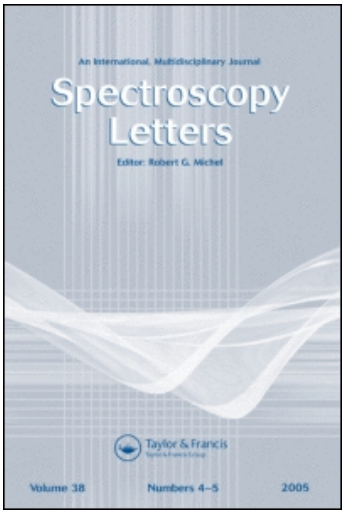

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ABSTRACT In this study, soil samples taken from a wetland from the surface to a depth of 160 cm were analyzed for water extracted organic matter (WEOM) fractions by UV/Vis and for lignin and polysaccharide components directly by Fourier transform infrared spectroscopy (FTIR). Our results indicate that lignin-derived phenolic compounds in the WEOM fractions decreased with increasing soil depth at three different wavelengths. This decrease was consistent with our FTIR results and was accompanied by a decrease of polysaccharides. Our study demonstrates the application of UV/Vis and FTIR to directly differentiate lignin and polysaccharide concentration gradients in a soil profile.

INTRODUCTION

Soil organic matter (SOM), as a sink and a source for carbon, is drawing increasing attention in studies of its chemical composition and structure, both of which are fundamental for making better predictions of SOM interactions in natural soil and water environments.[1] Primary constituents of SOM include altered and relatively unaltered plant biopolymer residues (such as polysaccharides, lignin, proteins, and cuticular materials), microbial tissues, and humic substances.[2,3] In SOM decomposition processes, SOM components experience a series of microbe-modified changes including structural alteration, incorporation into tissues of soil microorganisms, and mineralization.[2] The current lack of knowledge of SOM composition and structure hinders our understanding of SOM humification and decomposition processes, and thus the use of SOM as an environmental protection buffer.

The composition of SOM is so complex that it contains almost all naturally occurring organic compounds. Therefore, the study of SOM composition change is an extremely challenging task. However, various aspects of SOM have been widely studied. Water-extractable organic matter (WEOM) represents an active and mobile component of SOM,[4] and soluble organic compounds can be vertically transported by water. Analysis of functional

At the time of this research, Katherine E. Neff was an undergraduate student.

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groups through ultraviolet visible spectrometry (UV/Vis) for specific compounds, such as lignin-derived phenols and hydrolysable sugars, allows insight into processes that affect SOM decomposition and humification. For example, specific UV absorption at a wavelength of 254 nm has been used as an indicator of aromatic C content,[5] and absorbance ratios at wavelengths of 270 to 400 nm were used to characterize the degradation of phenolic/quinoid humic acids to simpler carboxylic aromatic compounds.[6] Properties of bulk soils or their components, such as lignin and polysaccharides, have been studied through the use of Fourier transform infrared spectroscopy (FTIR).[7,11] For example, Zimmermann et al.[8] used IR in combination with partial least-squares regression to quantify soil organic carbon in different particle size fractions. Currently, research exploring the use of UV/Vis and FTIR to differentiate SOM components along a soil profile is very limited.

The objectives of this study were to use UV/Vis spectrometry to differentiate WEOM that was extracted from soils at different depths and to explore the possibility of using FTIR to compare and differentiate functional groups for lignin and polysaccharides in wetland soils from different sampling depths. The results of this study help clarify SOM decomposition and humification processes in these soils.

MATERIALS AND METHODS

Soil Samples

Soil samples were collected from a wetland located in the Panthertown Valley section of the Nantahala National Forest in Western North Carolina (Fig. 1) in the spring of 2008. The studied wetland at Panthertown Valley receives some drainage from the surrounding mineral soil, is peat-accumulating, and supports marsh-like vegetation as well as several *Sphagnum* species. A 160-cm long core was extracted at the site using an Eijkelkamp hand corer (Eijkelkamp, Giesbeek, The Netherlands), which consists of a semi-closed tube of 1-m length and 6-cm diameter. This type of coring device does not compact sediments. The core was recovered in 1-m increments, and coring proceeded downward to refusal (bedrock). After recovery the collected core was logged, photographed, wrapped in aluminum foil, wrapped in plastic, and transported to Western Carolina University. Once in the lab the core was sub-sampled at 1-cm increments, and the sub-samples were stored in frozen state until analysis. Sub-samples were then combined to provide sufficient material for further analysis, as shown in Table 1.

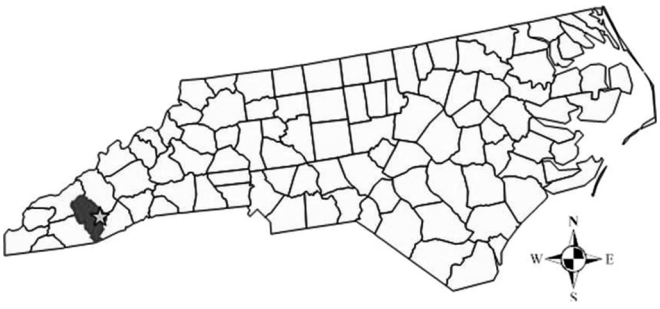

FIGURE 1 Location of the study site in Western North Carolina, USA.

Water-Extractable Organic Matter Fractions and UV/Vis

Mixed soil samples were dried at 105°C for 24 hr to remove hygroscopic water.[12] Two soil sub-samples (15 g) of each soil were soaked in deionized water at a ratio of 1:2 for 24 hr to break down all of the macro particles. The samples were then sonicated for 15 min. Each of the sub-samples was centrifuged at 2500 RPM for 2 hr, and the supernatant was decanted and filtered through a 0.45-μm glass fiber membrane filter.[13,14] The filtered liquid is designated as the water-extractable organic matter (WEOM) fraction. UV/Vis spectra of diluted WEOM fractions were collected on a Hewlett–Packard 8453 spectrophotometer (Hewlett–Packard Company, Wilmington, DE) in a 1-cm quartz cuvette and scanned from 190 to 700 nm. The dilution factors that were used are displayed in parentheses after the following sample designations: Sample 1 (4), Samples 2 and 3 (10), Samples 4 and 5 (5), and Samples 6 and 7 (1).

Soil KBr Pellets

Soil samples were first dried at 105°C for 24 hr to remove hygroscopic water.[12] A small amount of a soil sample was ground and then diluted with a pure, dry spectroscopic grade KBr powder at a concentration of about 0.3% sample in KBr (1% sample in KBr was tested and found to be too dark

TABLE 1 Soil Sample Depths in cm

Sample name		1	2	3	4	5	6	7	8	9	10
Soil depth (cm)	UV/Vis	0–25	26–55	56–80	81–109	110–134	135–149	150–160			
	FTIR	0–11	16–21	30–39	40–49	50–59	70–79	80–89	100–109	120–129	150–160

for FTIR). The sample was fully mixed with KBr using an agate mortar and pestle. The mixture was pressed into a semitransparent 13-mm diameter disk through a Perkin Elmer KBr pellet kit and Perkin Elmer hydraulic press (Carver Laboratory Press model C). We applied 15-tons-cm^{-2} pressure for 15 min under a vacuum. The KBr sample disk was then placed in the IR beam using a sample transmission holder. The spectrum scan was performed on a Perkin Elmer Spectrum One in the middle IR, ranging from 4000 to 400 cm^{-1}, with a scan number of four. Baseline corrections were applied at 1831 cm^{-1}, 1510 cm^{-1}, 1341 cm^{-1}, and 934 cm^{-1}. The intensities of absorption bands at 1598 cm^{-1} and 1105 cm^{-1} were measured using local baselines imposed between 1831 cm^{-1} and 1510 cm^{-1} and between 1341 cm^{-1} and 934 cm^{-1}.

Determination of Percentage Total Organic Carbon

Samples were ground and homogenized using a coffee grinder and a mortar and pestle and then were bagged and kept dry until further processing. Percentage total organic carbon (TOC) was determined by extracting sub-samples from each bag. Potential carbonate was removed by acidifying each sample with 10% HCl until effervescence ceased (all carbonate was consumed). TOC was measured using an Elementar Vario EL analyzer set on CNS mode, and we performed at least three runs for each sub-sample to calculate average values of TOC. The coefficient of variation was less than 5% for all triplicate analyses.

RESULTS AND DISCUSSION

UV/Vis Spectra of WEOM and Differentiation

The different spectra of soil WEOM are very similar as indicated in Fig. 2. Vertically, absorbance of WEOM decreases with increasing soil depth for all wavelengths from 200 to 400 nm, indicating a decrease in the amount of WEOM with depth. Horizontally, absorbance intensity of WEOM also decreases with increasing wavelength until about 250 nm, after which the absorbance increases, reaching a peak at about 276 nm and then decreases steadily, as illustrated in Fig. 2. UV/Vis absorption of WEOM between 250 and 280 nm is caused by $\pi \rightarrow \pi^*$ transition in phenolic compounds or aromatic compounds.[15] The absorbances at wavelengths of 254 nm, 260 nm, and 276 nm have been used in many studies for estimating the amount of aromatic C in natural aqueous organic matter.[15,16] We examined and correlated the absorbance of WEOM at all three wavelengths with soil depth data, and our results indicate that there are no significant differences in correlation coefficients among these wavelengths ($R^2 = 0.9442$ at 254 nm; $R^2 = 0.9217$ at 260 nm; and $R^2 = 0.9502$ at 276 nm). With increasing wavelength, the correlation between WEOM absorbance and soil depth remained similar, as indicated in Fig. 3. Again, dilution factors were included in the calculation of UV/Vis absorbance of the samples. In the visible

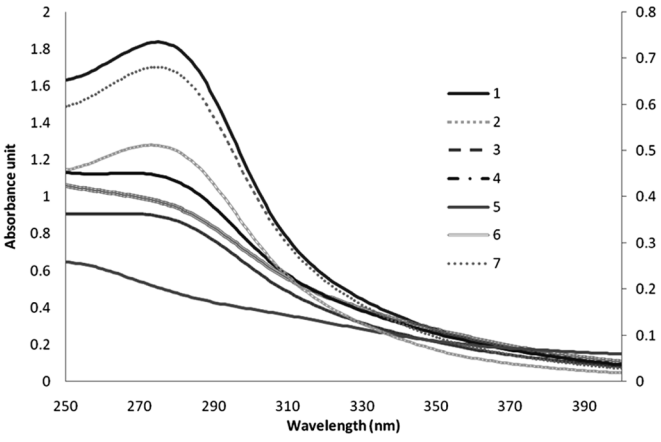

FIGURE 2 UV/Vis spectra of WEOM from samples 1 to 7 as in Table 1 (Sample 1 is plotted using the primary axis on the left side of the figure; all the other samples are plotted using the secondary axis on the right side).

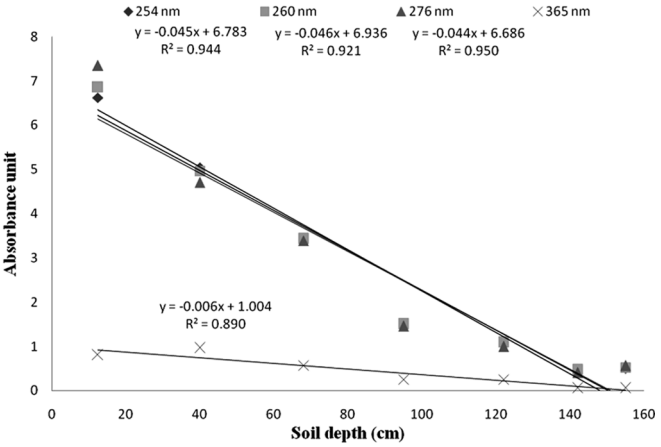

FIGURE 3 **Absorbance of WEOM in a soil profile.**

range (365 nm), the absorption of the WEOM was by the functional groups with quinoide and keto-enol systems.[17]

Lignin, which is the second most abundant component of plant residues, comprising up to 15% of the total dry matter in crop residues, is an amorphous phenolic polymer with a poorly characterized structure and is considered as making a significant contribution to SOM stability. It is known, as revealed by ^{14}C ages of aliphatic hydrocarbon and total organic carbon,[18] that the ages of SOM increase with increasing depth. If we assume that SOM in the deep soil is more stable than that in the surface and shallow layers, it is expected that more lignin would be found in the deeper soil. Our C analysis of the soil samples shows that percentages of soil organic C generally decrease with increasing depth, having a value of 20.98% from 1 to 25 cm, reaching the lowest value of 5.19% at 110–134 cm and then increasing to 5.42% at 135–150 cm and 7.74% at 151–160 cm. In combination with the C data, our UV/Vis results indicate that lignin-derived phenolic compounds and other water-extractable aromatic compounds decrease in the soil profile, suggesting a low contribution of lignin-derived phenolic compounds or water-extractable aromatic compounds to stable C pools. This result is consistent with the report from Kiem and Kögel-Knabner,[19] which suggests that lignin may not accumulate within the refractory C pool of soils. Aromatic components that come mainly from the surface may be preferentially retained on the soil mineral surface and may not easily migrate towards deeper soil horizons.[4,5]

FTIR Spectra of Soils and Their Differentiation

Typical spectra of the soil samples are shown in Fig. 4, demonstrating their superficial similarity. In general, the major vibrational frequency bands of SOM, as shown in Fig. 5, can be classified into four regions. The first region, above 3000 cm^{-1}, originates from N-H stretching of amides and O-H stretching of polysaccharides and/or water. The second region, from 3000 to 2850 cm^{-1}, originates from aliphatic C-H stretch. The vibrational stretching of the carbonyl functional group C=O in carboxylic acids, aldehydes, and ketones; and the vibrational stretching of C=C in aromatic compounds and other alkenes yield fingerprints in the range of 1780–1500 cm^{-1}. The C-O stretching vibrations in alcohols, carbohydrates, and phenolic compounds occur in the region of 1200–1000 cm^{-1}.[6,15,20]

In SOM decomposition and humification processes the main components, such as polysaccharides and lignin, are altered through microbial degradation and incorporated into humic substances.[3] Lignin is an amorphous complex phenolic polymer consisting of a variety of ether C-O-C and C-C bonds between the monomeric phenylpropanoid units.[11] Its absorptions at wavelengths of 1598 cm^{-1} and 1505 cm^{-1} have been used to quantify lignin contents in samples.[9,11] In our study, since lignin absorptions at 1505 cm^{-1} were not significant in all the soil

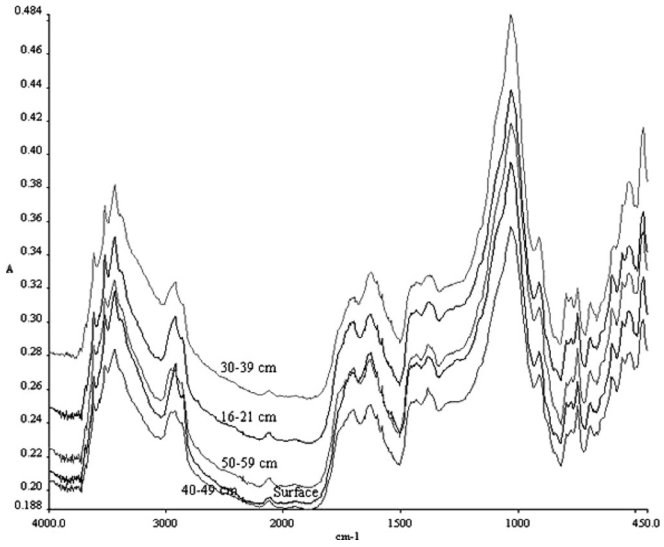

FIGURE 4 **Typical FTIR spectra of the soil samples from different soil layers (Soil: KBr = ~1:300).**

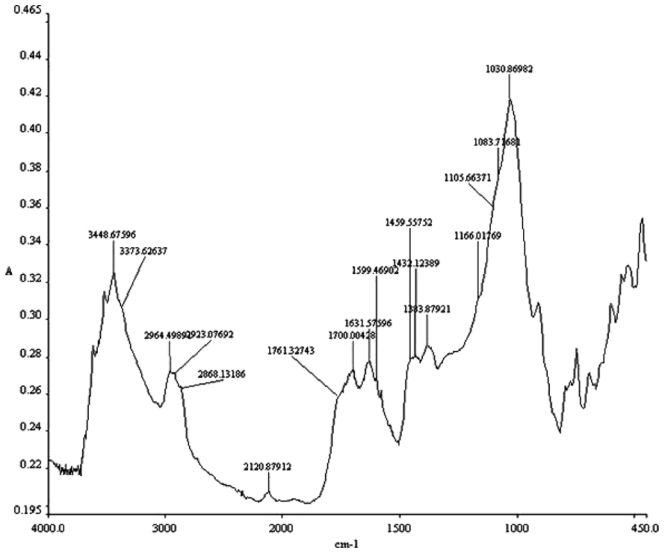

FIGURE 5 **FTIR absorption bands of the soil sample from 50–59 cm depth.**

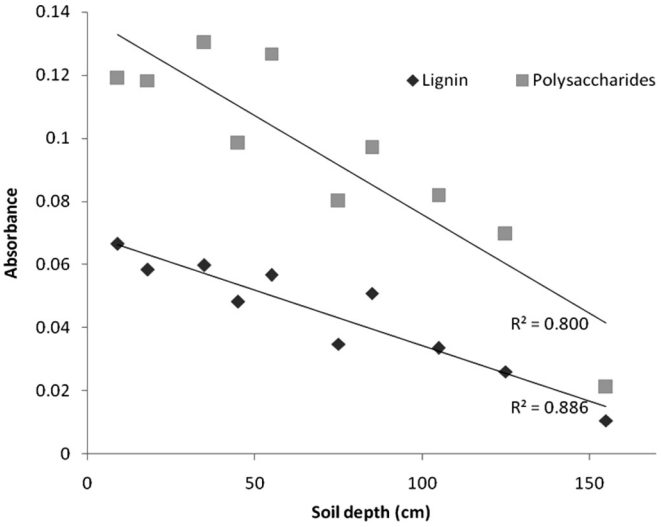

FIGURE 6 **Absorbance of lignin and polysaccharides in the soil profile.**

samples (see Fig. 5), 1598 cm^{-1} was chosen for quantifying the lignin content in the soil samples.

Polysaccarides are the major constituents in most cell walls. They provide a rigid, reinforcing layer around the cell membranes in plants, bacteria, and fungi. The major compositional units of polysaccharides are mono- or disaccharides that are joined together through glycosidic bonds. Among polysaccharide feature frequency bands at 1105, 1018, 1162, 1058, and 1000 cm^{-1},[10,20] 1105 cm^{-1} was selected for characterizing polysaccharides in the soil samples. The absorption bands of polysaccharides at 1018 and 1058 cm^{-1}, as illustrated in Fig. 5, are overlapped with the strong stretching band of Si-O at 1031 cm^{-1},[20] and the absorption band at about 1162 cm^{-1} was not significant in all the soils. Furthermore, the vibrational band at 1105 cm^{-1} is caused by glycosidic bonds, which distinguish the polysaccharides from lignin-derived phenols.

The absorbance of polysaccharides at 1105 cm^{-1} and the absorbance of lignin at 1598 cm^{-1} decreased with increasing soil depth, having a correlation coefficient of $R^2 = 0.8006$ for polysaccharides and a correlation coefficient of $R^2 = 0.8866$ for lignin, as indicated in Fig. 6. This result indicates that the contribution of both lignin and polysaccharides to SOM decreases with depth, suggesting less contribution of lignin and polysaccharides to old SOM, since major sources of SOM come from the surface, and the ages of SOM increase with soil depth.[18]

The absorption ratio of $A_{lignin}/A_{polysaccharade}$ is defined as the absorbance of lignin at 1598 cm^{-1} over the absorbance of polysaccharides at 1105 cm^{-1}. Our analysis of the absorption ratios of $A_{lignin}/A_{polysaccharade}$ for all of the soils did not give a clear result. Other research indicates that the depletion rate of lignin is faster than that of polysaccharides. Polysaccharides come from plant and microbial organisms. The plant-derived polysaccharides are easily degraded in soils as an energy source for soil organisms. However, microbial-originated polysaccharides act as binding agents between soil particles, making a contribution to the physical and chemical attributes of SOM,[21] which may explain the lack of a clear trend in the ratio of lignin over polysaccharides observed in this study. Further research is needed to investigate the concentrations of lignin and polysaccharides along a soil profile and the use of FTIR in their determination.

CONCLUSIONS

Soil samples were characterized for their water-extractable organic components through UV/Vis for lignin-derived phenolic and aromatic compounds to a depth of 160 cm below the surface. These soils were studied through transmission FTIR for lignin and polysaccharides. Our results indicate that lignin-derived phenolic (aromatic) compounds in WEOM fractions decrease with increasing soil depth

($R^2 = 0.969$) at a wavelength of 276 nm. There were no significant differences among the wavelengths of 254 nm, 260 nm, and 276 nm in characterizing water-soluble lignin-derived compounds. The decreasing absorbance of WEOM at the wavelength of 365 nm with depth indicates a decrease of quinoide and keto-enol compounds. The UV/Vis result indicated that both the total soluble organic C and the lignin-derived phenolic aromatic C of WEOM decreased with increasing soil depth. In combination with the percentage soil organic C data, the UV/Vis result suggests that lignin does not contribute significantly to the stable C pool, which was consistent with the FTIR data for lignin, which showed a decrease of polysaccharides in the soil profile.

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