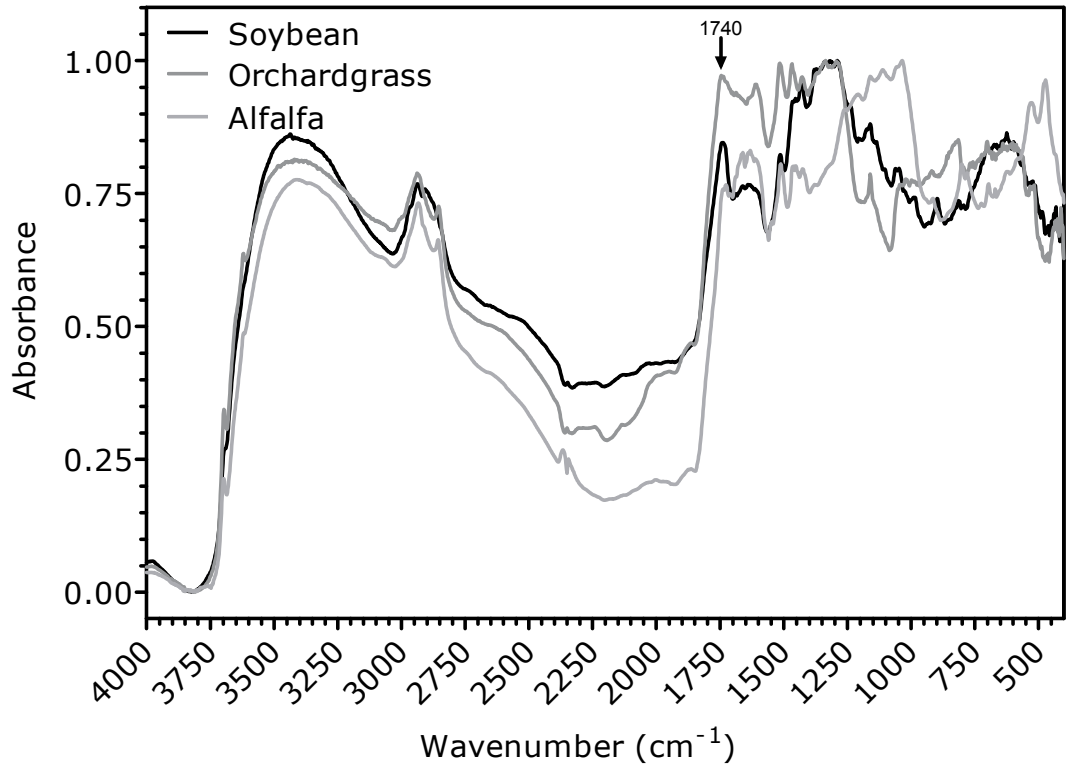

Figure 9: Mid-infrared spectra of the final residue of soybean, fescue, and alfalfa roots following all four sequential extraction steps (NDF+ADF+$KMnO_4$+H_2SO_4) (Spectra normalized to between 0 and 1).

CONCLUSIONS

This initial examination of the composition of the roots of a wide variety of plant species illustrates the potential of DRIFTS spectroscopy for the analysis of root composition. Results demonstrate that roots from the same species have similar composition and measurably differ from roots of other species as revealed by both DRIFTS and fiber analysis. Comparison of root spectral composition with established knowledge of above ground biomass composition indicate similarities in the two and may allow estimation of root contributions to SOM from extensive existing databases.

Diffuse reflectance infrared Fourier transform spectroscopy is sufficiently sensitive to detect variable and species dependent differences between fine and coarse roots. Comparison of spectra to fiber extraction data were consistent and reveal the potential to differentiate suberin in DRIFTS spectra of roots. The Van Soest extraction methods originally developed for forages indicate great promise for root compositional analysis, but additional research is needed to improve and verify complete extraction following each step. These preliminary results point to directions for further research into root analysis and illustrate that DRIFTS is a valuable tool to collect useful root composition data and focus efforts requiring further expensive and time consuming extraction procedures to the roots of greatest potential interest.

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Chapter 3: Cell wall compositional changes during incubation of plant roots measured by mid-infrared diffuse reflectance spectroscopy and fiber analysis

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.

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 1,000 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).

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 gauge 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 guaiacyl, respectively. In aboveground plant tissues lignins with a higher proportion of guaiacyl 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 guiacyl

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 hemicellulose 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).

METHODS

Root Samples

Alfalfa (*Medicago sativa* L.), soybean (*Glycine max* (L.) Merr.), conventional hybrid corn (*Zea mays* L.), tall fescue (*Festuca arundinacea Sheb.*), orchardgrass (*Dactylis glomerata* L.) and switchgrass (*Panicum virgatum* L.) 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). This timing was chosen to be close to onset of annual plant senescence or perennial plant dormancy. 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. Legume root samples included rhizobial nodules. 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).

Table 1: Results of preliminary sequential fiber extraction and total carbon (C) and nitrogen (N) analysis of roots from different species. NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber.

Species	NDF	ADF	Hemi-cellulose	Lignin	Cellulose	Wax	Total N	Total C	C:N
	----------	----------	----------	mg g^{-1}	----------	----------	----------	----------	
Corn	679	399	279	42.1	301	23.3	6.53	389	59.5
Switchgrass	468	272	197	59.1	198	12.8	4.23	434	103
Soybean	732	561	170	82.5	406	50.5	14.2	430	30.2
Orchardgrass	729	400	329	62.3	291	36.5	12.9	427	33.1
Fescue	651	389	262	55.5	285	22.4	8.21	412	50.1
Alfalfa	308	213	94.9	42.7	165	6.47	23.1	432	18.7

Incubation

Triplicate ground root samples were sealed in ANKOM Technology (Macedon, NY, USA) 5.0 cm x 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 x 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 a single soil sample collected from the 0 to 15 cm depth from a BARC crop field. The containers were placed in a dark incubator at 25˚ C. Containers were weighed twice weekly and deionized water added as needed to maintain a consistent moisture content. Triplicate root bags for each species were removed from the incubation chambers at 1, 2, 3 and 4 weeks, dried at 60˚ C and weighed to determine mass loss. The bags were then opened and the remaining root tissue collected for DRIFTS and fiber analysis.

Spectroscopy

All infrared spectra were obtained using DRIFTS on a Digilab FTS-7000 Fourier Transform Spectrometer (Digilab/Varian/Agilent, Santa Clara, CA, USA) equipped with a glow bar source, KBr beam splitter and Peltier cooled DTGS detector. Spectra were obtained from 4000 to 400 cm^{-1} at 4 cm^{-1} resolution using a

Pike Autodiff autosampler (Pike Technologies, Watertown, WI, USA) with KBr as the background and each sample scanned as ground non-KBr diluted material. Each spectrum consists of 64 co-added, background corrected scans. Reference spectra of starch, cellulose and octadecyl octadecanoate were recorded using materials obtained from Sigma-Aldrich.

Spectral Analysis

Individual spectra of each triplicate sample were normalized between 0 and 1 and then averaged using irAnalyze ver. 3.0.19.0 (LabCognition, Analytical Software GmbH & Co, KG). Spectra were compared and qualitatively analyzed using irAnalyze ver. 3.0.19.0 (LabCognition, Analytical Software GmbH & Co, KG) or GRAMS/AI ver. 8.0 (Thermo Electron Corp., Waltham, MA, USA).

Fiber Analysis

Fiber analysis was carried out in triplicate in sealed bags using an Ankom Technology A200 Fiber Unit (Macedon, NY, USA). Samples were analyzed sequentially for neutral detergent and acid detergent fiber (NDF and ADF). The NDF fraction was determined gravimetrically following extraction under pressure at 100 ˚C for 75 min. in a solution containing 0.1M sodium lauryl sulfate + 0.05M Disodium EDTA + 0.02M sodium borate decahydrate + 0.03M Na_2HPO_4 + 0.07M triethylene glycol. The ADF fraction was similarly determined following extraction under pressure at 100 ˚C for 60 min. in 0.5M H_2SO_4 + 0.05M cetyl trimethyl ammonium bromide. Further information on the NDF and ADF methods used may be found at http://www.ankom.com/analytical-procedures.aspx. The ADF was then analyzed for permanganate lignin and cellulose by treatment of the permanganate lignin residue

with 72% H_2SO_4 (Goering and Van Soest, 1970; Van Soest, 1994), but using the fiber bags as opposed to crucibles as outlined in the original method. Briefly, following NDF and ADF extraction with the Ankom unit, the bags were placed in a large beaker and covered with a permanganate solution [0.21M $KMnO_4$ + 1.1 x 10^{-4}M Ag_2SO_4 + 0.005M $Fe(NO_3)_3 \cdot 9\ H_2O$ + 3.0 x 10^{-4}M $AgNO_3$ + 2.9M $C_2H_4O_2$ + 0.017M CH_3CO_2K + 1.4M $(CH_3)_3COH$]. A smaller beaker was placed inside the large beaker to keep the bags submerged. Samples were stirred by hand and then sonicated for 2 minutes every 5 min for a total extraction time of 90 min. Sonication was performed to break up the formation of manganese oxide coatings on the exterior of the sample particles allowing continued access by the permanganate extracting solution. The H_2SO_4 extraction was conducted similarly using a 72% by weight solution for 180 min with sample mixing and stirring every 30 min. The residue remaining following 72% H_2SO_4 extraction is composed of waxes and minerals. In roots this wax may be suberin which was quantified by subtracting the weight of the ash remaining following sample combustion at 550 ˚C. Additional research is necessary to definitively confirm that wax composition is suberin. Differences in fiber fractions were tested using ANOVA and means separations were performed by LSD at the $P<0.05$ level of significance using SAS System for Windows Version 9.3 Copyright © 2011 SAS Institute Inc., Cary, NC.

RESULTS and DISCUSSION

Reduction in Sample Mass During Incubation

Figure 1 illustrates the loss of sample mass as a percentage of the mass on Day 0 over a 28 day incubation period. Both switchgrass and alfalfa roots differed

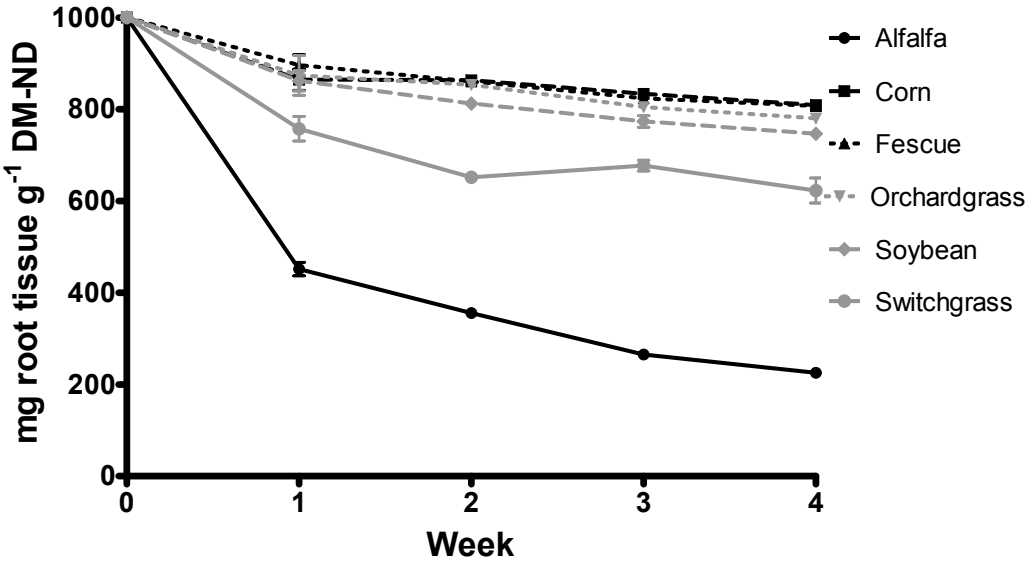

Figure 1: Changes in decomposing alfalfa, corn, fescue, orchardgrass, soybean and switchgrass root sample mass expressed as a fraction of the starting un-incubated sample mass over a four week incubation period. Bars indicate standard error.

significantly ($P<0.05$) from each other and from all the other species. Corn, fescue, orchardgrass and soybean roots all followed a similar trend, though the mass of soybean root was significantly different ($P<0.05$) after Week 1. The three grasses were not significantly different ($P<0.05$) from one another at any sampling interval. All species decomposed more rapidly during the first seven days, likely as soluble and easily mineralized root tissue constituents were utilized by decomposing microorganisms. Initial root mineralization during the first week is correlated with water soluble root compounds (Abiven et al., 2005; Machinet et al., 2011). In Week 1 alfalfa lost 54.8% of its starting mass, far exceeding the rate of decomposition of the other species. Corn, fescue, orchardgrass and soybeans lost 13.6%, 10.4%, 12.6% and 13.8%, respectively, while switchgrass exhibited a comparatively moderate loss of 24.3% of its starting mass. Previous authors have also found differences in root mineralization between legumes and grasses, as well as differences in decomposition

rate among species (Abiven et al., 2005; De Neergaard et al., 2002; Lindedam et al., 2009; Redin et al., 2014). For example, Lindedam et al. (2009) report an average of 68% of white clover root C was respired after 120 days compared to 31% of fescue root C.

The rate of decomposition slowed following Week 1 and was linear for all species. Alfalfa continued to decompose at the fastest rate losing an average of 7.5% $week^{-1}$. Alfalfa losses were significantly different ($P<0.05$) with each progressive week. Soybean roots lost an average of 3.8% $week^{-1}$ during the remaining 21 days of incubation with significant differences ($P<0.05$) between Weeks 1 and 2 and between Weeks 3 and 4. Orchardgrass and fescue lost a similar average of 3.1 and 3.0% $week^{-1}$, respectively. Corn roots lost an average of 1.8% $week^{-1}$. These three grass species didn't differ significantly ($P<0.05$) from Week 1 until Week 4. Switchgrass roots lost 10.6% between Week 1 and Week 2 and then lost an average of 1.0% $week^{-1}$ thereafter and decomposition did not decline significantly ($P<0.05$) after Week 2. In this study the roots of the cool season grasses (fescue and orchardgrass) lost mass at a similar rate during the incubation, however, the roots of the warm season grasses (corn and switchgrass) and the legumes (alfalfa and soybean) did not. These results clearly show differences in decomposition among roots of different crop species possibly due to differences in the soluble and readily degradable compounds and the relative degradability of the cell wall components in each species.

Loss of cell wall constituents during incubation

Neutral Detergent Fiber (NDF) has long been considered the equivalent of plant cell walls in forage studies and NDF decomposability (digestibility in animal

nutrition studies) is a measure of how easily cell wall materials (hemicellulose, cellulose, lignin and wax) are decomposed. In Figure 2 the rapid reduction in the mass of alfalfa root cell walls throughout the incubation period follows the trend in root mass loss, indicating that sample mass loss was due to the degradation of root cell walls in addition to mineralization of soluble components. Alfalfa root

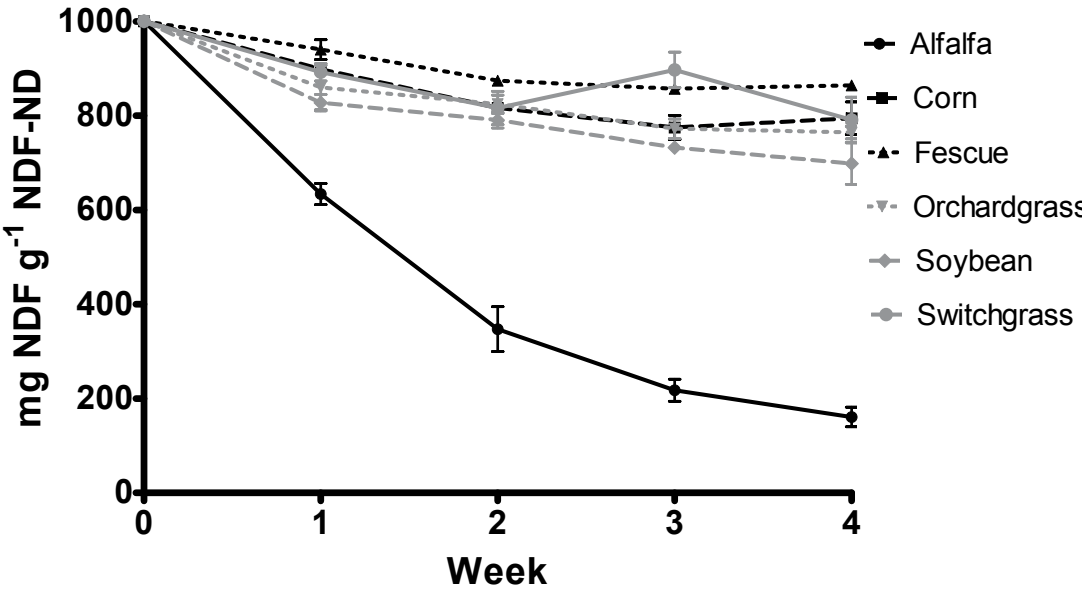

Figure 2: Decomposability of decomposing alfalfa, corn, fescue, orchardgrass, soybean and switchgrass total cell wall constituents as indicated by Neutral Detergent Fiber (NDF) fractions of each root sample expressed as a fraction of the total cell constituents in the un-incubated root samples over a four week incubation period. Bars indicate standard error.

significantly ($P<0.05$) lost 37% of its cell wall mass in the first week and an additional 84% over the entire four weeks. In contrast, much smaller amounts of cell wall decomposition was observed for all of the other species. Losses between Week 1 and Week 2 were significantly different ($P<0.05$) ranging from 17% to 7.0% of initial cell wall mass. No significant differences ($P<0.05$) were found between later weeks for any species consistent with the relatively flat decomposition curves after Week 2.

The greater loss in switchgrass sample mass relative to cell wall loss may indicate a greater degree of readily decomposable non-cell wall components (e.g. sugars, proteins and starch) in switchgrass roots than in the other grass roots and soybean roots. Both alfalfa and switchgrass are perennial plants and utilize root system starch for energy storage to a greater extent than do annual species (Chapin et al.,1990).

Figure 3 illustrates the decomposability of alfalfa (a) and switchgrass (b) cell wall hemicellulose, cellulose and lignin relative to their initial mass in the incubated roots. The data reveals large and rapid decomposition of alfalfa root cellulose and hemicellulose, as well as some decomposition of root lignin during the four week incubation period. In contrast, minimal decomposition of switchgrass root cell walls occurred during the four week incubation.

Alfalfa root cellulose degraded rapidly for the first three weeks, losing a significantly different ($P<0.05$) 25% and 30% a week between Weeks 1 and 2 and Weeks 3 and 4, respectively. Decomposition slowed between weeks 3 and 4, ultimately declining by 91% of the original concentration. Only 16% of switchgrass root cellulose decomposed in four weeks, losses that were not significant. Similar trends in decomposition and statistical significance were found for the other studied species. Alfalfa root hemicellulose lost 61% within the first week of incubation, consistent with the expected ease of digestibility of this root fiber fraction. Significantly different ($P<0.05$) losses of 12% and 13% were observed in Weeks 2 and 3, respectively. Switchgrass lost 26% of its root hemicellulose to decomposition in the first two weeks. Losses were significantly different ($P<0.05$) between Weeks 1 and 2, but not thereafter. Like switchgrass, corn, fescue, orchardgrass and soybeans

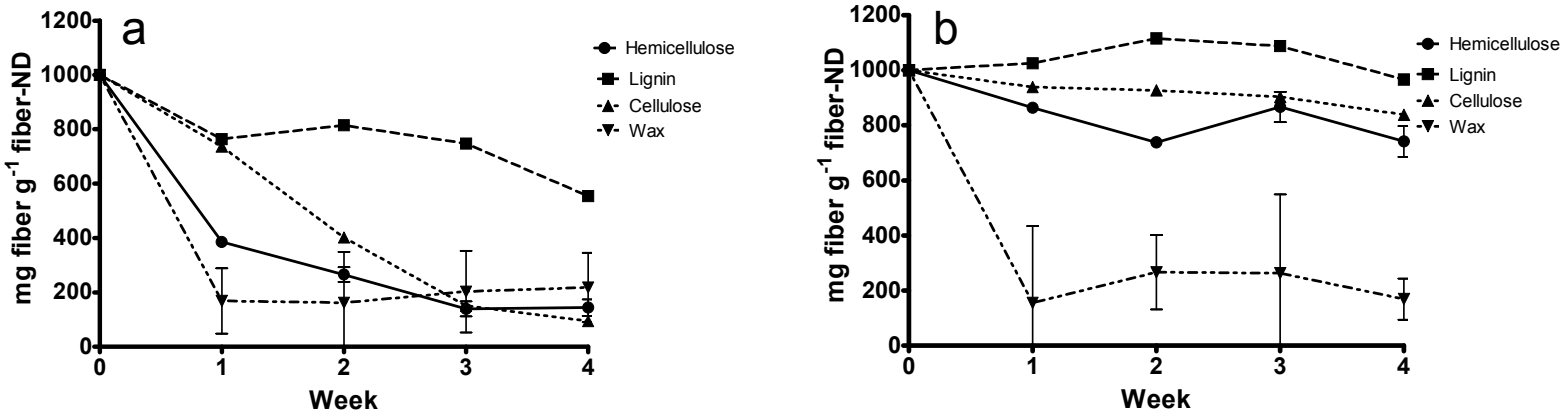

Figure 3: Decomposability hemicellulose, lignin and cellulose in decomposing alfalfa root (a) and switchgrass root (b) samples expressed as a fraction each cell wall constituent in the un-incubated root samples over a four week incubation period. Bars indicate standard error.

lost significant ($P<0.05$) amounts of hemicellulose during the first one to two weeks of incubation and not thereafter. This was surprising because in animal nutrition studies hemicellulose is generally considered readily digestible (Keys et al., 1969; Van Soest, 1994). It is possible that less substituted hemicelluloses were degraded leaving more substituted molecules and crosslinked molecules to become a greater proportion of the samples collected over time (Amin et al., 2014; Machinet et al., 2011). Machinet et al. (2009) noted differences in root hemicellulose degradation in different lines of brown midrib corn depended on the degree of arabinose substitution to xylose within the hemicellulose structure. Studies have shown that esterification with ferulic and *p*-coumaric acid can inhibit the activity of hemicellulases (Puls and Schuseil, 1996) and thus the degradation of hemicellulose. Ferulic acid cross-linking between hemicellulose and lignin also limits root C mineralization (Amin et al., 2014). Previous forage analysis has revealed higher concentrations of *p*-coumaric acid in corn stover relative to alfalfa hay (Reeves, 1986). It is likely that the differences in hemicellulose decomposition are due to differences in the structure of root cell wall hemicellulose. The leveling off of decomposability in the latter part of the incubation period suggests that after the first 2-3 weeks primarily the more resistant highly substituted and cross-linked hemicellulose remained. Future research should include analysis of sugars in ADF residues to explore differences in hemicellulose decomposition among species. Hemicellulose has been found to be important in the formation of soil organic matter, for example, Russell et al. (2004) reported that long term C accrual in long-term forest ecosystems was most influenced by root lignin and hemicellulose. The decrease in decomposition for Week 3 is due to the decrease in overall switchgrass root cell wall decomposability.

Some alfalfa root lignin (24%) was lost during the first week, but little additional decomposition was observed until Week 4 and losses were not significant ($P<0.05$). Likewise no significant ($P<0.05$) decomposition of switchgrass root lignin or the other studied roots occurred during the entire four week incubation.

Wax losses were similar in the first week with both alfalfa and switchgrass losing 83% and 84% of the initial mass, respectively. The amounts of wax present in these roots was very small. Prior to incubation, these species contained only 6.47 mg g^{-1} and 12.8 mg g^{-1} of wax, respectively (White et al., 2011), and large standard errors in the decomposability data prevent statistical inference. However, the trend seems to be essentially no change in decomposition for either species between Week 1 and Week 4.

Changes in root cell wall composition measured by DRIFTS

White et al. (2011) demonstrated that the peaks at wavenumbers 1510 cm^{-1} and 897 cm^{-1} were indicative of root lignin and cellulose, respectively, consistent with the findings of other researchers (Reeves, 1993, Séné et al., 1994; Pandey, 1999; Dokken and Davis, 2007.) Table 2 lists the ratios of the lignin:cellulose DRIFTS peak height for all studied species at each sampling interval. Changes in this ratio over time can indicate that the relative concentration of these constituents is changing while no change can indicate either that they are not, or that they are changing at the same rate. However, when viewed in conjunction with the fiber data, Table 2 illustrates the link between the quantitative fiber data and the qualitative DRIFTS data. Changes in the ratio of the heights of the lignin and cellulose peaks of samples at each week are consistent with the digestibility of these root cell wall constituents as indicated by fiber analysis (Figure 3 and Table 2). The large reduction in alfalfa root cellulose relative to lignin illustrated in Figure 3a is

Table 2: Ratio of the DRIFTS peak heights at 1510 cm^{-1} (lignin) and 897 cm^{-1} (cellulose) for undecomposed root samples and decomposing root samples.

Week	Alfalfa	Corn	Fescue	Orchardgrass	Soybean	Switchgrass
	Lignin Peak Height:Cellulose Peak Height					
0	1.19	1.30	1.19	1.26	1.28	1.10
1	1.21	1.32	1.24	1.29	1.36	1.19
2	1.32	1.31	1.25	1.26	1.40	1.24
3	1.40	1.30	1.23	1.32	1.38	1.22
4	1.47	1.29	1.26	1.31	1.40	1.24

reflected in the DRIFTS data. Reduction in both lignin and cellulose during the first week is clear, as there is no change in the peak ratio for this period. Following Week 1, the greater reduction in cellulose concentration relative to lignin concentration is seen as an increase in the lignin:cellulose peak ratio. In comparison there is minimal change in the lignin:cellulose ratio for switchgrass (as well as the remaining species) over time, consistent with the data presented in Figure 3b. This agreement with the fiber data further illustrates the utility of DRIFTS as a tool for root analysis (White et al., 2011).

The focus of this study was to investigate changes in cell wall composition, so analyses of non cell wall materials such as soluble sugars, proteins and starch was not performed. However, mid-infrared analysis also provides a possible explanation of the differences in sample mass loss of alfalfa and switchgrass roots relative to the other species illustrated in Figure 1. Both alfalfa and switchgrass had lower concentrations of cell wall materials relative to soluble constituents than the other species in this study. Alfalfa root cell wall material only comprised 308 mg g^{-1} of the undecomposed sample, while in switchgrass roots the concentration was 468 mg g^{-1}, compared to an average of 698 mg g^{-1} for the other species. Alfalfa and switchgrass were the only perennial species included in the study and, as previously stated, both species utilize starch in their root systems for energy storage. Roots for this study were collected in September and

October, therefore starch storage in their root systems was not unexpected and was reflected in the DRIFTS spectra of both alfalfa and switchgrass (Figure 4a and b). Starch has peaks arising from the glycosidic linkage and C-O-C ring bonds at 930 cm^{-1}, 861cm^{-1} and 767 cm^{-1} (Pigorsch, 2009) which rapidly decrease with incubation time as shown in Figure 4. The spectra of the other species did not have peaks at these three locations; represented by the spectrum of fescue (Figure 4c). Also shown in this figure is the spectrum of cellulose with the diagnostic absorbance at 897 cm^{-1}. Figure 4a and 4b compare the spectra of starch, cellulose and alfalfa root or switchgrass root at Week 0 and Week 1, the time period corresponding with the rapid reduction in sample mass. As Figures 4a and 4b clearly illustrate, the undecomposed root samples exhibit absorbance peaks corresponding to both starch and cellulose. After only one week of incubation the three peaks indicating the easily degraded starch has completely disappeared from the Week 1 spectral trace, while the cellulose peak remained. The latter is consistent with the cellulose data presented in Figure 3 and Table 2, indicating little change in root cell wall cellulose for this time period.

The resistance of lignin and, potentially, also wax compounds to decomposition is evident in a comparison of the DRIFTS spectra of alfalfa roots at Week 0 and Week 4 (Figure 5). The spectrum of octadecyl octadecanoate is included in Figure 5 as a reference for root waxes such as suberin (Zeier and Schreiber, 1999). Changes between1800 cm^{-1} and 1500 cm^{-1} (Figure 5b) reveal the relative increase in lignin (1510 cm^{-1}) (White et al., 2011; Dokken and Davis; 2007, Pandey, 1999; Reeves, 1993) and carbonyl compounds (1742 cm^{-1} and 1663 cm^{-1}) (White et al., 2011; Dokken and Davis, 2007, Pandey, 1999, Reeves, 1993; Séné et al., 1994) as a proportion of the incubated

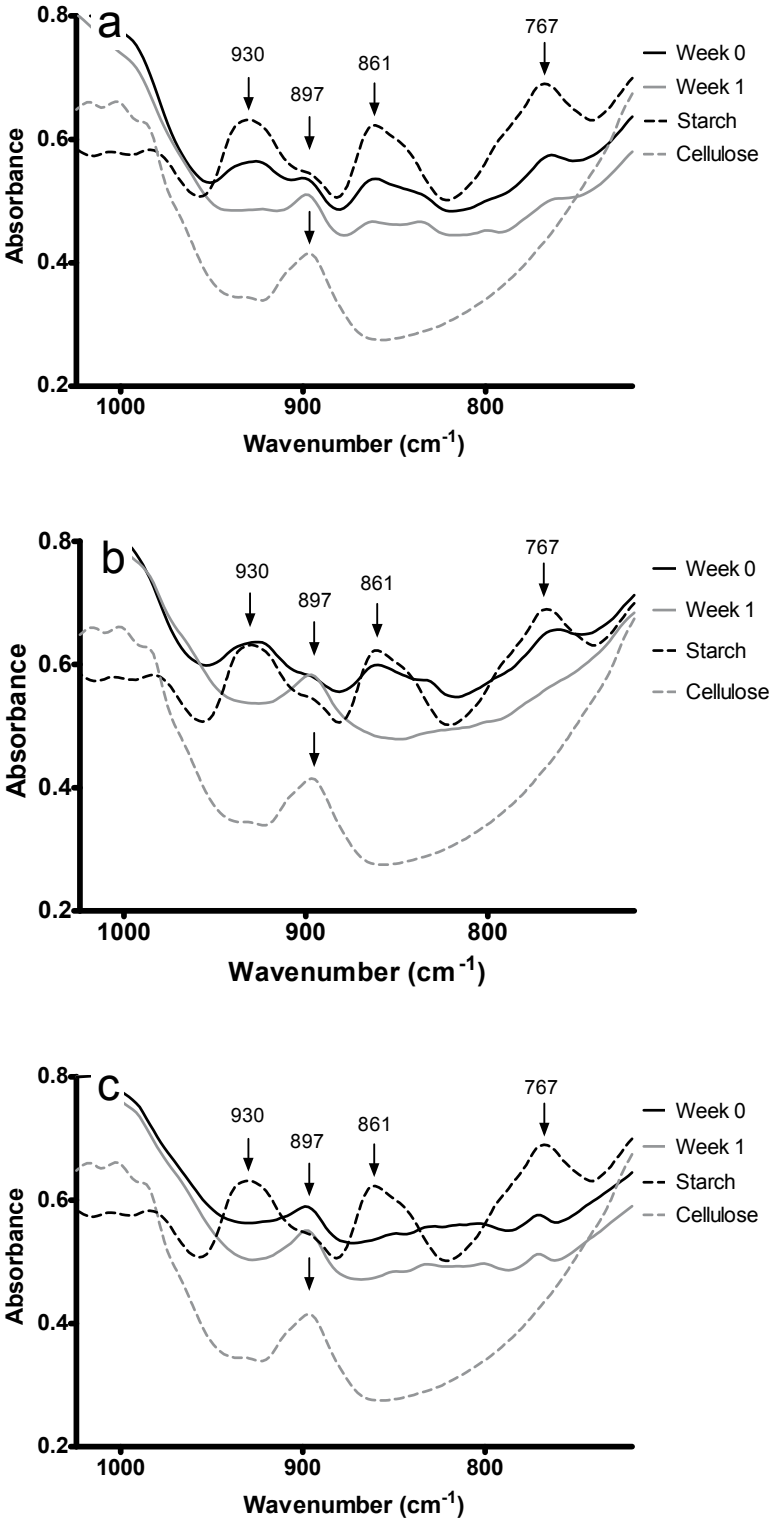

Figure 4: Comparison of the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of alfalfa (a), switchgrass (b) and fescue (c) roots at Week 0 and Week 1 of a four week incubation with the spectra of starch and cellulose.

root samples, consistent with the data presented in Figure 4. Given the qualitative nature of the respective spectra, the difference in magnitude between the two peaks should not be interpreted as evidence of increased concentration. However, when viewed in conjunction with the quantitative fiber results and the increase in lignin:cellulose peak height (Table 2) the sharper and better defined lignin peak at 1510 cm^{-1} was further evidence for the relative recalcitrance of lignin. The peak at 1640 cm^{-1} in the Week 0 spectrum was indicative of protein amides (White et al., 2011, Dokken and Davis, 2007, Séné et al., 1994) and its disappearance from the Week 4 spectrum is evidence of protein degradation. The preservation of hemicellulose shown in Figure 3a is supported by the changes in the peaks at 1740 cm^{-1} and 1663 cm^{-1} arising from the acid carbonyls of hemicellulose (White et al., 2011), both of which become more prominent as alfalfa root samples were degraded. The peak at 1740 cm^{-1} may also be indicative of suberin, as this peak has been attributed to the carbonyl stretching in the esters of this compound (Dokken and Davis, 2007; Lopes et al., 2000; Schreiber et al., 2005; Séné et al., 1994; Zeier and Schreiber, 1999). A strong peak in the spectrum of octadecyl octadecanoate was also recorded at this location consistent with the findings of Zeier and Schreiber (1999) lending further evidence for the presence of waxes such as suberin.

Further spectral evidence for the presence of suberin as an increasing proportion of the incubated samples over time can be seen between 3000 cm^{-1} and 2850 cm^{-1} (Figure 5a). The fiber data shows that wax is preserved in the decomposing root samples (Figures 3a and 4a) and this is supported by the DRIFTS spectra. Peaks in this region are a product of symmetric and asymmetric C-H stretching, such as those found in the aliphatic moiety of waxes such as suberin. Three prominent peaks at 2856 cm^{-1}, 2930 cm^{-1} and 2959 cm^{-1}

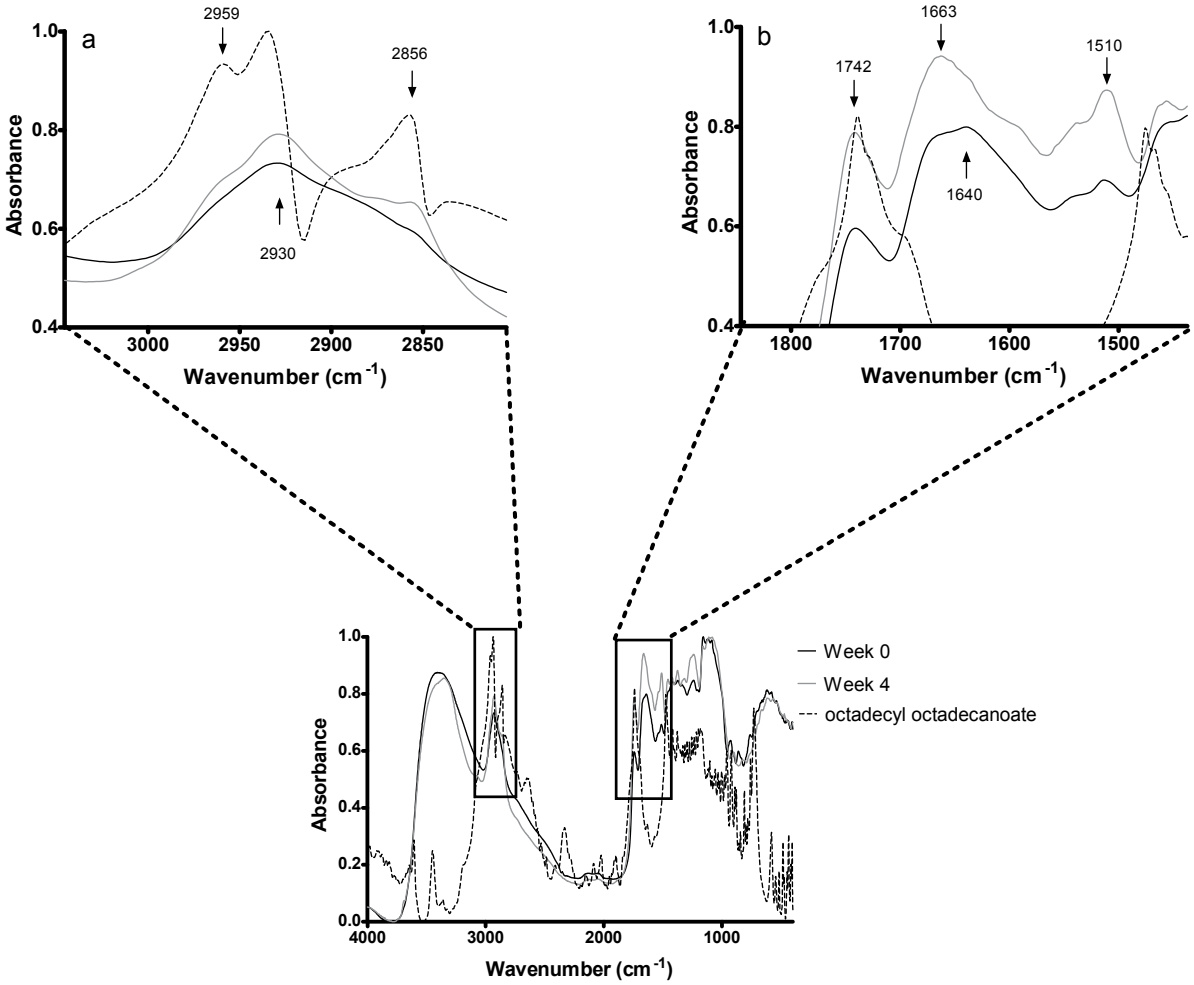

Figure 5: Comparison of changes in the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of alfalfa roots at Week 0 and Week 4 of a four week incubation and with the spectrum of octadecyl octadecanoate.

are evident in the octadecyl octadecanoate spectrum. These peaks correspond to asymmetric CH_3 stretching, asymmetric CH_2 stretching and symmetric CH_2 stretching, respectively, which have been attributed to suberin (Dokken and Davis, 2007; Lopes et al., 2000; Zeier and Schreiber, 1999). At Week 0 the peak at 2930 cm^{-1} is evident, as is a slight shoulder at 2856 cm^{-1}. As other root compounds degrade by Week 4 the peak at 2930 cm^{-1} is better defined and the shoulder at 2856 cm^{-1} has become more prominent. A shoulder at 2950 cm^{-1} is also evident. Similarly, Zeier and Schreiber (1999) found a strong correspondence between these peaks in octadecyl octadecanoate and transesterified root endodermal cell walls. Lopes et al. (2000) also report strong absorbances in this region in the spectrum of suberin extracted *Quercus suber* L. cork.

Figure 6 illustrates the same spectral regions in switchgrass and octadecyl octadecanoate. Less pronounced differences were seen in the relative magnitude of peak heights between Week 0 and Week 1. Comparing Figures 5 and 6, one can detect differences in the pattern of peaks due to variations in the molecular structure of root cell wall materials between the two species (White et al., 2011); differences that remain consistent as the roots decompose. In addition to the lignin peak at 1510 cm^{-1}, a peak at 1603 cm^{-1} has become more prominent by Week 4 (Figure 6b). This peak may be due to aromatic C=C stretching in lignin (Séné et al., 1994; Zeier and Schreiber, 1999), ester linked aromatics in suberin (Zeier and Schreiber, 1999) or stretching of the carboxyl groups on pectic polysaccharides (Dokken and Davis, 2007; Séné et al., 1994). No change is evident in the peak at 1633 cm^{-1}, also indicating C=C stretching in either lignin or hydroxycinnamic acids (Dokken and

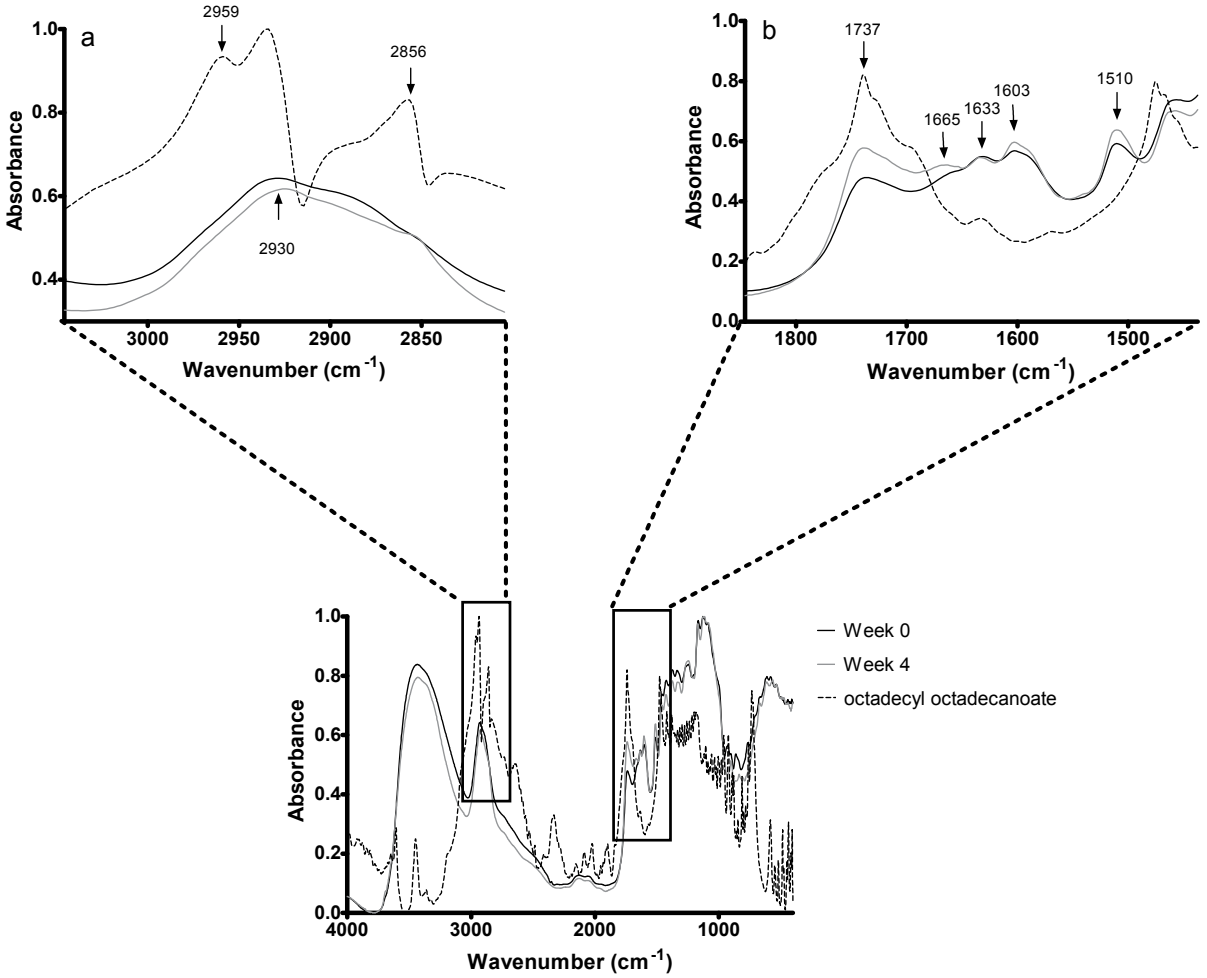

Figure 6: Comparison of changes in the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of switchgrass roots at Week 0 and Week 4 of a four week incubation and the spectrum of octadecyl octadecanoate.

Davis, 2007; Séné et al., 1994) such as those present in the structure of suberin (Lopes et al., 2000). A peak at 1665 cm^{-1} becomes more prominent by Week 4, due to the preservation of hemicellulose (White et al., 2011) as shown in Figure 3b. Finally, the peak at 1737 cm^{-1} becomes relatively more prominent, likely due to the preservation of additional carbonyl containing compounds such as hemicellulose and waxes like suberin consistent with the data in Figure 3b (Dokken and Davis, 2007; Lopes et al., 2000; Schreiber et al., 2005; Séné et al., 1994; Zeier and Schreiber, 1999; White et al., 2011). Again, a corresponding peak is evident in the spectra of octadecyl octadecanoate.

Comparison of the switchgrass root spectra with the spectrum of octadecyl octadecanoate revealed features similar to those observed in alfalfa (Figure 6a). The peak at 2930 cm^{-1} from aliphatic C-H bonds and the shoulder at 2856 cm^{-1} is apparent. However, the shoulder seen at 2959 cm^{-1} in the alfalfa root spectrum was not present. Many aliphatic compounds and aromatic compounds such as lignin contribute to CH stretching vibrations. Given the lesser degree of degradation observed in the switchgrass roots compared to alfalfa roots during this study (Figures 1 and 4b), it was possible that vibrations arising from other compounds are obscuring this feature in the switchgrass spectra.

Taken together, the spectral data presented in Table 2 and Figures 4, 5 and 6 further illustrated the capability and utility of DRIFTS for the analysis of decomposing root tissue, expanding upon the work presented in White et al. (2011). In addition, spectral data provided important information about the molecular structure of root cell walls and changes in the relative proportion of different

compounds during decomposition. Comparison of peak height ratios revealed information about the decomposability and preservation of different root cell wall constituents. Specifically, in the spectra of both alfalfa and switchgrass roots there was clear evidence for the preservation of lignin, hemicellulose and wax as well as the loss of more easily degradable protein and starch. Measurement by DRIFTS was able to reveal the presence of waxy root compounds, such as suberin, that were present in minute amounts and not effectively measured by wet chemical methods.

CONCLUSIONS

The rate at which alfalfa roots decompose was different relative to the other studied crop species. These differences appear to arise primarily from dissimilarities in the relative decomposability of alfalfa root constituents, especially that of starch, hemicellulose, cellulose and lignin. Alfalfa and switchgrass roots began to decompose quickly due to the presence of cellular starch reserves, as indicated by DRIFTS analysis of the decomposing residues. Alfalfa root tissue continued to decompose rapidly over the course of the four week incubation because cellulose was easily degraded relative to lignin. In contrast, none of these three cell wall constituents were appreciably decomposable in the other plant species studied. This observation was supported by DRIFTS, as the ratio of the diagnostic peaks for cellulose and lignin increased over time in the alfalfa root spectra but not in the spectra of the other species. Possible evidence for the preservation of wax compounds (e.g. suberin) was also seen in the alfalfa DRIFTS data though this was difficult to quantify by fiber analysis. The large changes in alfalfa root cell wall composition quantified by fiber analysis was confirmed in the DRIFTS spectra illustrating the utility of this analytical

method for assessing changes in decomposing organic materials. Taken together, the results of this study revealed that even over a relatively brief four week time interval following root senescence, the chemical composition of root cell wall constituents and the nature of their interconnection exerted an important influence on tissue degradation and mineralization. Knowledge of the chemical composition of plant roots, and especially their cell wall composition, is necessary to understand and predict short term SOM dynamics. This knowledge will aid in the prediction of changes in SOM accretion versus degradation due to changes in cropping practice or soil disturbance such as tillage, logging or fire and the possible impact of such changes on soil health and productivity as well as long term organic C stabilization and the potential for C sequestration.

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Chapter 4: Degradation changes in plant root cell wall structural molecules during extended decomposition of important agricultural crop and forage species

ABSTRACT

Little is known about the changes in the cell wall structural molecules lignin, cellulose and hemicellulose as plant roots decompose, despite their importance in the formation of soil organic matter. The objectives of this study were to quantify changes in root composition during 270 d incubations of the roots of ten important grain and forage crop species utilizing forage fiber analysis techniques and to characterize the changes in root cell wall composition and molecular structure using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). The roots samples were incubated at 25˚ C and sampled at 30, 60, 90, 180 and 270 days. Large species dependent variations were observed in the extent of root tissue decomposed over time, ranging from 82.5% of initial mass for alfalfa to 21.5% for switchgrass. Following an initial period of rapid decomposition, fiber analysis revealed that lignin, cellulose and hemicellulose degraded proportionally over time. These results were supported by DRIFTS as similar trends were found in the ratios between the diagnostic peaks for cellulose, the ester and acid carbonyls of hemicellulose and waxes, and for lignin. The DRIFTS spectra revealed spectral features illustrating molecular changes in root composition as roots decomposed. These features were most pronounced in the more extensively decomposed roots. Features potentially indicative of suberin preservation were found in the spectral region between 2800 cm^{-1} to 3000 cm^{-1}. Examination of the polysaccharide fingerprint region between 1000 cm^{-1} to 1300 cm^{-1} revealed changes in decomposing roots possibly indicative of

hemicellulose structural changes as more degradable polysaccharides are preferentially degraded. The results demonstrate the effect of differences in cell wall composition and structure on the extent of root tissue decomposition and expand understanding of the role of roots in soil organic matter dynamics. Given the variability in root tissue degradation and changes in cell wall composition observed among species, these results illustrate the necessity for characterization of a broad range of individual species and their decomposition characteristics in order to predict root contributions to soil C cycling and potential C sequestration.

INTRODUCTION

Knowledge of the sources of carbon (C) and their decomposition to form soil organic matter (SOM) is crucial for understanding the terrestrial C cycle. The decomposition of plant roots are important for soil nutrient cycling and are an important source of soil C (Rasse et al., 2005). Root C persists in soil an average of 2.4 times longer that shoot C (Rasse et al., 2004). Experimental data has revealed that 42% and 49% of root derived C from legume and small grains, respectively, remained in soil one year following harvest (Gale and Cambardella, 2000; Puget and Drinkwater, 2001). Effective C sequestration requires that C be converted into SOM with long soil residence times. Evidence from nuclear magnetic resonance studies has revealed that stable SOM consists of a mixture of the degradation products of plant, animal and microbial residues such as proteins, carbohydrates, lignin and aliphatic biopolymers from fatty acids and waxes rather than as a distinct chemical category (Kelleher and Simpson, 2006). Root lignin and hemicellulose are important contributors to long-term SOM in forest surface soils (Russell et al., 2004), while

aliphatic lipids and cellulose-derived sugars predominate in subsoils (Vancampenhout et al., 2012). Aliphatic compounds derived from suberin have been found to be major contributors to grassland and agricultural subsoils (Allard, 2006; Feng and Simpson, 2007; Mendez-Millan et al., 2010). However, there is little available research into the chemical structure of roots and their degradation products (Lindedam et al., 2009). Knowledge of crop root composition during degradation and conversion to stable SOM are essential to determine the role of agricultural systems in the terrestrial C cycle and to develop management practices that promote C sequestration, as well as promoting soil quality, nutrient cycling and increased agricultural productivity (Lal, 2009).

Root residues consist of soluble and readily degradable compounds such as sugars and proteins and also insoluble and more resistant cell wall structural compounds, in particular, hemicelluloses, cellulose, lignin and suberin (Aulen et al., 2012; Machinet et al., 2009, 2011; Picon-Cochard et al., 2012; Redin et al., 2014; White et al., 2011). The cell wall compounds exhibit structural characteristics that are resistant to microbial attack and are intertwined in a complex network that limits the accessibility of these compounds to extracellular enzymes for degradation (Amin et al., 2014; Bertrand et al., 2006). Root composition differs by species, and root cell wall decomposition is species dependent (White et al., 2011; Redin et al., 2013). Understanding these differences among a wide range of species over time is important for discerning the role of root inputs to SOM, in terms of SOM cycling and soil fertility and soil health, as well as for organic C stabilization. Therefore knowledge of root composition and its relative recalcitrance to degradation are

necessary to gauge the potential impact agricultural cropping systems will have on soil health, soil quality, and the potential for long term C storage in SOM.

Lignin structure and interactions with soluble cell substances as well as with hemicellulose, cellulose and polyphenols play a vital role in the resistance of plant material to decomposition (Melillo et al., 1982; Bertrand et al., 2006; Machinet et al., 2011; Talbot and Treseder, 2012; Talbot et al., 2012). In aboveground plant residues, lignin chemistry determines the rate of decay through varying degrees of cross-linking with hemicellulose and lignin and by the structural stability of the lignin molecule itself (Bertrand et al., 2006; Talbot et al., 2012). For example, lignins with a higher ratio of guaiacyl to *p*-hydroxyphenyl monomers were more resistant to degradation due to a greater degree of recalcitrant linkages between subunits (Talbot et al., 2012). Studies of corn root tissue decomposition have found poor correlations between C mineralization and the ratio of syringyl (a more labile lignin monomer) to guaiacyl and long-term C mineralization rates (Abiven et al., 2005; Machinet et al., 2009). Carbon mineralization in corn roots was negatively correlated with root hemicellulose concentration, hemicellulose arabinose substitution and the amount of esterified *p*-coumaric acid (Machinet et al., 2009). These results indicate that root decomposition is regulated by cell wall lignin, hemicellulose substitution and the degree of cross-linking between the lignin and hemicellulose by hydroxycinnamic acids.

Hemicellulose is a complex polysaccharide with a backbone of β-1-4 linked xylan chains with acetyl and glucouronic branches and varying degrees of arabinose substitution in grasses or rhamnose substitution in legumes (Hatfield, 1989; Dehority,

1993; Brett and Waldron, 1996). Increasing arabinose substitution results in increasing hemicellulose resistance to degradation (Amin et al., 2014; Machinet et al., 2009). The hydroxycinnamic acids ferulic and *p*-coumaric acid form esterified linkages between lignin and hemicelluloses (Ralph and Helm, 1993), which in turn forms a complex network of lignin and hemicellulose within which cellulose is embedded (Boerjan et al. 2003). These linkages limit enzyme access to cellulose and hemicellulose, resulting in decreased rates of root decomposition (Amin et al., 2014).

Suberin is a waxy component of cell walls comprised of both aliphatic and aromatic moieties (Bernards, 2002). The aromatic domain consists of a polymeric mixture of hydroxycinnamic acids and lignin monomers embedded within the cell wall (Bernards, 2002). This domain is covalently linked to the aliphatic component which is a glycerol-based polyester compound composed of long chain fatty acids and fatty alcohols as well as esterified ferulic acid that extend outward from the cell wall (Bernards, 2002). Suberin-derived aliphatic compounds are preserved in soil without significant alteration from humification processes (Nierop et al., 2003, Allard, 2006), but little is known about suberin preservation or degradation in decomposing root tissues.

Previous research has shown that root decomposition of a selection of important agricultural crops differs among plant species with notable differences in short-term degradation of root cell wall components among species (White et al., 2015). Longer-term studies of plant root degradation are necessary to assess the relative recalcitrance of the root cell wall constituents. Given the paucity of information on the cell wall composition of most species (White et al., 2011), it is

necessary to characterize the composition of a wider range of grain and forage crop species and examine changes in composition during root tissue degradation to improve understanding of the contribution of root derived compounds to SOM. The objectives of this study were to i) quantify changes in root composition during long-term incubations of ten important grain and forage crop species utilizing fiber analysis techniques, and ii) characterize changes in root composition and molecular structure using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS).

METHODS

Root Samples

Alfalfa (*Medicago sativa* l.), sorghum sudangrass (*Sorghum bicolor* (L.) Moench), soybean (*Glycine max* (L.) Merr.), conventional hybrid corn (*Zea mays* L.), tall fescue (*Festuca arundinacea* Scheb.), orchardgrass (*Dactylis glomerata* L.), winter rye (*Secale cereal* L.), wheat (*Triticum aestivum* L.), gammagrass (*Tripsacum dactyloides* (L.) L), and switchgrass (*Panicum virgatum* L.) root samples were collected in June at the Beltsville Agricultural Research Center (BARC) in Beltsville, Maryland, USA (39.0° N, 76.9° W). Initially, the use of roots grown in sand culture in a greenhouse was planned to allow for easier collection of complete root systems. Root collection from the field was necessitated when DRIFTS analysis of the greenhouse grown roots revealed structural features, such as higher protein content, inconsistent with field grown roots. Samples were collected from randomly selected locations within a single production field or research plot. Roots and soil were collected to a depth of 15 to 20 cm and soil was gently washed from the roots with water. Legume root samples

Table 1: Results of sequential fiber extraction and total carbon (C) and nitrogen (N) analysis of roots from different species.

Sample ID	Cell Walls	Hemicellulose	Lignin	Cellulose	Wax	Total N	Total C	C:N
	----------	----------	----------	----------	mg g^{-1} ----------	----------	----------	
Switchgrass	773	287	197	266	22.0	3.50	458	131
Gammagrass	782	257	206	292	25.9	5.86	451	76.9
Wheat	781	338	146	292	5.84	7.58	403	53.1
Winter Rye	724	318	151	252	2.20	6.88	373	54.3
Orchardgrass	776	355	136	277	8.70	11.1	421	37.7
Fescue	802	333	162	198	9.42	11.6	439	38.0
Soybean	715	174	251	284	6.06	10.2	450	44.0
Hybrid Corn	783	334	138	310	3.03	9.64	418	43.4
Sorghum x sudangrass	708	274	126	297	3.12	9.93	378	38.1
Alfalfa	527	116	123	263	3.16	14.8	431	29.2

included rhizobial nodules. Samples were dried at 60˚ C and ground in a cyclone grinder (UDY Corporation, Fort Collins, CO, USA) to pass a 20 mesh (0.841 mm) screen. Characterization data for the root samples including hemicellulose, cellulose, lignin and wax concentrations for each species are shown in Table 1..

Incubation

Ground root samples were sealed in ANKOM Technology (Macedon, NY, USA) 5.0 cm x 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 in order to only assess the biochemical recalcitrance of root cell wall constituents. The root bags were placed in acid-washed coarse quartz silica sand in individual 7.6 cm x 7.9 cm vented polyethylene incubation containers (Corning Snap-Seal, Corning, NY, USA). Deionized water was added to each container to simulate gravimetric water content at field capacity (0.137 g g^{-1} moisture). Nitrogen was added with the water as KNO_3 to simulate N concentrations found in subsoils (2 mg N kg^{-1}). The incubation containers were inoculated with 2 μL of a 2:1 deionized water:soil extract using a single soil sample collected from a BARC crop field and placed in a dark incubator at 25˚ C. Deionized water was added twice weekly as needed to maintain consistent moisture content. Triplicate root bags for each species were removed at 30, 60, 90, 180 and 270 days, dried at 60˚ C and weighed to determine mass loss. The dried root tissue was then used for fiber analysis and DRIFTS.

Spectroscopy

All infrared spectra were obtained using DRIFTS on a Digilab FTS-7000 Fourier Transform Spectrometer (Digilab/Varian/Agilent, Santa Clara, CA, USA) equipped with

a glow bar source, KBr beam splitter and Peltier cooled DTGS detector. Spectra were obtained from 4000 to 400 cm^{-1} at 4 cm^{-1} resolution using a Pike Autodiff autosampler (Pike Technologies, Watertown, WI, USA) with KBr as the background and each sample scanned as ground non-KBr diluted material. Each spectrum consists of 64 co-added, background corrected scans. Spectra of the triplicate root samples at each sampling interval were normalized to between 0 and 1 and averaged prior to spectral analysis.

Spectral Analysis

Spectra were compared and analyzed using irAnalyze ver. 3.0.19.0 (LabCognition, Analytical Software GmbH & Co, KG) or GRAMS/AI ver. 8.0 (Thermo Electron Corp., Waltham, MA, USA).

Fiber Analysis

Fiber analysis of Day 0 and incubated samples was carried out using an Ankom Technology A200 Fiber Unit (Macedon, NY, USA). Samples were analyzed sequentially for neutral detergent and acid detergent fiber (NDF and ADF). Protocols are available at: http://www.ankom.com/analytical-procedures.aspx. Briefly, the NDF fraction was determined gravimetrically following extraction under pressure at 100 ˚C for 75 min. in a solution containing 0.1M sodium lauryl sulfate + 0.05M Disodium EDTA + 0.02M sodium borate decahydrate + 0.03M Na_2HPO_4 + 0.07M triethylene glycol. The ADF fraction was determined gravimetrically following extraction under pressure at 100 ˚C for 60 min. in 0.5M H_2SO_4 + 0.05M cetyl trimethyl ammonium bromide. The ADF was then analyzed for permanganate lignin and then for cellulose by treatment of the permanganate lignin residue with 72% H_2SO_4 (Goering and Van Soest, 1970; Van Soest, 1994), but using the fiber bags as opposed to crucibles as outlined in the original method. Following

NDF and ADF extraction with the Ankom unit, the bags were placed in a large beaker and covered with a permanganate solution [0.21M $KMnO_4$ + 1.1 x 10^{-4}M Ag_2SO_4 + 0.005M $Fe(NO_3)_3 \cdot 9\ H_2O$ + 3.0 x 10^{-4}M $AgNO_3$ + 2.9M $C_2H_4O_2$ + 0.017M CH_3CO_2K + 1.4M $(CH_3)_3COH$]. Samples were stirred by hand and then sonicated for 2 minutes every 5 min for a total extraction time of 90 min. Sonication was used to break up manganese oxide coatings formed on the exterior of the sample particles allowing continued access by the permanganate extracting solution. The H_2SO_4 extraction was similarly conducted in a large beaker using a 72% by weight solution for 180 min with sample mixing and stirring every 30 min. Differences in fiber fractions were tested using ANOVA and means separations were performed by LSD at the $P<0.05$ level of significance using SAS System for Windows Version 9.3 Copyright © 2011 SAS Institute Inc., Cary, NC.

RESULTS and DISCUSSION

Sample Mass Loss During Incubation

Widely different mass loss and decomposition rates were observed among species between Day 0 and Day 30 (Figure 1). Mass loss (and percent loss) at Day 30 followed the order alfalfa (58.4%) > sorghum-sudangrass (34.7%) > hybrid corn (29.2%) > soybean (29.2%) > fescue (25.1%) > orchardgrass (19.3%) > winter rye (13.3%) > wheat (12.8%) > gammagrass (12.3%) > switchgrass (10.5%). This order remained consistent at each sampling interval throughout the 270 day incubation period. The rate of mass loss for all species was most rapid during the initial 30 day period likely as easily degradable soluble constituents were mineralized. Previous research has demonstrated links between initial root tissue mineralization and soluble root compounds (Abiven et al., 2005; Machinet et al., 2011).

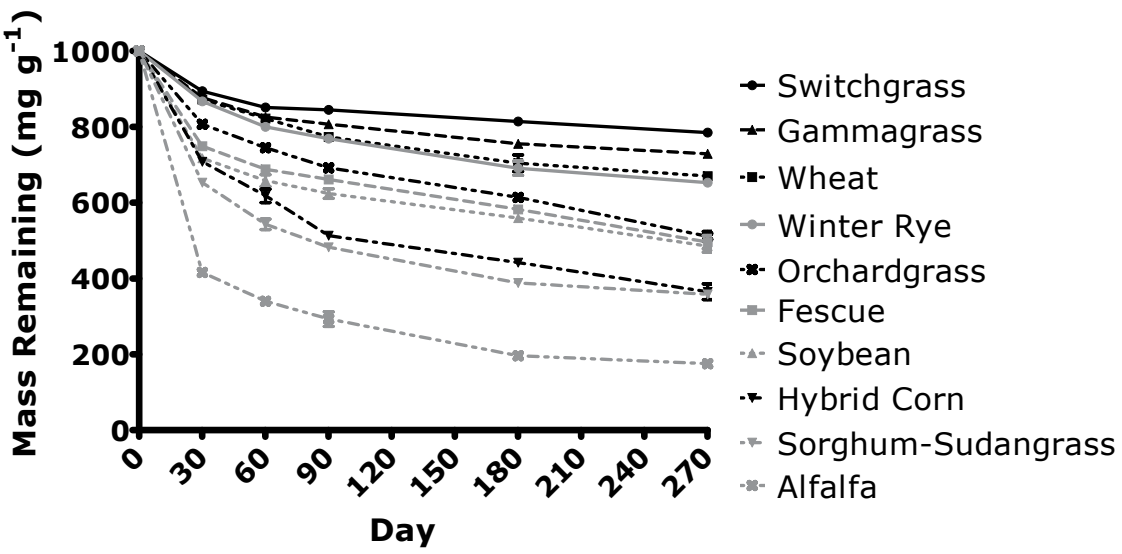

Figure 1: Changes in decomposing root sample mass over a 270 day incubation period. Bars indicate standard error.

Following Day 30 the rate of mass loss for the crop species roots was greater than that of the two native grass species, switchgrass and gammagrass. After Day 30 the rate of mass loss slowed, with the average daily rate of loss following the order: hybrid corn (1.4 mg g^{-1}), sorghum-sudangrass (1.2 mg g^{-1}), orchardgrass (1.2 mg g^{-1}), fescue (1.1 mg g^{-1}), alfalfa (1.0 mg g^{-1}), soybean (1.0 mg g^{-1}), winter rye (0.89 mg g^{-1}), wheat (0.84 mg g^{-1}), gammagrass (0.62 mg g^{-1}) and switchgrass (0.45 mg g^{-1}). Significant differences ($P<0.05$) in rate of mass loss among species followed botanical classifications with rate of mass loss for the warm season perennial grasses, switchgrass and gammagrass, being significantly less ($P<0.05$) than all other species, but not different from each other. Similar results were observed for the rate of mass loss for the cool season perennial grasses, orchardgrass and fescue, which were also not significantly different ($P<0.05$) from soybean. Likewise, the rate of mass loss for the cool season annual grasses, wheat and winter rye, were not significantly different ($P<0.05$) from one another. The rate of mass loss for hybrid corn and sorghum-sudangrass, both warm season annuals, were not

significantly different ($P<0.05$) from one another. Finally, the rate of alfalfa mass losses were significantly different ($P<0.05$) from all other studied species. For this reason the remaining discussion will focus on a representative species from each of these six botanical classification divisions (alfalfa, hybrid corn, soybean, fescue, winter rye and switchgrass) which correspond to important crop and forage species. Though soybean and fescue had similar rates of mass loss, both are included in the following discussion in order to represent another legume in addition to alfalfa.

Changes in Cell Wall Composition During Incubation

In all plant species, over time, root cell wall structural compounds made up nearly the entirety of the sample collected at each sampling interval (Figure 2). In alfalfa and corn, the most decomposable species (Figure 2a and 2b) samples were nearly 100% cell walls by Day 90, however, no significant differences ($P<0.05$) in sample cell wall concentration were found after Day 60. Among the moderately decomposable species, soybean root samples were almost entirely cell walls by Day 60 with no significant difference ($P<0.05$) noted thereafter, while fescue root samples did not approach 100% cell walls until day 90 with no significant differences ($P<0.05$) thereafter (Figure 2c and 2d). The least decomposable of the representative species, winter rye and switchgrass, seemed to maintain a higher proportion of non-cell wall composition until Day 90 and 180, respectively (Figure 2e and 2f). Root cell walls concentration was not significantly different for either species at later samplings.

Examination of the changes in individual cell wall constituents does not reveal any preferential resistance to decomposition for either lignin or cellulose (Figure 2). Sample lignin concentrations in all species increased and were significantly different

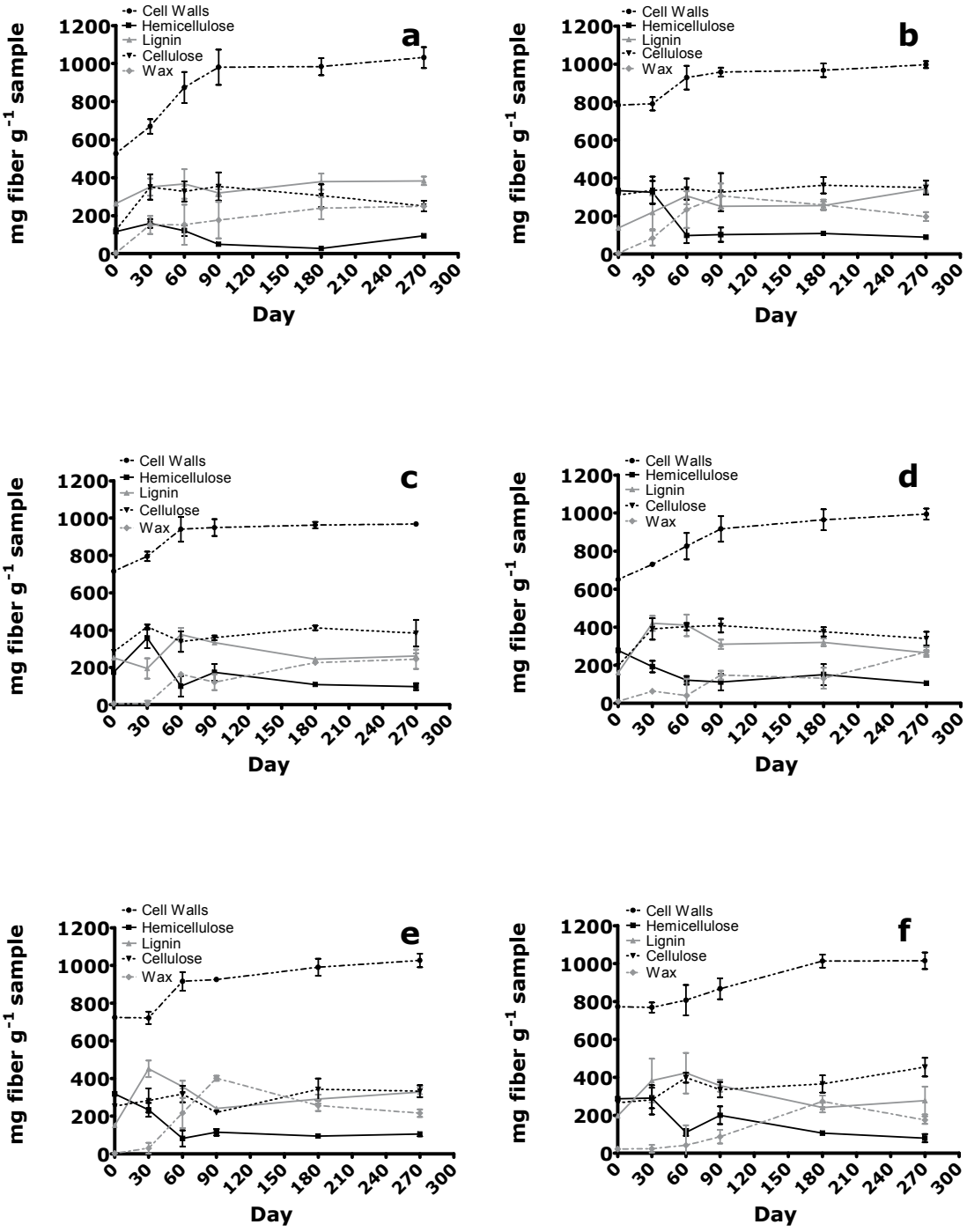

Figure 2: Decomposing root sample cell wall concentration and composition over a 270 day incubation period. a = Alfalfa, b = Hybrid Corn, c = Soybean, d = Fescue, e = Winter Rye, f = Switchgrass. Bars indicate standard error.

($P<0.05$) between Day 0 and Day 30 as easily degraded carbohydrate and protein compounds were decomposed, consistent with the findings presented in Chapter 3. Similar results were seen for cellulose in most species, but the increases were not significant ($P<0.05$). Lignin concentrations declined to levels similar to their Day 0 concentration between Day 30 and Day 90 and no significant differences ($P<0.05$) were noted after Day 60. No significant differences ($P<0.05$) in cellulose concentration were found throughout the incubation period. Initial losses of hemicellulose in all species except alfalfa occurred between Day 0 and Day 60. No significant differences ($P<0.05$) were found after Day 60.

Alfalfa hemicellulose concentrations were significantly different ($P<0.05$) at each sampling interval up to Day 90 and not thereafter. These initial losses were likely due to preferential degradation of more easily accessible and less substituted hemicellulose components. The remaining hemicellulose was likely more extensively cross-linked with lignin and cellulose and possibly more highly substituted (Amin et al., 2014; Machinet et al., 2011), though additional analyses would be required to confirm this. This cross-linking may also explain the stability of the cell wall constituents as a fraction of the sample at each interval throughout the incubation.

These results indicate that following the initial rapid degradation between Day 0 and Day 30 (Figure 1), lignin and cellulose each decompose similarly, maintaining a constant proportion in the samples collected over time. This result is interesting as one would expect hemicellulose and cellulose to be more degradable relative to lignin and the samples to become enriched in lignin over time. Once the more accessible and degradable hemicellulose fraction has been mineralized, it too follows this same trend.

Finally, for all species an initial increase in wax concentration resulting from the decomposition of readily degradable cell components resulted in significant differences ($P<0.05$) at each sampling interval until Day 90. After Day 90 no significant differences ($p<0.05$) in wax concentration were found for any species. For all species except hybrid corn and winter rye the trend over time appears to be for slowly increasing wax concentrations as a proportion of residual cell wall constituents.

Compositional Changes Revealed by DRIFTS

Figure 3 illustrates the ratios of important peak heights in the DRIFTS spectra of the incubated root samples at each sampling interval. Peaks at about 1510 cm^{-1} and 897 cm^{-1} are indicative of root lignin and cellulose (Reeves, 1993, Séné et al., 1994; Pandey, 1999; Dokken and Davis, 2007; White et al., 2011), while peaks at about 1740 cm^{-1} and 1660 cm^{-1} are indicative of carbonyl compounds contained in lipids and waxy compounds such as suberin and hemicellulose, respectively (White et al., 2011; Dokken and Davis, 2007, Pandey, 1999, Reeves, 1993; Séné et al., 1994). In addition, a portion of the peak at 1740 cm^{-1} is also attributable to hemicellulose (White et al., 2011). The diagnostic lignin peak seemed little changed in all of the DRIFTS spectra and as lignin is likely the cell wall fraction that has the greatest control over decomposition rates (Mellilo et al., 1982, Talbot et al., 2012) changes in the peaks for the two sources of carbonyls and cellulose (1740 cm^{-1}, 1660 cm^{-1} and 897 cm^{-1}) were measured relative to lignin (1510 cm^{-1}). Evaluation of these ratios provides information about whether the relative concentrations of these compounds are changing over time. It is important to note that a static ratio might indicate either no change in concentration of the particular constituent, *or* that its concentration is changing at the same rate as that of lignin. Previous research has shown

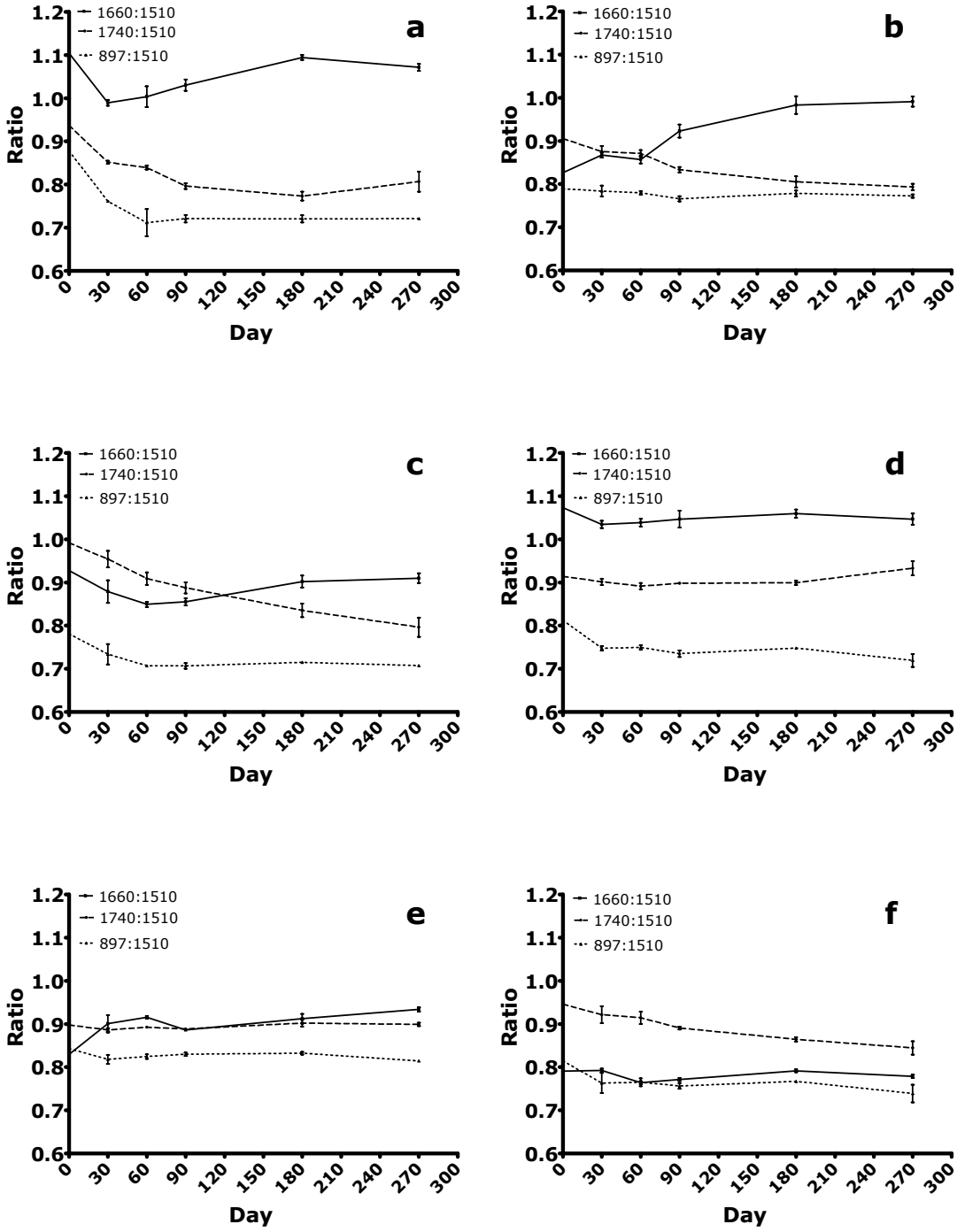

Figure 3: Peak intensity ratios (1660 cm^{-1}:1510 cm^{-1}, 1740 cm^{-1}:1510 cm^{-1}, 897 cm^{-1}:1510 cm^{-1}) resulting from differences in DRIFTS absorbance at wavenumbers indicative of lignin, cellulose, hemicellulose and wax. a = Alfalfa, b = Hybrid Corn, c = Soybean, d = Fescue, e = Winter Rye, f = Switchgrass. Bars indicate standard error.

that changes in these ratios correspond to changes in decomposability of cell wall constituents (White et al., 2015*).*

In general the DRIFTS peak ratio data indicate little change in the cell composition of the incubated roots at each sampling interval over time (Figure 3). The greatest changes are seen in alfalfa, hybrid corn and soybean (Figure 3a, 3b and 3c). In all three of these species, the ratio of the ester carbonyls (1740 cm^{-1}) to the lignin (1510 cm^{-1}) decreased over time. This is in contrast to the trend seen in the fiber data (Figure 2a, 2b and 2c) in which only soybean wax appeared to decrease relative to lignin. In alfalfa (Figure 3a) the decline was significantly different ($P<0.05$) between Day 0 and Day 30 and Day 30 and Day 90. After Day 90 the change in ratio leveled off and no further significant differences were detected. The first significant difference ($P<0.05$) in this ratio in hybrid corn (Figure 3b) was found at Day 90 and again at Day 180. In soybean the ratio was significantly different ($P<0.05$) at each sampling interval throughout the incubation. This data suggests that wax ester compounds are steadily degraded; however as will be discussed below, this data might also indicate the decomposition of the easily degradable fraction of hemicellulose consistent with the fiber data in Figure 2.

Alfalfa cellulose also initially declined as evidenced by the decreasing ratio of the diagnostic cellulose peak (897 cm^{-1}) to lignin (1510 cm^{-1}) between Day 0 and Day 60. The ratio was significantly different ($P<0.05$) at Day 30 and Day 60, but not thereafter. No significant differences ($P<0.05$) were found in this ratio for either hybrid corn or soybean and the curves remained essentially flat (Figure 3b and 3c).

Interestingly, the ratio of the hemicellulose carbonyl peak (1660 cm^{-1}) to lignin (1510 cm^{-1}) increases over time in all three of these species in contrast to the decline in

hemicellulose concentration measured over time (Figure 2a, 2b and 2c). In alfalfa and soybean (Figure 3a and 3c) this ratio declines after Day 0, but then increases by Day 180 to again match the Day 0 value. Days 30, 60 and 90 remained relatively similar for both species but became significantly different ($P<0.05$) from Days 180 and 270. In hybrid corn this ratio began to increase following Day 60 with significant differences ($P<0.05$) noted at Day 90 and Day 180 (Figure 3b). For all three species the ratio approached 1.0 by the end of the incubation period. Though the concentration of hemicellulose and lignin in the samples remained essentially unchanged after Day 60 (Figure 2), the greater degree of tissue degradation (Figure 1) and loss of non-cell wall constituents possibly allowed for greater contribution of residual hemicellulose to the prominence of the peak at 1660 cm^{-1}.

Taken together, this data suggests that in readily degradable roots, some fraction of hemicellulose is preserved relative to lignin. However, considering the low concentration of hemicellulose measured relative to lignin in these samples, particularly in the latter days of the incubation period (Figure 2), this result is unexpected. Absorbances at 1660 cm^{-1} can also be assigned to microbial amides (Movasaghi et al., 2008; Naumann, 1998). Increases in this peak relative to lignin might also indicate colonization of the root tissue by microbial communities. Microbial colonization seems likely given the degree of degradation of these roots (Figure 1). These results also indicate that cellulose is initially degraded, leaving a less degradable fraction cross-linked to lignin and the remaining hemicellulose (Amin et al., 2014; Talbot et al., 2012).

In contrast, the roots from the remaining plant species exhibited minimal changes in any of the DRIFTS peak height ratios. In fescue and winter rye roots (Figures 3d and

3e) no significant differences ($P<0.05$) were found for any of the ratios at any sampling interval. Switchgrass roots (Figure 3f) were similar with the exception of the wax carbonyl: lignin ratio (1740 cm^{-1}:1510 cm^{-1}) which declined steadily over time with significant differences ($P<0.05$) noted between both Day 180 and Day 270 and the rest of the sampling intervals. This observation seems more consistent with the trends for lignin and wax concentrations apparent in the fiber data. This decline corresponds to decreasing hemicellulose concentrations in the samples (Figure 2f). Apart from switchgrass hemicellulose, these less degradable roots (Figure 1) appear to undergo little decomposition of cell wall constituents relative to their starting concentrations on Day 0.

Alfalfa root lost 82.5% of its starting mass by Day 270 (Figure 1) and the effect of that degree of degradation is evident in the DRIFTS spectra of the root residues measured at different sampling intervals (Figure 4). Figure 4 and each of the following figures illustrate the complete DRIFTS spectra from 400 cm^{-1} to 4000 cm^{-1} as well as three regions of particular interest, from 2800 cm^{-1} to 3000 cm^{-1} (a), from 1450 cm^{-1} to 1800 cm^{-1} (b) and from 1000 cm^{-1} to 1300 cm^{-1} (c). These regions represent changes in aliphatic CH_2, contributions from ester and acid carbonyl as well as lignin, and the polysaccharide fingerprint region, respectively.

Supporting evidence for the preservation of wax as measured in fiber analysis (Figure 2) is found in the DRIFTS spectra. Peaks at 2930 cm^{-1} and 2855 cm^{-1} (Figure 4a) are produced by asymmetric and symmetric CH_2 stretching, respectively, which have been attributed to suberin (Dokken and Davis, 2007; Lopes et al., 2000; Zeier and Schreiber, 1999). Over time the peak at 2930 cm^{-1} becomes better defined by Day 270, as does the shoulder at 2855 cm^{-1} which is not present on Day 0. These peaks are found in

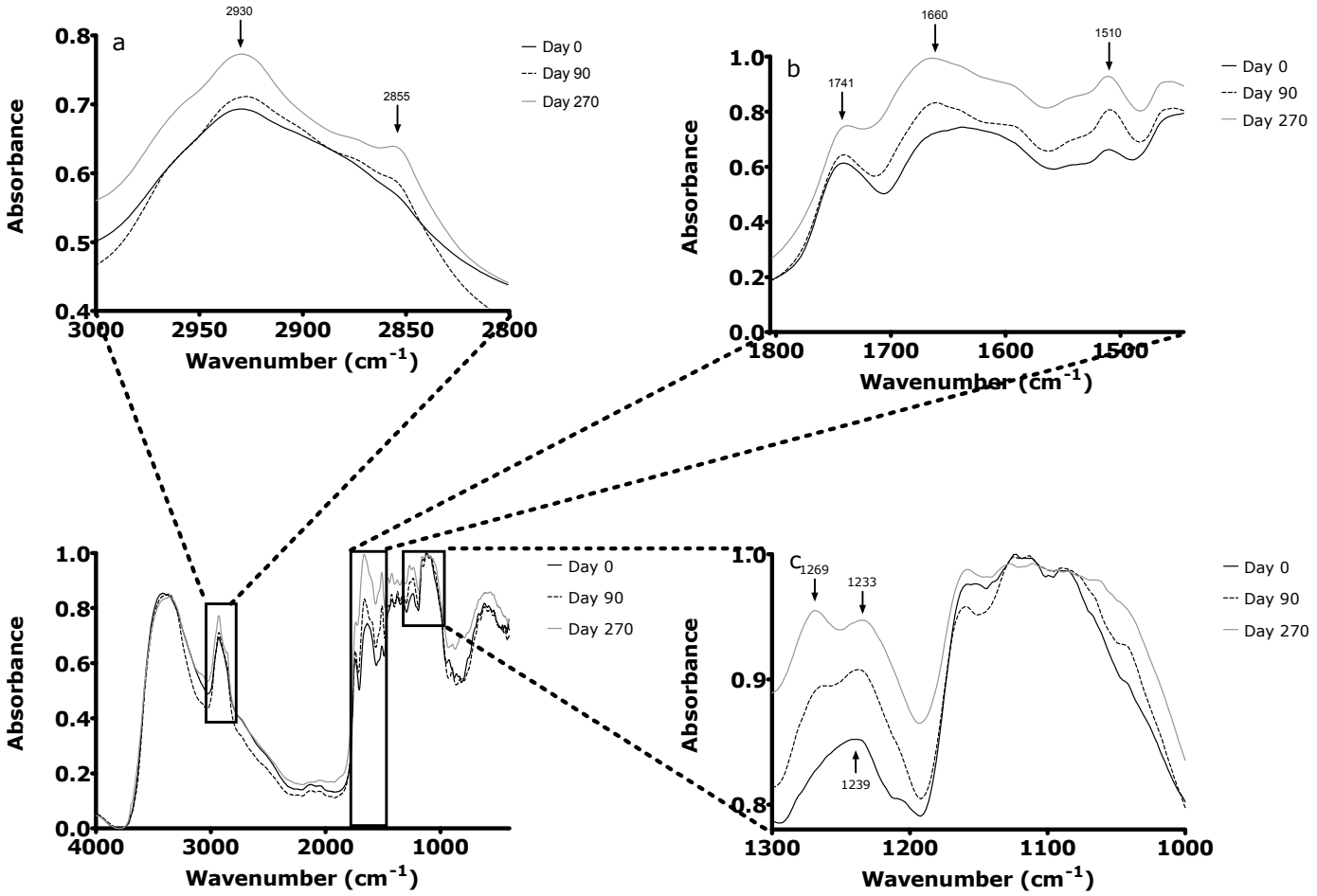

Figure 4: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of decomposing alfalfa root tissue recorded at Day 0, 90 and 270 and regions illustrating changes potentially indicative of suberin (a), peaks arising from suberin and hemicellulose carbonyls as well as lignin (b), and the polysaccharide fingerprint region (c).

transesterified root cell walls and are evident in the spectrum of suberin extracted *Quercus suber* cork (Lopes et al., 2000). The increasing prominence of these peaks suggest a greater contribution from aliphatic lipids in suberin as other root compounds are degraded.

In Figure 4b the peak at 1741 cm^{-1} remains evident throughout. This peak is the result of carbonyl stretching in lipid esters (Dokken and Davis, 2007; Lopes et al., 2000; Zeier and Schreiber, 1999; Séné et al. 1994, Schreiber et al., 2005; Movasaghi et al., 2008). As stated above, the acid carbonyls of hemicellulose also contribute to this peak (White et al., 2011). This peak initially declines in intensity relative to lignin (Figure 3a), reflecting hemicellulose degradation prior to Day 90 (Figure 2a). The stability of this peak relative to lignin after Day 90 (Figure 3a), combined with the spectral evidence for suberin aliphatics (Figure 4a), may be another indication of the preservation of suberin during root decomposition.

The lignin peak at 1510 cm^{-1} remains distinct throughout the incubation period and becomes better defined as degradation progresses, supporting the consistent and ultimately predominant lignin concentration in samples throughout the incubation (Figure 2a). The increasing prominence of the peak at 1660 cm^{-1} is also evident. At Day 0 this peak isn't apparent at all, but by Day 270 it equals the intensity of the lignin peak suggesting increasing contributions of hemicellulose carbonyls as the alfalfa roots decompose. However, as was discussed above this increase is also likely due to the microbial colonization of the highly degraded root tissue.

Evidence for the potential preservation of a more resistant fraction of hemicellulose can be seen in the polysaccharide fingerprint region between 1000 cm^{-1} to

1300 cm^{-1} (Figure 4c). The pattern of the peaks in this region arises from difficult to interpret, complex interactions of vibrations from polysaccharide backbones (Dokken and Davis, 2007; Séné et al., 1994; Kačuráková et al., 1994; Robert et al., 2005). Different polysaccharides exhibit distinctive peak maxima in this region related to their backbone structure and substituents (Kačuráková et al., 2000, Roberts et al, 2005). As a result differences in this fingerprint for a given species over time are indicative of changes in hemicellulose structure during decomposition even though specific peaks are difficult to assign. Peaks in the region between 1185 cm^{-1} to 1280 cm^{-1} are attributed to C-O-H deformation and C-O stretching of phenolics coupled with C-C-O stretching of esters (Séné et al., 1994) and have been cited as diagnostic for hemicellulose (Dokken and Davis, 2007). Peaks in the region between 1000 cm^{-1} to 1190 cm^{-1} have been assigned to C-O and C-O-C and C-C stretching as well as ring vibrations and C-OH bending in hemicellulose arabinoxylans (Kačuráková et al., 1994; Kačuráková et al., 2000). Changes are evident in each of these regions as decomposition progresses over time. For example, the peak at 1239 cm^{-1} in the Day 0 spectrum shifts toward 1233 cm^{-1} and a second peak at 1269 cm^{-1} begins to appear in Day 90 spectrum, becoming prominent by Day 270. Further evidence can be seen in the distinct changes in the shape of the peaks in the region between 1000 cm^{-1} and 1190 cm^{-1} over time. Taken together, this evidence suggests changes in hemicellulose structure during decomposition, potentially arising from the disappearance of easily degraded hemicellulose structures and the preservation of more substituted and difficult to degrade polysaccharides. This would explain the persistence of hemicellulose throughout the incubation period (Figure 2a).

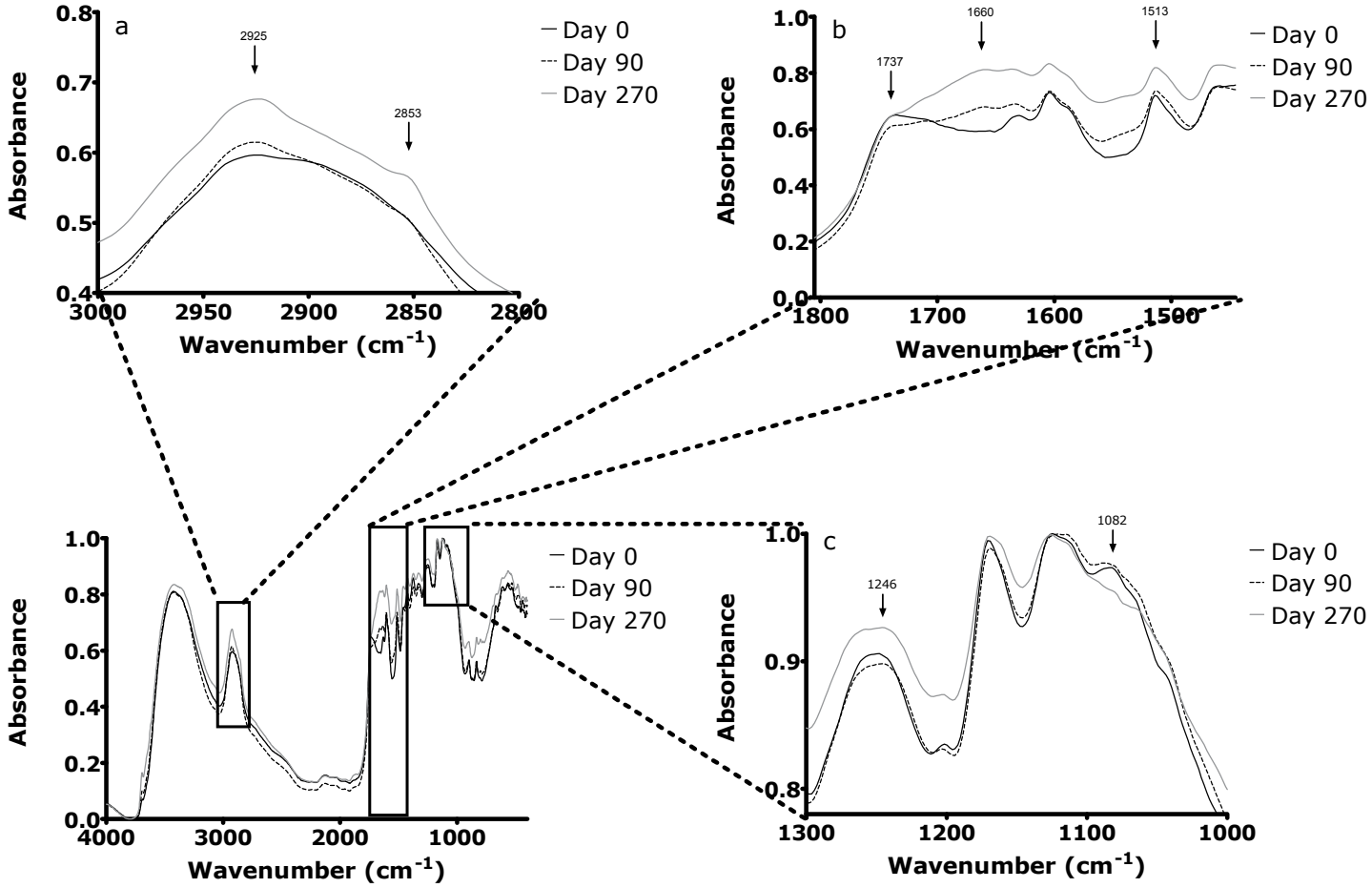

Figure 5: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of decomposing hybrid corn root tissue recorded at Day 0, 90 and 270 and regions illustrating changes potentially indicative of suberin (a), peaks arising from suberin and hemicellulose carbonyls as well as lignin (b), and the polysaccharide fingerprint region (c).

Similar trends are seen in the spectra of hybrid corn which lost 63.5% of its initial mass (Figure 1) during the course of the incubation period. Like alfalfa peaks indicative of aliphatic CH_2, in this case 2925 cm^{-1} and a shoulder at 2853 cm^{-1} become more pronounced by Day 270 (Figure 5a) as wax concentration in the samples increased (Figure 2b). Unlike alfalfa the shoulder was not apparent at Day 90, perhaps as result of the lesser degree of decomposition at this date relative to alfalfa. The lignin peak at 1513 cm^{-1} remains distinct and little change is seen in its shape at any interval (Figure 5b) consistent with the fiber data (Figure 2b). The intensity of the suberin carbonyl peak at 1737 cm^{-1} appears similar at each interval, but steadily decreases in intensity relative to lignin (Figure 3b) as more degradable hemicellulose is decomposed (Figure 2b). As with alfalfa the peak at 1660 cm^{-1} isn't apparent at Day 0, but increases over time along with increasing degradation and microbial biomass. Change in the polysaccharide fingerprint region was less pronounced than that found in alfalfa (Figure 5c). The band from 1211 cm^{-1} to 1298 cm^{-1} had a consistent maximum at 1246 cm^{-1} and did not split into two distinct peaks as occurred in alfalfa, though the top of the band did become broader with time. The shape of the region between 1000 cm^{-1} and 1190 cm^{-1} differed from that of alfalfa and unlike alfalfa there was little change save for the disappearance of a peak at 1082 cm^{-1} in the Day 270 spectrum. This peak may arise from arabinoxylose ring vibrations and has been reported to be strongly influenced by the degree of branching and hydration in the polysaccharide (Kačuráková et al., 1994). This could indicate changes in hemicellulose structure and substitution as the more easily degraded portions are decomposed, but sugar analysis would be required to confirm this. The differences in the response between alfalfa and hybrid corn may be due to differences in hemicellulose

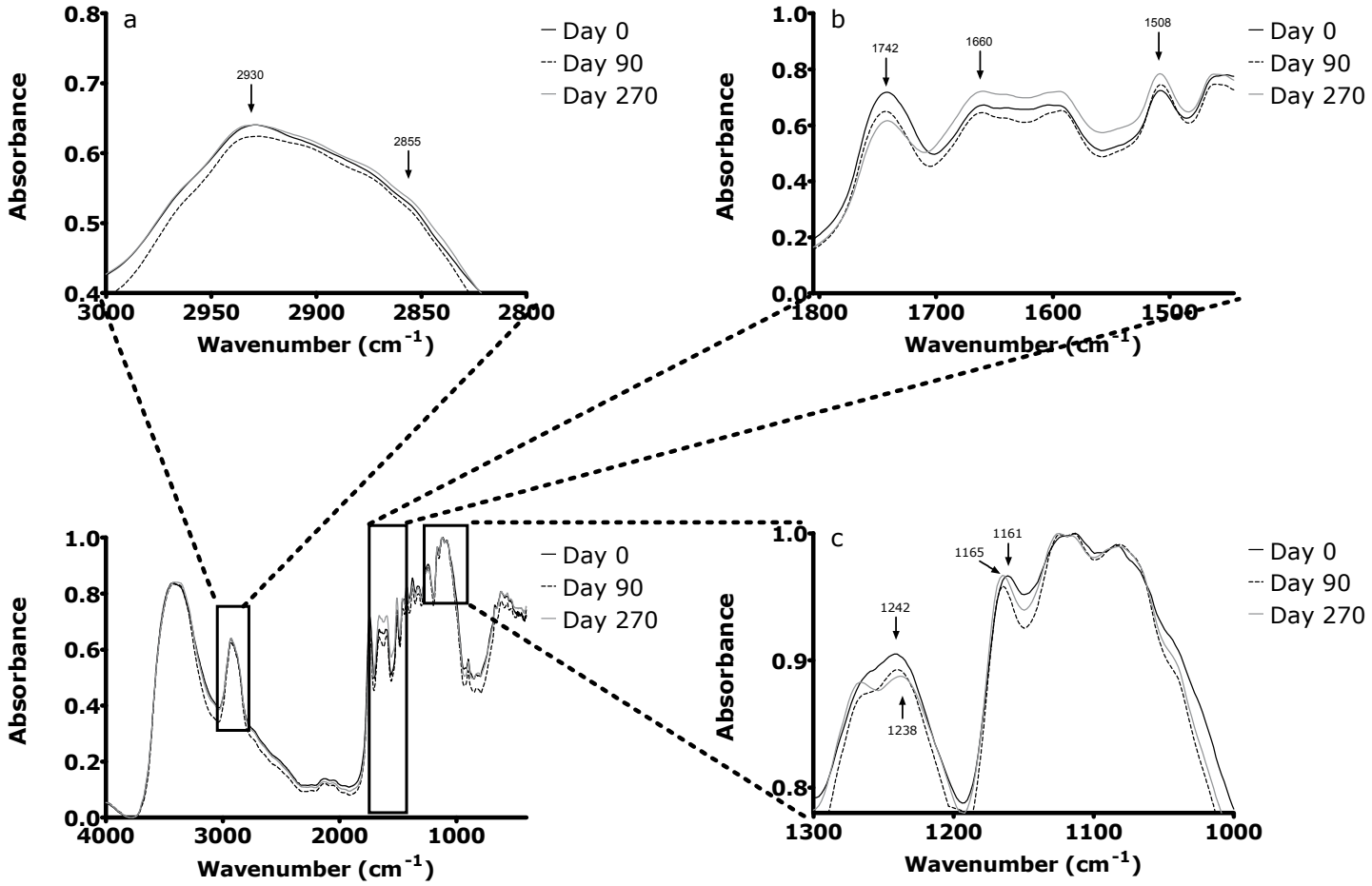

Figure 6: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of decomposing soybean root tissue recorded at Day 0, 90 and 270 and regions illustrating changes potentially indicative of suberin (a), peaks arising from suberin and hemicellulose carbonyls as well as lignin (b), and the polysaccharide fingerprint region (c).

structure between the two species. Alfalfa hemicellulose is principally composed of rhamnoxylose, while grasses such as corn are predominantly arabinoxylose (Dehority, 1993; Machinet et al., 2009) which would result in different DRIFTS fingerprints.

Despite losing 51.5% of its original mass (Figure 1) little change is evident in the DRIFTS spectra of decomposing soybean roots (Figure 6). In contrast to alfalfa, the other legume, there was no change in the peak at 2930 cm^{-1} and no shoulder at 2855 cm^{-1} was evident at any sampling interval (Figure 6a). Stretching vibrations in this region arise from C-H groups attached to many compounds. Given the lower degree of degradation, this may have obscured the specific peaks arising from the aliphatic CH_2 at these wavenumbers. The peak at 1742 cm^{-1} steadily decreases in intensity relative to lignin (1508 cm^{-1}), while the peak intensity at 1660 cm^{-1} increases (Figure 6b, Figure 3c). Little change is seen in the intensity of the diagnostic lignin peak. The change at 1660 cm^{-1} is not as pronounced as was seen in alfalfa or hybrid corn. If the hypothesis that the increase in this peak is due to microbial colonization rather than hemicellulose preservation, one would expect less microbial biomass and therefore less change at lower levels of degradation. Very little change in the polysaccharide fingerprint region is also apparent in these less decomposed roots. The overall shape of the peaks in the region from 1000 cm^{-1} to 1298 cm^{-1} is similar to that seen in alfalfa. Since both species are legumes, they likely have similar hemicellulose structure. The only change evident is the peak at 1161 cm^{-1} possibly arising from C-O-C stretching of the glycosidic link (Kačuráková et al., 1994; Robert et al 2005) which shifts to 1165 cm^{-1}. There is some change in the shape of the band between 1298 cm^{-1} and 1211 cm^{-1} over time, with a shift in the peak at 1238 cm^{-1} at

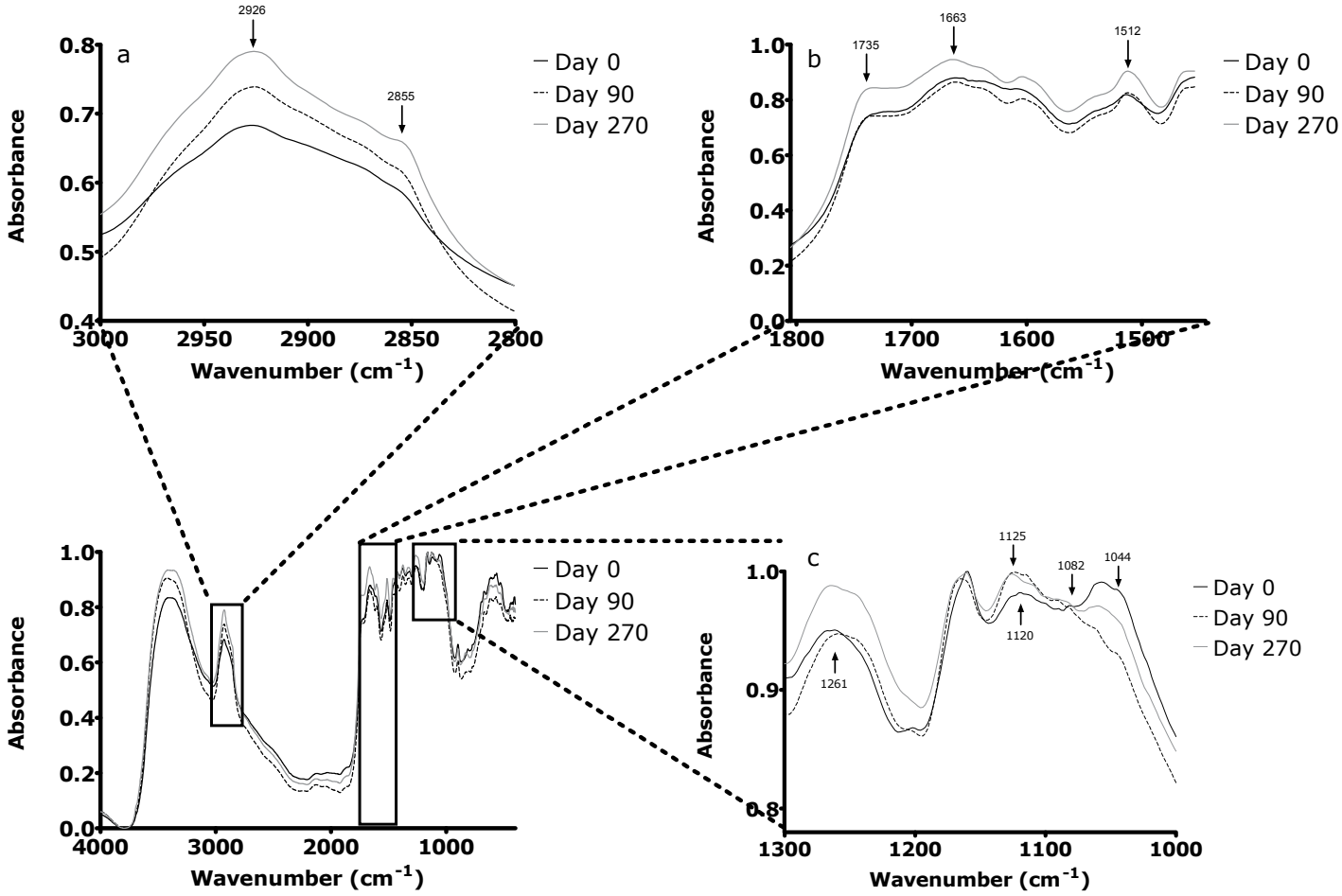

Figure 7: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of decomposing fescue root tissue recorded at Day 0, 90 and 270 and regions illustrating changes potentially indicative of suberin (a), peaks arising from suberin and hemicellulose carbonyls as well as lignin (b), and the polysaccharide fingerprint region (c).

Day 0 to 1242 cm^{-1} at Day 270. Taken together this data suggests a change in residual hemicellulose structure, but not to the extent seen in the more decomposable alfalfa roots.

Slightly different effects are seen in the DRIFTS spectra of fescue, despite a similar degree of decomposition (50.4% of Day 0 mass). Changes in the peak at 2926 cm^{-1} and the shoulder at 2855 cm^{-1} are similar to those seen in hybrid corn roots (Figure 7a) reflecting the increase in wax concentration (Figure 2d). Yet unlike hybrid corn, the suberin carbonyl peak at 1735 cm^{-1} does not decrease in intensity relative to lignin at 1512 cm^{-1} over time. Instead it maintains essentially a constant ratio (Figure 7b, Figure 3d). As with soybean roots, the intensity of the hemicellulose carbonyl peak (1663 cm^{-1}) remains constant suggesting less microbial colonization. Little change was found in hemicellulose or lignin concentration in the root tissue after Day 60 and Day 30, respectively (Figure 2d,) which is supported by the near constant intensity of the two peaks. The only changes in the structure of hemicellulose were evident in the polysaccharide fingerprint region (Figure 7c). The band from 1211 cm^{-1} to 1298 cm^{-1} changes shape but the maxima remains constant at 1261 cm^{-1}. Substantially more change was seen in the region from 1000 cm^{-1} to 1190 cm^{-1} than was measured in hybrid corn, the more decomposable grass. For example, the peak at 1120 cm^{-1} which arises from C-O and C-C stretching (Kačuráková et al., 1994) shifts to 1025 cm^{-1} after Day 90. Unlike hybrid corn no appreciable peak is seen at 1082 cm^{-1}, but there is a shoulder at 1044 cm^{-1} from C-OH bending (Kačuráková et al., 1994) that was not present in the hybrid corn spectra. This shoulder becomes less distinct by Day 270. These differences may represent

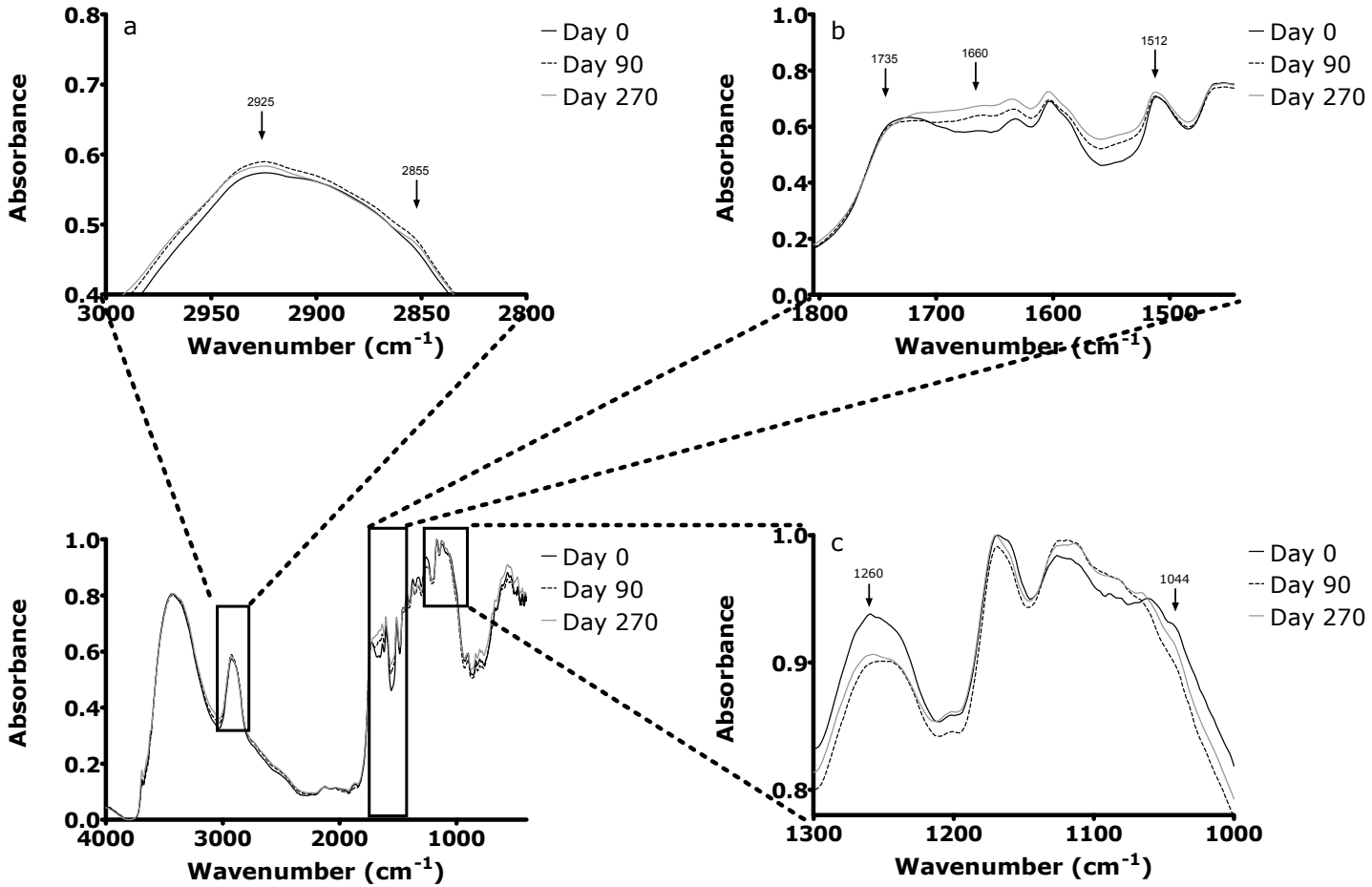

Figure 8: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of decomposing winter rye root tissue recorded at Day 0, 90 and 270 and regions illustrating changes potentially indicative of suberin (a), peaks arising from suberin and hemicellulose carbonyls as well as lignin (b), and the polysaccharide fingerprint region (c).

differences in hemicellulose structure and substitution resulting in differences in the preservation of hemicellulose in perennial cool season fescue vs. annual warm season hybrid corn.

The trend for fewer changes in the DRIFTS spectra as the degree of decomposition decreases is clear in the spectra of winter rye roots (34.7% Day 0 mass lost). In fact the trace of each spectrum from widely separated sampling days nearly overlaps one another (Figure 8). Like soybean there were no changes in the peak at 2925 cm^{-1} and no shoulder at 2855 cm^{-1} (Figure 8a) as minimal root decomposition resulted in CH vibrations from many sources that obscured these features. There is no change in the suberin carbonyl peak at 1735 cm^{-1}, or in the lignin peak at 1512 cm^{-1} (Figure 8b). There is a slight increase in intensity at 1660 cm^{-1}, but the peak is negligible. All of this data suggests little change in root cell wall composition. Only a few slight changes in the polysaccharide fingerprint region are evident (Figure 8c). Like fescue, which is also a cool season grass, there is a shoulder present at 1044 cm^{-1} that becomes less distinct by Day 270, as the peak at 1260 cm^{-1} becomes sharper and more pronounced. There was little change in winter rye cell wall composition throughout the study (Figure 2e) and little overall degradation, and this is reflected in the DRIFTS spectra.

Switchgrass was the least decomposed of all the studied species, losing only 21.5% of its starting mass by Day 270 (Figure 1). Like rye, the individual spectra plot nearly overlap illustrating little change in root composition throughout the incubation. However, unlike winter rye, some distinct changes were noted in the region corresponding to suberin aliphatic CH_2 vibrations (Figure 9a). The peak at 2926 cm^{-1} at

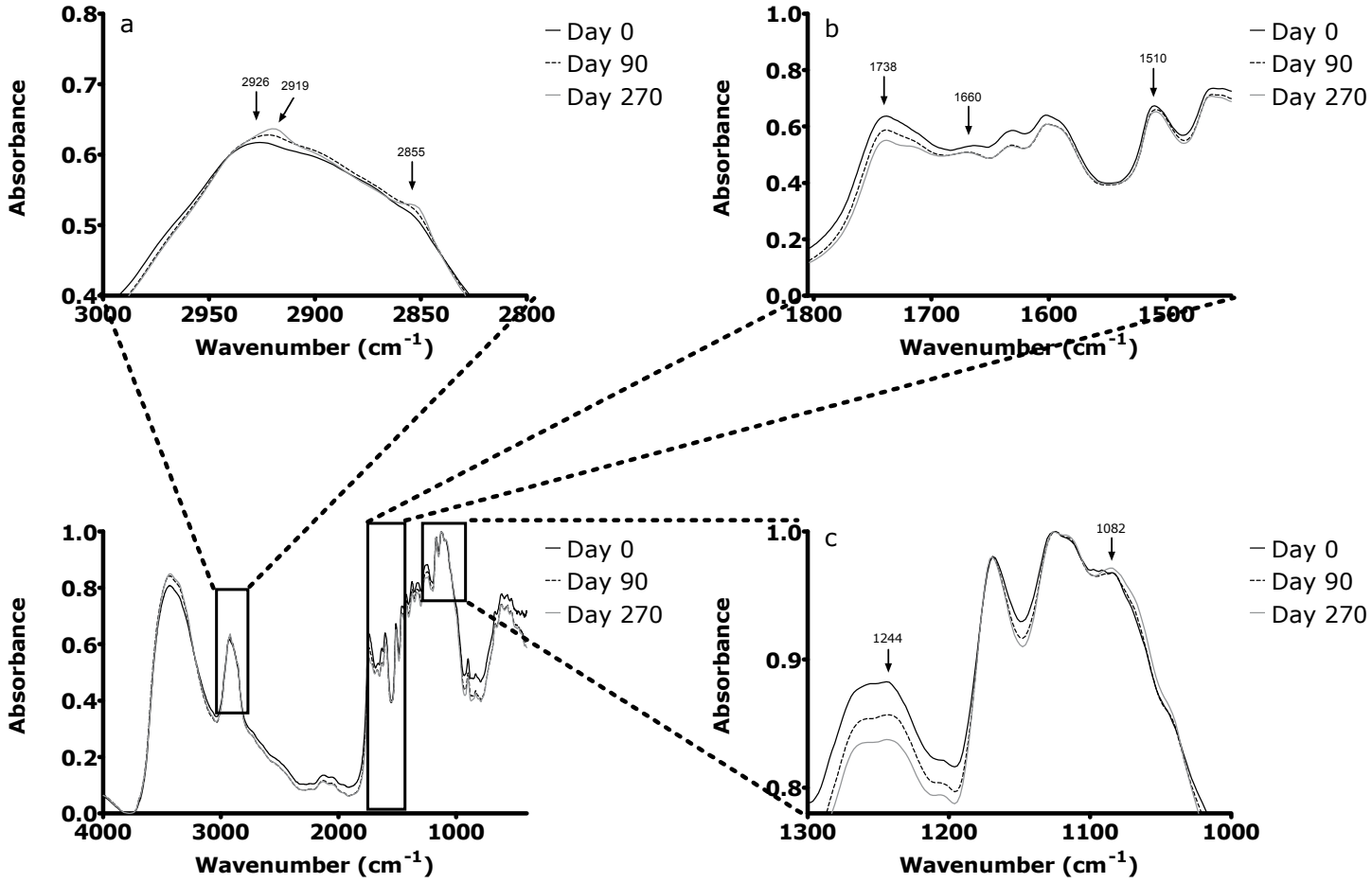

Figure 9: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of decomposing switchgrass root tissue recorded at Day 0, 90 and 270 and regions illustrating changes potentially indicative of suberin (a), peaks arising from suberin and hemicellulose carbonyls as well as lignin (b), and the polysaccharide fingerprint region (c).

Day 0 shifts to 2919 cm^{-1} by Day 270 with a shoulder at 2855 cm^{-1}. Even with very little overall degradation of root tissue, there is evidence for the potential preservation of suberin aliphatics suggested by the increasing wax concentration in the root residue over time (Figure 2f). The intensity of the suberin carbonyl peak at 1738 cm^{-1} declines relative to the unchanged lignin peak (1510 cm^{-1}) (Figure 9b, Figure 3f) but given the evidence for suberin aliphatics this decline is likely caused by the decomposition of readily degradable fractions of hemicellulose consistent with the decline in fiber concentration (Figure 2f). There is also minimal change in the peak at 1660 cm^{-1} consistent with the relative slight degree of decomposition despite the fact that hemicellulose concentration in the samples declined with time (Figure 2f) perhaps indicating the preservation of a more substituted and cross-linked fraction. The polysaccharide fingerprint region looks very similar to that of hybrid corn, also a warm season grass (Figure 9c). The shape of the band from 1211 cm^{-1} to 1298 cm^{-1} becomes slightly broader at the top, but the maxima remain constant at 1246 cm^{-1}. Like hybrid corn there is a peak at 1082 cm^{-1} at Day 0, but unlike those spectra the peak becomes more slightly more pronounced as decomposition progresses. Similar to corn changes in this peak could be an indicator of changes in hemicellulose structure as this peak is influenced by the degree of branching and hydration of the polysaccharide polymer (Kačuráková et al., 1994).

CONCLUSIONS

There are large species dependent differences in the rate, extent and nature of root components that are degrading over time. After an initial rapid period, root decomposition can be characterized as the slow degradation of root cell walls. Lignin, cellulose and resistant hemicellulose appear to degrade in proportion to one another

throughout this latter degradation period. The IR data supports this observation as the peak ratios indicative of these molecules follow trends consistent with the wet chemical data. Changes in characteristic features in the DRIFTS spectra of decomposing roots revealed details of changes in root cell wall composition. In particular, revealing the preservation of suberin and potentially indicating changes in hemicellulose structure as the molecule becomes more resistant to degradation over time. Changes in the features of the polysaccharide fingerprint spectral region appear species dependent possibly due to differences in hemicellulose composition and degradability. Chemical analysis of sugar composition is necessary to confirm these differences. These results demonstrate that differences in root composition and cell wall structural characteristics among species strongly influence the rate and degree of root tissue degradation and mineralization. Differences in the extent of decomposition and the nature of the cell wall components degraded among warm season grasses, cool season grasses and legumes suggests that no broad conclusions regarding soil C cycling and potential contribution to C sequestration can be made on this basis. This further confirms the necessity of detailed knowledge of root composition and structure and decomposition characteristics to effectively predict SOM dynamics following changes in agricultural cropping practice, efforts to increase soil C sequestration, or soil disturbance such as tillage, logging or fire. This ability is crucial for farmers and land managers seeking to maximize soil health, productivity and long term organic C stabilization.

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Chapter 5: Conclusions

The cell wall composition of plant roots varies by species. Both fiber analysis and DRIFTS reveal measurable differences in root composition both prior to and during root decomposition. Fiber analysis and DRIFTS data are consistent with one another and are complementary methods for the quantification and characterization of root cell wall composition both prior to and during root decomposition. The results of this study demonstrate that DRIFTS is a valuable tool for root analysis. In particular, DRIFTS is sufficiently sensitive to reveal details of root composition, such as suberin content, that are present in amounts too minute to be effectively measured by fiber analysis. In addition, DRIFTS provides insight into structural changes in cell walls such as changes in structural polysaccharide composition.

The rate and extent of root tissue degradation in both the short and long term is highly variable among species. Differences in initial decomposition appear to largely be the result of differences in readily degradable non-cell wall root components such as proteins, soluble sugars and starch. In addition, alfalfa root cell wall lignin, cellulose and hemicellulose are much more degradable than was observed for any of the other studied species in both short and long term incubations. In the short term, alfalfa root samples were characterized by increasing lignin concentrations relative to more degradable cellulose and hemicellulose. The concentrations of root cell wall constituents of the other studied species did not change in the short term. This was supported by DRIFTS data as the relative difference of the diagnostic lignin and cellulose peaks increased over time. In addition, DRIFTS was potentially able to detect the preservation of suberin despite the large degree of uncertainty in measurements of wax concentrations in the decomposing

root tissues using traditional fiber analysis techniques. This further illustrates the utility of DRIFTS for the assessment of cell wall structural changes in decomposing roots.

For all studied species, long term decomposition was characterized by slow degradation of cell wall constituents. Surprisingly, root residues do not appear to become enriched with lignin relative to the other constituents, rather lignin, cellulose, hemicellulose and wax all appear to degrade proportionally. As with the short term data, DRIFTS revealed finer details of changes in cell wall composition that supported and expanded upon the trends seen in the fiber data. The DRIFTS data indicate the potential preservation of suberin. In addition, differences in the polysaccharide fingerprint region illustrate differences in hemicelluloses structure among species. Changes in the polysaccharide fingerprint as roots decompose appear to be species dependent and indicate changes in hemicellulose structure as easily degraded hemicelluloses are preferentially decomposed. The results demonstrate that differences in root composition and cell wall structural characteristics among species influence the rate of root tissue decomposition.

Taken together, the results of these studies demonstrate that root degradation is highly variable and species dependent. Differences in root tissue composition and particularly cell wall composition exert a strong influence on tissue degradation both immediately after root senescence and over longer time scales. Characterization of root composition and the nature and extent of the interconnection of root cell wall macromolecules for a wide variety of species will be necessary to understand and predict their role in SOM cycling and as well as the extent to which a given species will potentially contribute to atmospheric C sequestration. The results of these studies expand

knowledge of the role of root cell wall macromolecules in the formation of SOM. The analytical tools evaluated have demonstrated the utility of DRIFTS for the characterization of the composition of both fresh and decomposing roots enabling simplified collection of data essential for modeling the terrestrial C cycle. This research provides a needed assessment of the influence of root composition on root C recalcitrance. The information on root composition and changes during mineralization will aid researchers studying SOM formation and stabilization and help to develop a more complete understanding of soil C dynamics. This is essential to understand and predict the potential impact of changes in land management practices, such as a change in cropping system, as well as the impact of soil disturbance on soil health and productivity. In addition, this research has contributed to understanding the role of root-derived compounds in the long term stabilization of organic C and the potential role of crop roots in atmospheric C sequestration. This information is vital to farmers and land managers seeking to maximize soil health and productivity and to those seeking to model, measure and enhance long-term organic C stabilization.

Appendix: Additional data

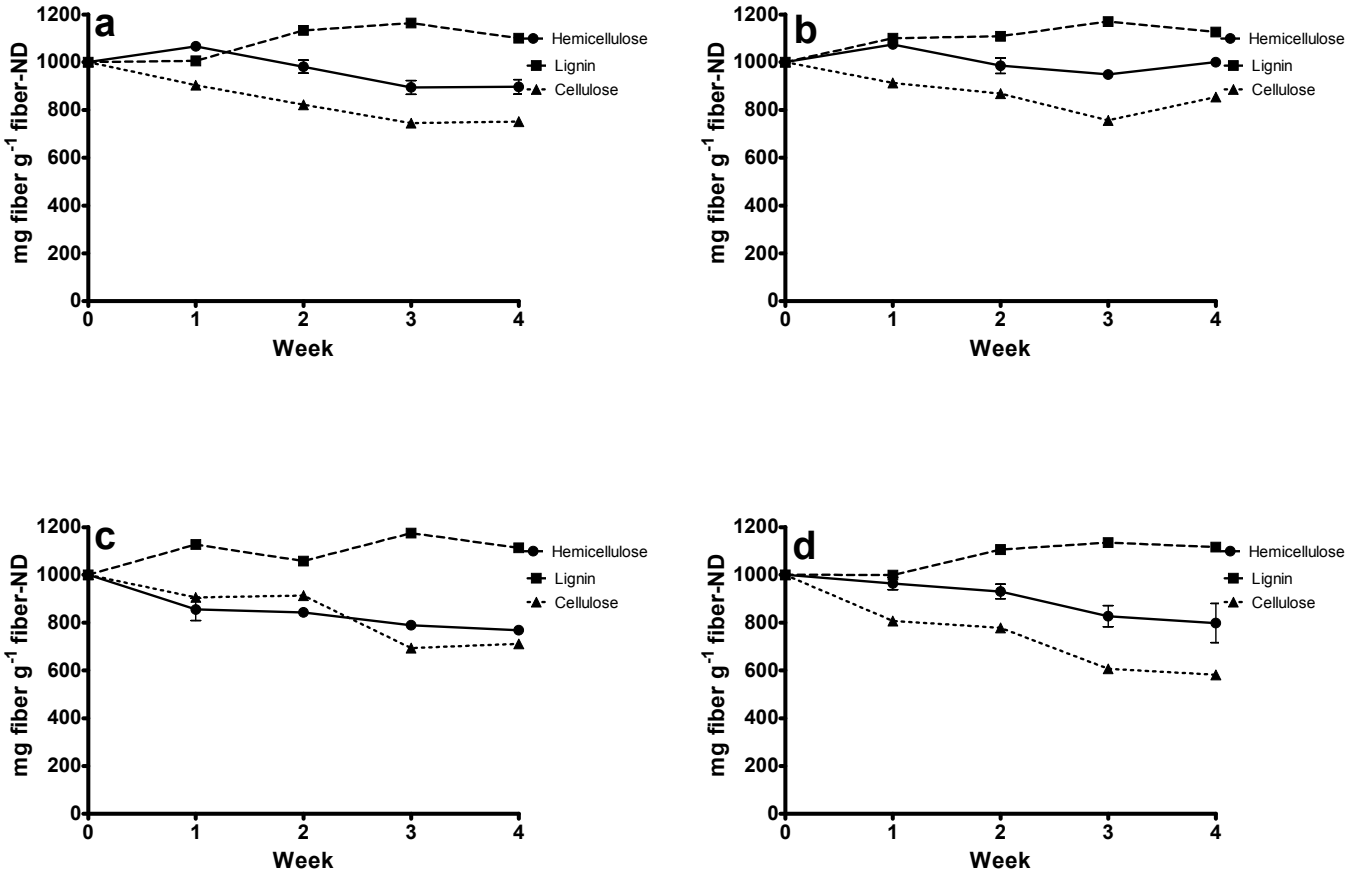

Figure 1: Decomposability hemicellulose, lignin and cellulose in decomposing corn root (a), fescue root (b), orchardgrass root (c), and soybean root (d) samples expressed as a fraction each cell wall constituent in the un-incubated root samples over a four week incubation period. Bars indicate standard error.

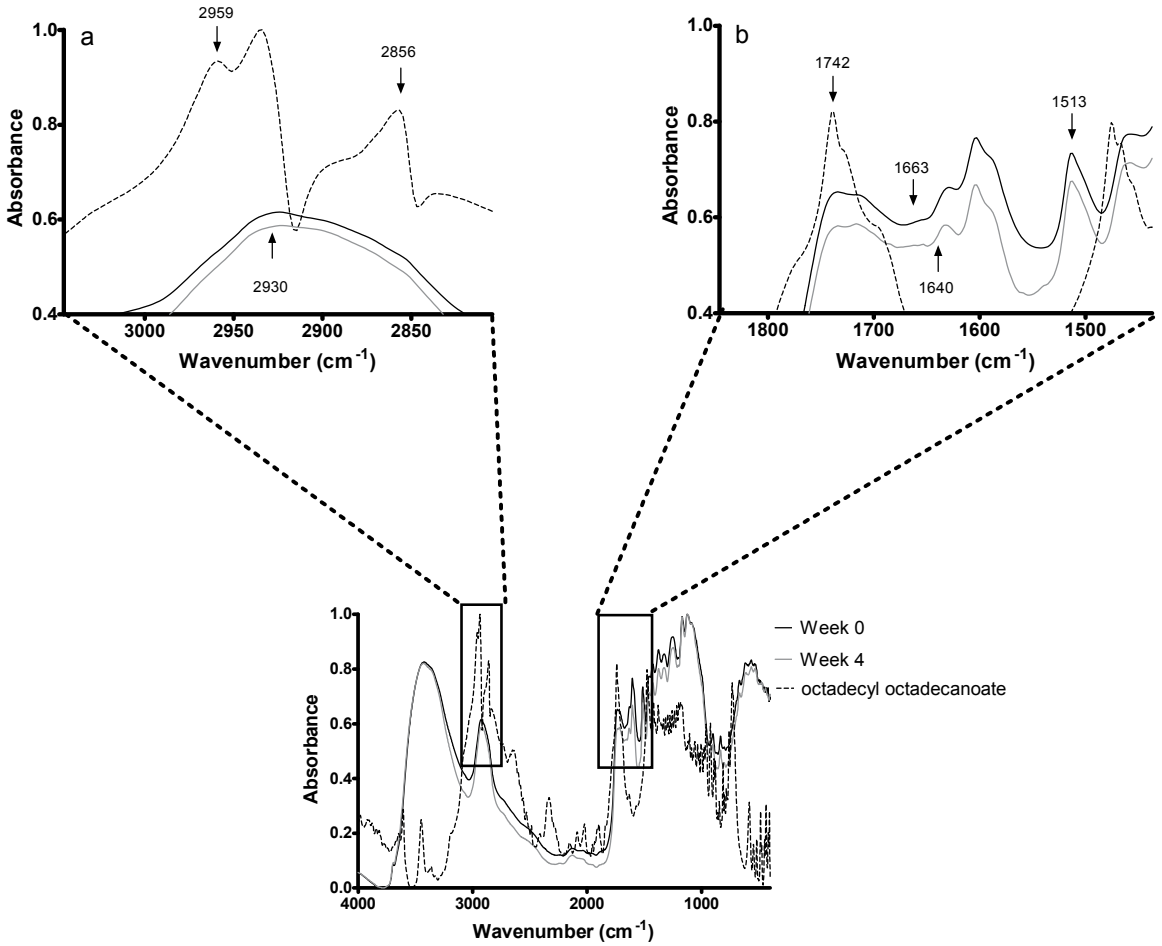

Figure 2: Comparison of changes in the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of corn roots at Week 0 and Week 4 of a four week incubation and the spectrum of octadecyl octadecanoate.

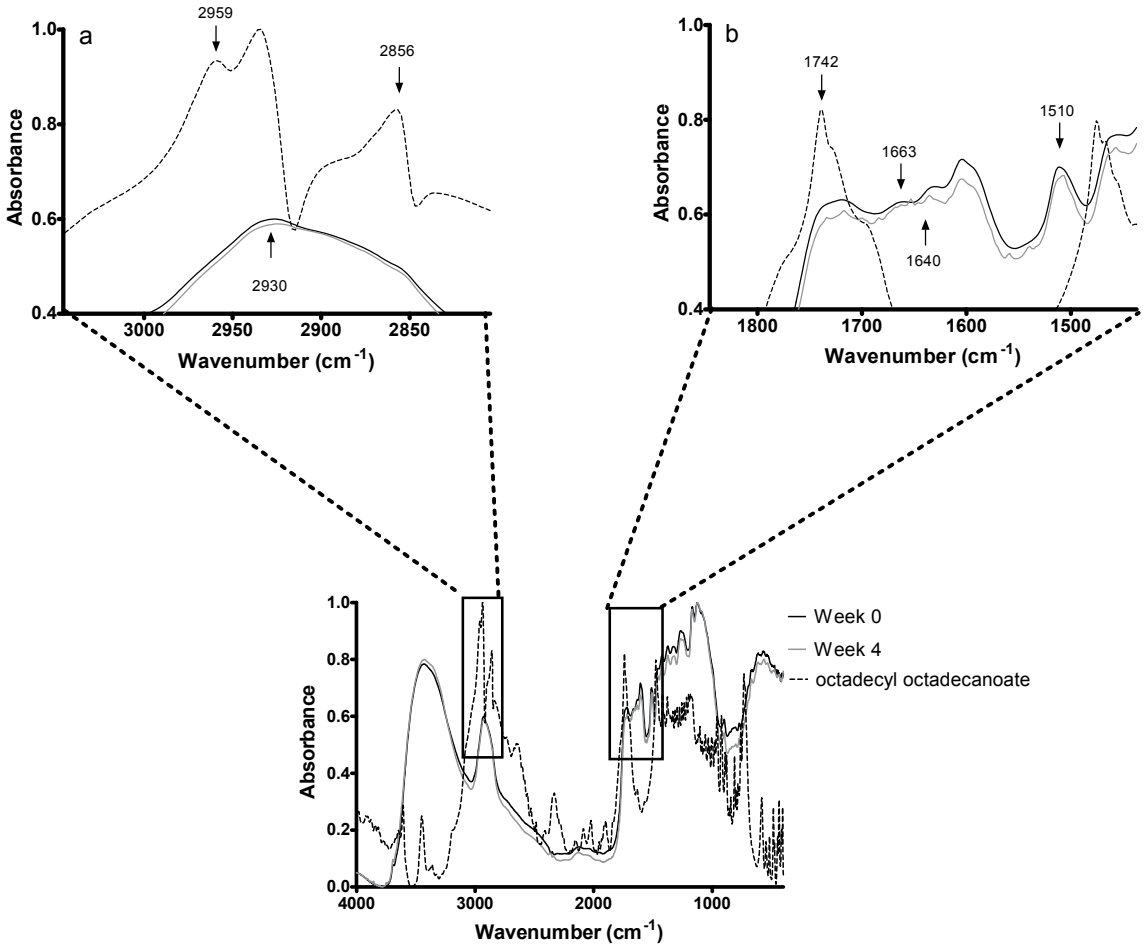

Figure 3: Comparison of changes in the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of fescue roots at Week 0 and Week 4 of a four week incubation and the spectrum of octadecyl octadecanoate.

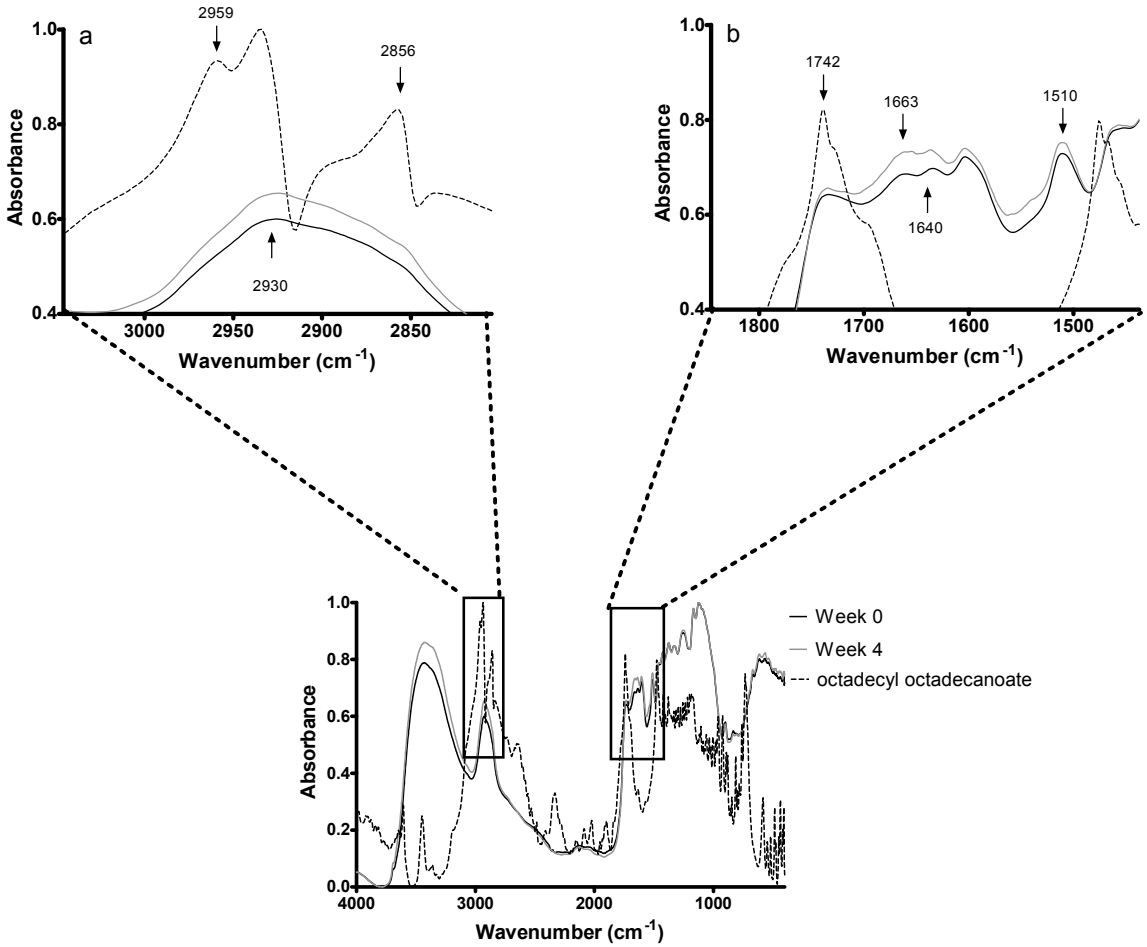

Figure 4: Comparison of changes in the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of orchardgrass roots at Week 0 and Week 4 of a four week incubation and the spectrum of octadecyl octadecanoate.

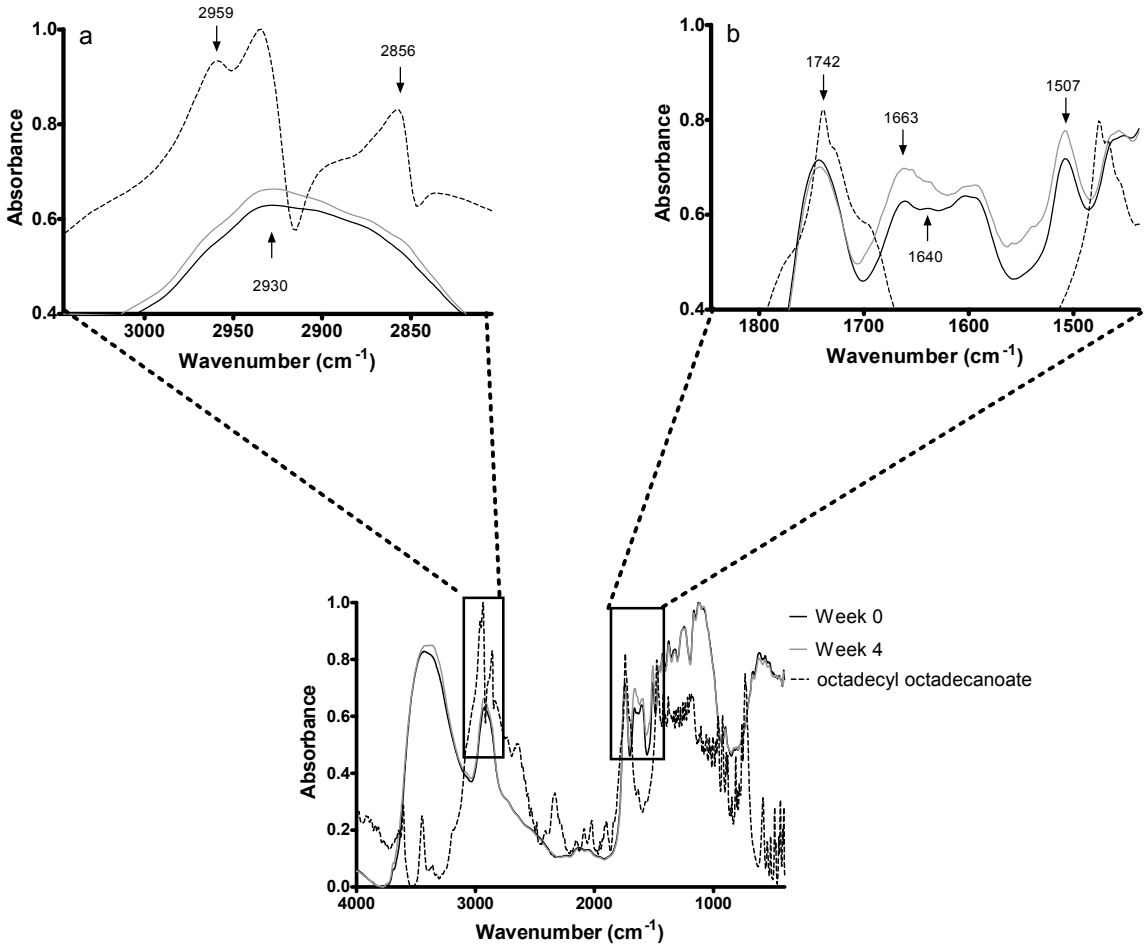

Figure 5: Comparison of changes in the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of soybean roots at Week 0 and Week 4 of a four week incubation and the spectrum of octadecyl octadecanoate.

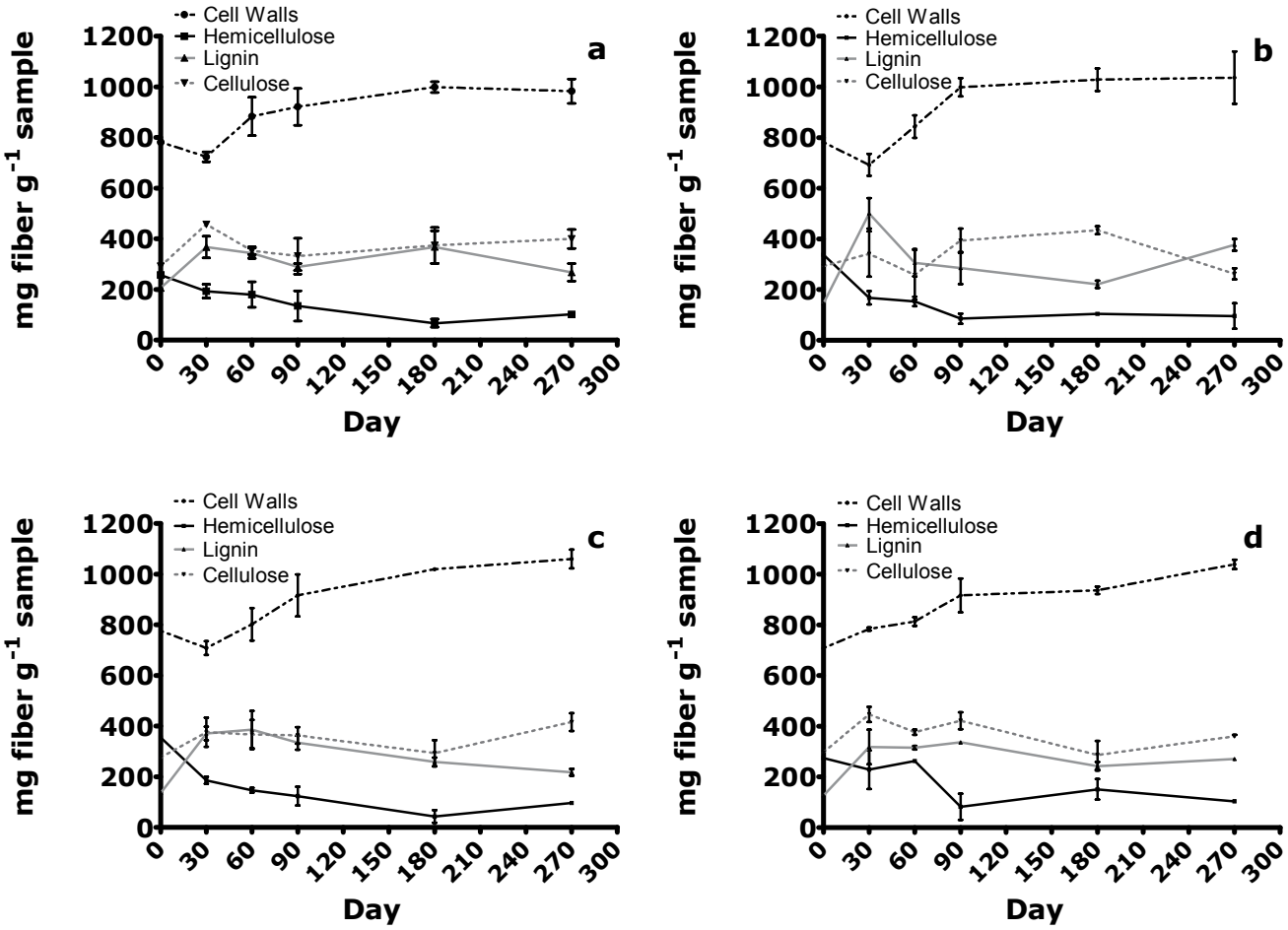

Figure 6: Decomposing root sample cell wall concentration and composition over a 270 day incubation period. a = Gammagrass, b = Wheat, c = Orchardgrass, d = Sorghum x sudangrass. Bars indicate standard error.

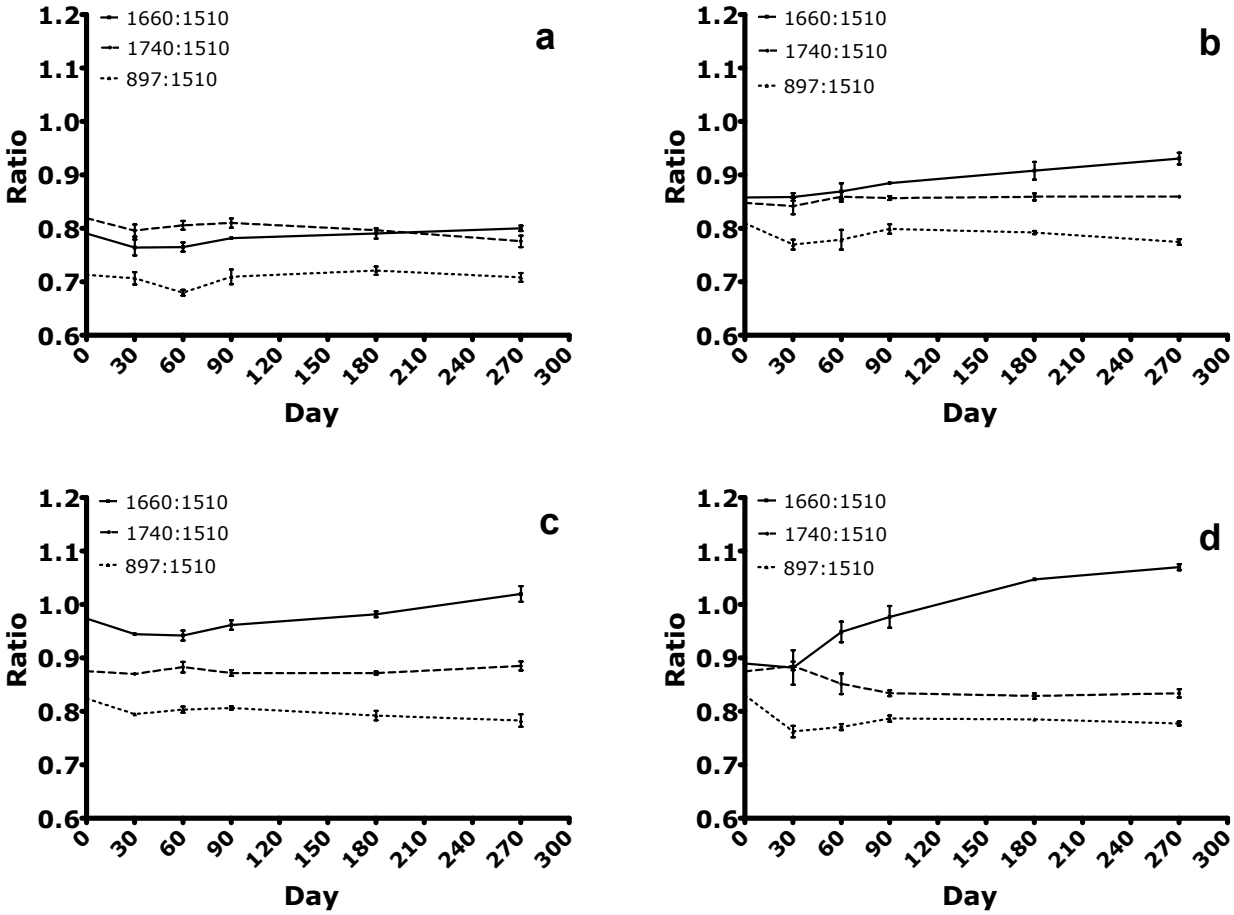

Figure 7: Peak intensity ratios (1660 cm^{-1}:1510 cm^{-1}, 1740 cm^{-1}:1510 cm^{-1}, 897 cm^{-1}:1510 cm^{-1}) resulting from differences in DRIFTS absorbance at wavenumbers indicative of lignin, cellulose, hemicellulose and wax. a = Gammagrass, b = Wheat, c = Orchardgrass, d = Sorghum, x sudangrass. Bars indicate standard error.

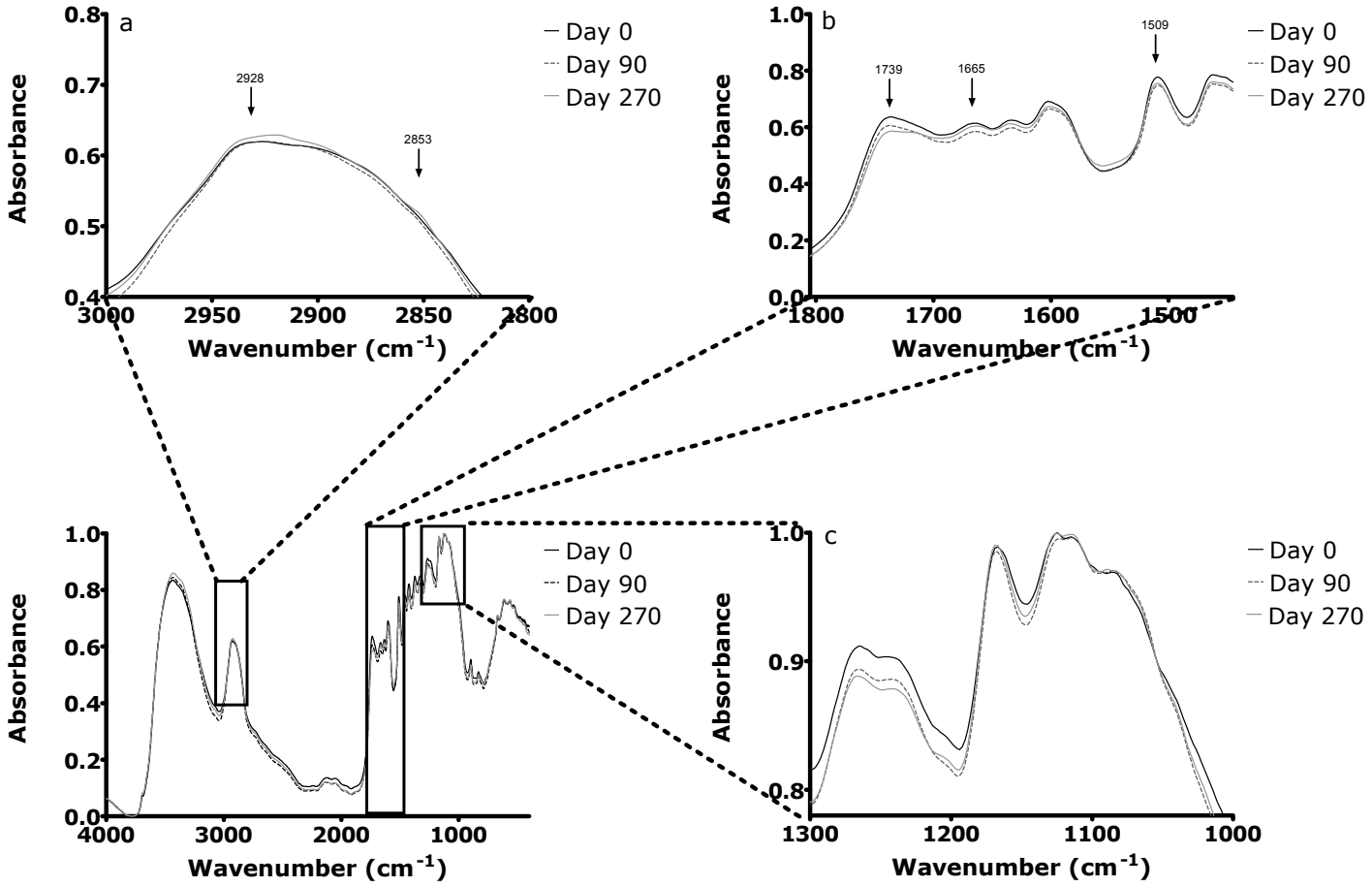

Figure 8: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of decomposing gammagrass root tissue recorded at Day 0, 90 and 270 and regions illustrating changes potentially indicative of suberin (a), peaks arising from suberin and hemicellulose carbonyls as well as lignin (b), and the polysaccharide fingerprint region (c).

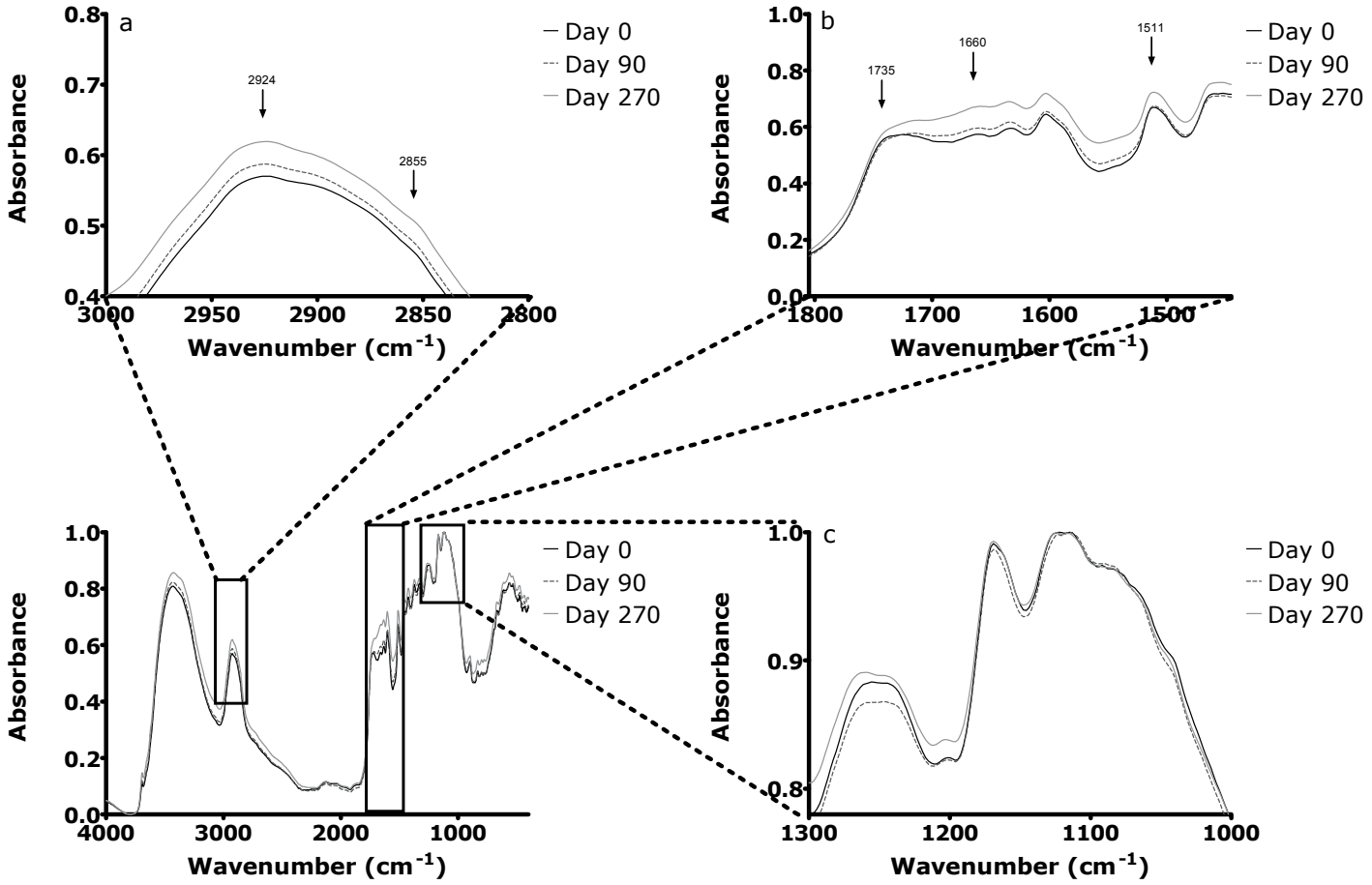

Figure 9: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of decomposing wheat root tissue recorded at Day 0, 90 and 270 and regions illustrating changes potentially indicative of suberin (a), peaks arising from suberin and hemicellulose carbonyls as well as lignin (b), and the polysaccharide fingerprint region (c).

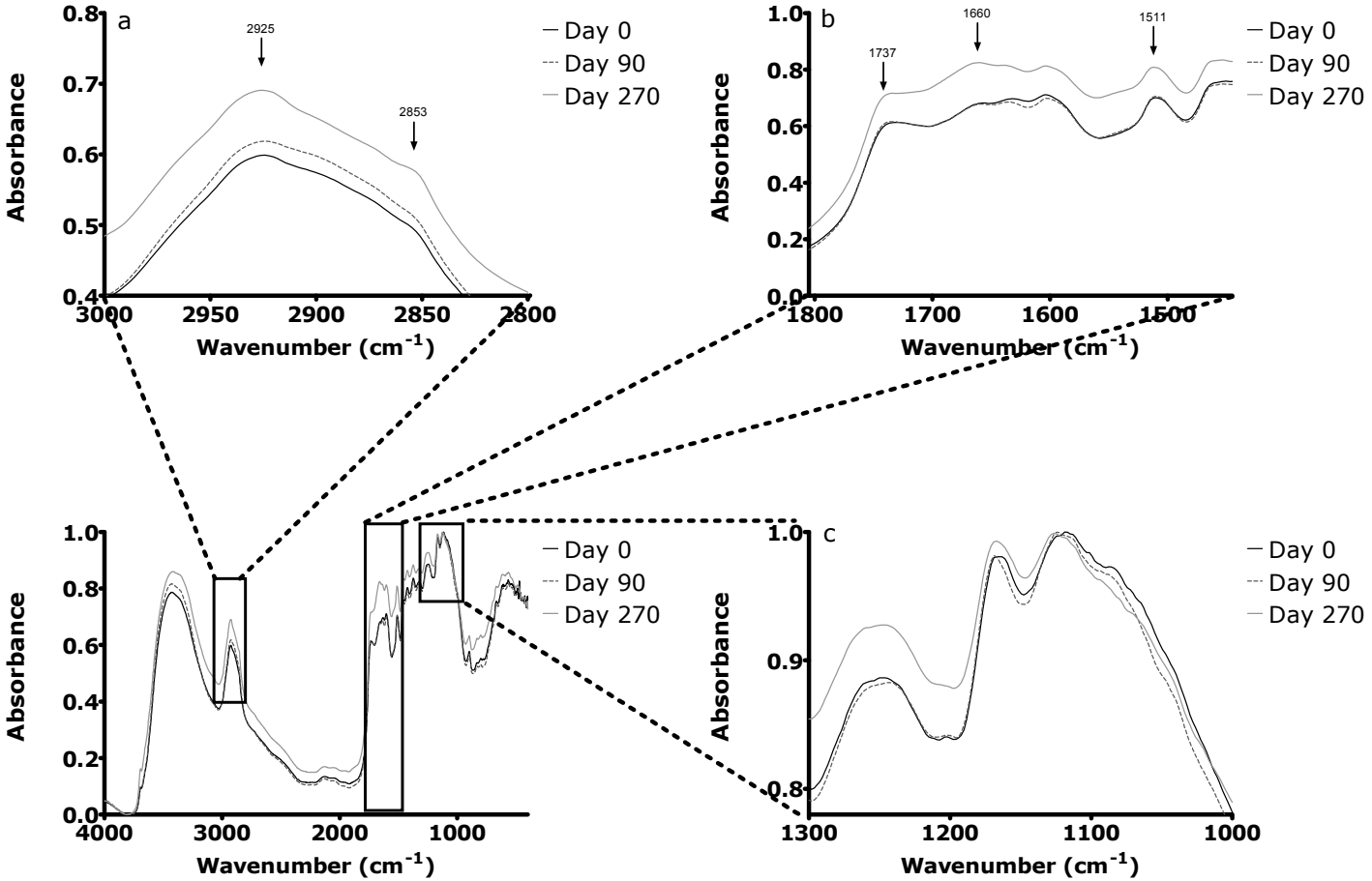

Figure 10: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of decomposing orchardgrass root tissue recorded at Day 0, 90 and 270 and regions illustrating changes potentially indicative of suberin (a), peaks arising from suberin and hemicellulose carbonyls as well as lignin (b), and the polysaccharide fingerprint region (c).

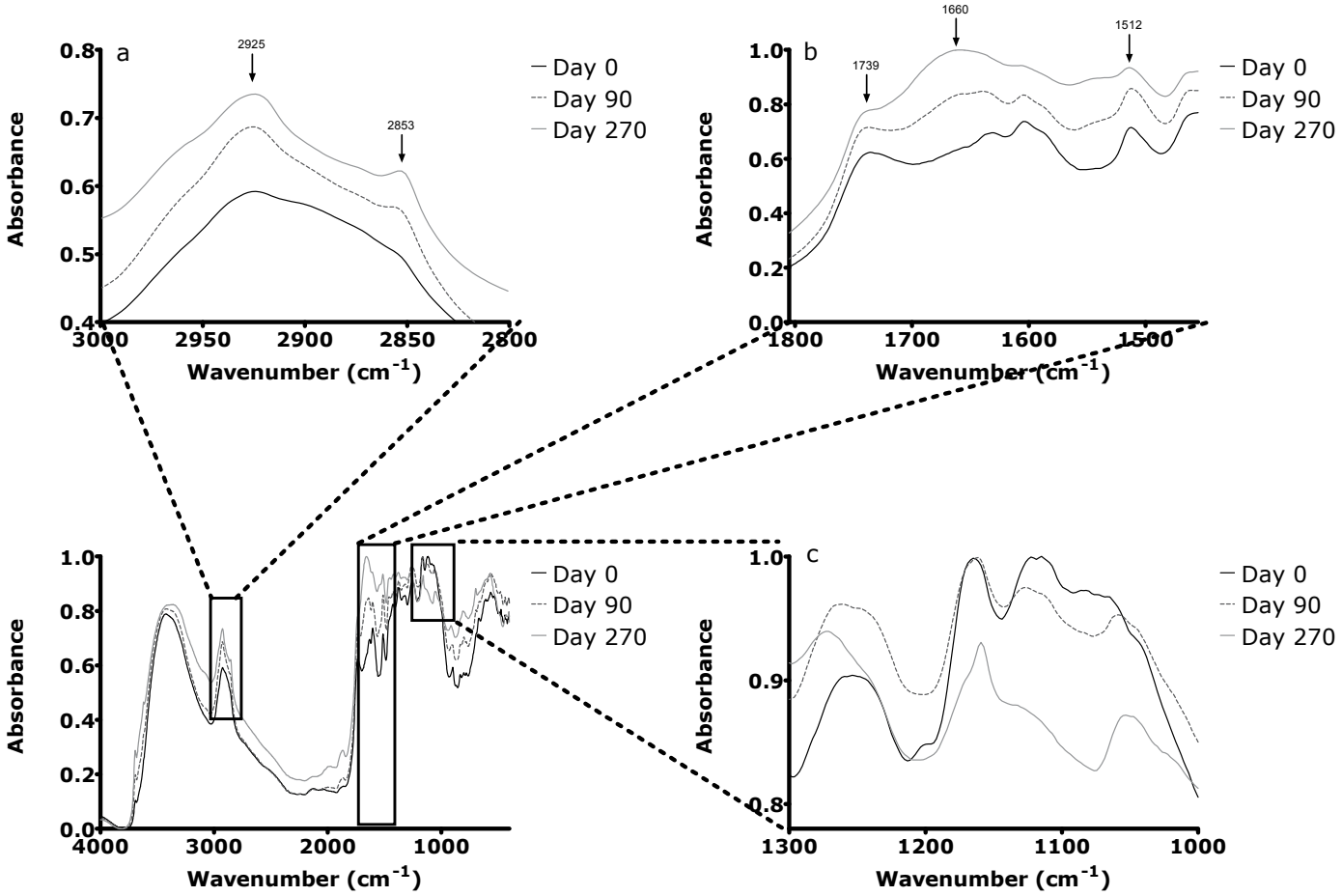

Figure 11: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra of decomposing sorghum x sudangrass root tissue recorded at Day 0, 90 and 270 and regions illustrating changes potentially indicative of suberin (a), peaks arising from suberin and hemicellulose carbonyls as well as lignin (b), and the polysaccharide fingerprint region (c).

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