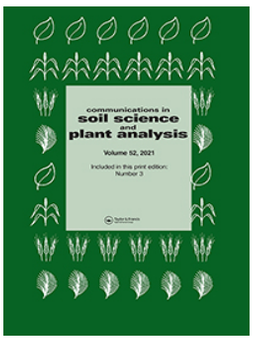

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A Rapid Approach to Determine Soil Carbon Quality and Its Relationship to Soil Greenhouse Gas Emissions

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ABSTRACT

Soil organic matter composition controls many microbial processes in the soil matrix. How these processes interact to drive carbon cycling through greenhouse gas fluxes or carbon stabilization through biochemical transformations continues to evolve. From laboratory incubations, it is clear that low molecular weight compounds (LMWCs; e.g. dextrose, mannitol, and trehalose) can have a profound effect on microbially mediated fluxes. However, relationships between LMWCs and soil efflux have been largely unproven in field studies due to methodological constraints. In the current study we developed a novel use of Near Infrared Reflectance Spectroscopy (NIRS) to quantify LMWCs (explaining 38–51% of the variance). Paradoxically, we found dextrose concentrations to be negatively correlated with carbon dioxide fluxes in residential sites while mannitol was positively correlated with carbon dioxide fluxes in agricultural sites. Methane fluxes were strongly correlated with trehalose indicating a potential fungal interaction with bacterial methanogens. We found no clear link between LMWCs on nitrous oxide emissions suggesting inorganic nitrogen is a stronger limiting factor. The results from this study showed how a NIRS-based approach can improve mechanistic understanding of the drivers of soil greenhouse gas fluxes.

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Carbon; near infrared reflectance spectroscopy; methane; nitrous oxide; carbon dioxide

Introduction

Soil organic matter degradation is a pivotal part of ecosystem functioning and controls many ecological features such as organic matter formation, nutrient cycling, and greenhouse gas emissions (Schlesinger and Bernhardt 2013). Floodplains and riparian forests can be hotspots for these processes due to high productivity and can have a profound effect on watershed scale soil nutrient cycling, greenhouse gas emissions (Baas et al. 2017; Knoepp et al. 2018), and regional stream water quality (Webster et al. 2012). In addition, changing land use in riparian zones from forest to agriculture to residential development can have widely divergent influences on CO_2 (Vose and Bolstad 2007; Vose et al. 2005), CH_4 (Goldman et al. 1995) and N_2O fluxes (Baas et al. 2017; Groffman, Gold, and Jacinthe 1998; Hefting, Bobbink, and de Caluwe 2003).

Theoretical and empirical studies can explain a part of the variance in microbial-mediated releases of greenhouse gases using temperature, pH, (in)organic nitrogen, and organic carbon concentrations (Baas et al. 2017; Davidson, Belk, and Boone 1998; Werner, Kiese, and Butterbach-Bahl 2007). In some cases, bulk carbon concentrations have been found to result in a mitigation (i.e., a decrease) of CH_4 and N_2O emissions (Merino, Pérez-Batallón, and Macías 2004). Different types of carbon, however, interact with microbial processes in a variety of manners (van Hees et al. 2005). Growing evidence suggests that soil organic matter (SOM) formation and cycling does not progress simplistically from

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the easily soluble components (i.e., low molecular weight compounds [LMWCs] such as amino acids and sugars) to progressively more complex compounds (i.e., high molecular weight compounds [HMWCs] such as cellulose and lignin) resulting in recalcitrant forms of SOM (Melillo, Aber, and Muratore 1982; Rovira and Rovira 2010). A diversity of microbially driven pathways can result in greenhouse gas efflux and SOM formation and may be specifically dependent on microbial turnover of labile LMWCs (Calderón et al. 2011; Cotrufo et al. 2013; Kleber et al. 2015; Preston, Nault, and Trofymow 2009).

This shifting paradigm suggests that models of carbon cycling focusing on the effect of HMWCs on greenhouse gas emissions or SOM formation (Aber, Melillo, and McClaugherty 1990; McKee et al. 2016; Talbot et al. 2012) is in need of more attention to LMWC substrate availability and cycling. Indeed, LMWCs have been found essential for many processes producing the three major greenhouses gasses (Denman et al. 2007); CO_2 (Eilers et al. 2010; van Hees et al. 2005), CH_4 (Lu et al. 2000; Rothfuss and Conrad 1992) and N_2O (Hill et al. 2000; Richardson and Ferguson 1992). For example, additions of the LMWC glucose has resulted in spikes in CO_2 and N_2O effluxes in wide ranging ecosystems like rice paddies (Wang et al. 2005), forest soils (Baas et al. In prep), and agricultural cropping systems (Henderson et al. 2010; Sanchez-Martin et al. 2008; Shelp, Beauchamp, and Thurtell 2000). Further, in wetland ecosystems, glucose additions are associated with increased methane efflux (Aerts and de Caluwe 1999; Peng et al. 2015). Understanding temporal and spatial heterogeneity (Baas et al. 2014; Groffman et al. 2009; McClain et al. 2003) is a critical challenge, particularly as it relates to microbial-mediated greenhouse gas fluxes. Meeting this challenge, however, will require greater knowledge of the abundance of LMWCs, which is currently limited by the lack of high throughput, cost-effective measurement approaches.

Innovative uses of ^{13}C Nuclear Magnetic Resonance (NMR), Fourier Transformed Infrared Reflectance Spectroscopy (FTIR), and Near Infrared Reflectance Spectroscopy (NIRS) provide some new alternatives for measuring carbon forms. Of these technologies, NIRS is the only option that is field-deployable and it has a limited need for soil preparation (Peltre et al. 2011). NIRS has been used frequently to predict total soil carbon and organic nitrogen concentrations (Chang and Laird 2002; Ladoni et al. 2010) and to distinguish between different carbon pools (Cozzolino and Morón 2006; Vasques, Grunwald, and Sickman 2009) as well as estimating microbial biomass (Coûteaux, Berg, and Rovira 2003). Success has also been achieved in using NIRS in determining beet (Roggo et al. 2002) and fruit sugar concentrations (Ji, Li, and Shigefuji 2008). However, to our knowledge, no literature exists that has utilized NIRS to predict specific ecologically relevant LMWC concentrations in the soil matrix. The goal of the current study was to determine if NIRS spectra could be calibrated to common LMWCs and, secondly, if the predicted carbon composition could explain greenhouse gas effluxes for different riparian land-uses and times of the year. Given the previous success with NIRS for predicting total soil carbon we expected that common sugars such as dextrose, trehalose, and mannitol could be well predicted ($r^2 > 0.8$). Further, we expected that these sugars would be well related to greenhouse gas efflux (i.e., CO_2, CH_4 and N_2O) across a range of land uses (forest, agriculture, and residential).

Materials and methods

Site description

The study was conducted in Macon County, North Carolina, in the Blue Ridge physiographic province in the southern Appalachian Mountains. This region receives an average of 1300 mm of precipitation a year (NOAA 1950-2013). The highest temperatures are between May and September (20°C) and the lowest temperatures are between December and February (5°C). The growing season starts in May and ends in September (Swift and Cunningham 1988).We selected 8 study sites that included forest cover (N = 3), undisturbed since the 1920s; agricultural use (N = 3), comprised of managed pasture with and without grazing, and low-density residential development (N = 2). All sites were adjacent to a first

or second order headwater stream. At each site we established a riparian zone sampling area 3 m wide and 20 m long perpendicular to the stream. For more site information see Baas et al. (2017).

Carbon composition

We estimated the available C content of mineral soils present as lignin, cellulose, dextrose, trehalose, and mannitol in the lab using a NIRS for July and November 2012, and March and May 2013 samples. This required creation of a NIRS calibration model for mineral soil (0–15 cm) from each of the eight sites. Soils were combusted (500°C for 24 h) to remove all organic matter, we then added and mixed concentrations of specific carbon compounds (0–5% w/w range) in the form of lignin (Lignin, alkali Sigma-Aldrich 370959, batch #: 0801288), cellulose, dextrose, trehalose, or mannitol. Soil/C mixtures were scanned with an ASD FieldSpec 3 (Analytical Spectral Devices [ASD], Inc., Boulder, CO). The NIRS scans from 350 to 2500 nm in 1-nm increments using a contact probe with a 2-cm-diameter window. The scans were completed by first performing a baseline scan using a Spectralon® white blank (Labsphere, North Sutton, NH). The lens of the contact probe was pressed firmly against the surface of the soil within its sample bag so that no light from the lens was visible. Soil within the sample bag was mixed, and the refreshed surface was scanned again. Each spectrum was averaged from a compilation of 50 readings during each scan. This was performed three times per sample, and the three scans were then averaged. Between each sample, the contact probe was cleaned. A baseline scan was performed after every 10 samples. Spectra were transformed to the first-derivative before statistical analysis. The samples were divided into a calibration (70%) and validation (30%) dataset. Using The Unscrambler software (CAMO PROCESS AS, Oslo, Norway) partial least squares best cross-validated models were developed with the training dataset for each of the specific carbon compounds. The models were validated by regression analysis using the validation dataset.

We conducted additional validation of this technique using traditional soil extraction and analysis approaches on a subset of air-dried soil samples (N = 10) at the Complex Carbohydrate Research Center at the University of Georgia. For carbohydrate analysis, about 3.0 g of each soil sample was weighed into a pre-rinsed screw-cap glass tube and extracted with 80% ethanol for mannitol, trehalose, and glucose through an end to end shaker/rocker. Thereafter, the tubes were spun and the supernatant/extract of each sample was transferred into another glass tube, dried under a stream of nitrogen gas. The dried extracts were dissolved with water and transferred into high-performance liquid chromatography vials for analysis. Four concentrations of mannitol, trehalose, and glucose standard mixture were prepared serially to establish a calibration equation. The quantity of each analyte in the sample was calculated by linear interpolation of respective residue area units into the calibration equation. The soil carbohydrates were analyzed by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) using a Dionex ICS3000 system equipped with a gradient pump, an electrochemical detector, and an autosampler. The residues were separated by a Dionex CarboPac PA20 (3 x 150 mm) analytical column with pre-installed amino trap and eluted with degassed nanopure water and 200 mM NaOH. Injection was made every 32 min. For cellulose analysis between 15 and 20 mg of each of the samples was used for the analysis. The samples were mixed with 1 ml of 2 M trifluoracetic acid (TFA) for 2 hours at 121°C to hydrolyze all non-cellulosic polysaccharides. The samples were then washed multiple times with DI water to remove the TFA and hydrolyzed monosaccharides. The amount of cellulose in the samples was then calculated using a variation of the method of Dubois et al. (1956) in which a portion of between 420 and 530 ug of the dried samples was mixed with 5% phenol and subjected to concentrated sulfuric acid. Absorbance of the solution was then read at 500 nm and estimations are made in relation to cellulose standards. For lignin analysis each sample was prepared in duplicate by weighing ~5.0 mg and single-shot pyrolyzed (Frontier Lab) at 500°C. The volatile compounds were separated by HP-5 MS column (30 m x 0.25 mm, Agilent Technologies, Inc.) fitted to a 6890 N gas chromatography system, which was interfaced to 5975B inert MSD (Agilent Technologies). Oven temperature was initially set at 50°C and ramped to 280°C over a period of 53 min. Helium was the carrier gas for the volatile compounds and the split ratio was set at 50:1.

Greenhouse gas fluxes

We measured net soil CO_2, CH_4 and N_2O fluxes from May 2012 and May 2013 between 0900 and 1600 h using static PVC flux chambers as described in Baas et al. (2017).

Statistics

We conducted tests for treatment effects on respiration rates using standard least squares analysis of variance (ANOVA) to determine differences in predicted soil carbon concentrations with regards to different land-use. The relationship between drivers of processes and gas flux rates were determined using Spearman correlation analysis on non-transformed data to determine the strongest predictors for greenhouse gas fluxes and N cycling rates. All statistical analyses were conducted in JMP 11 (SAS Institute Inc., Cary, NC) and significant differences are indicative of $p < .05$ unless otherwise stated.

Results

Carbon composition

Calibrations of carbon compound concentrations using NIRS varied with carbon type (Table 1; Figures 1 and 2). The carbon compound concentrations in the synthesized mixed samples were relatively well-validated, explaining between 48 and 88% of the variation. However, external validation using traditional extraction methods proved less successful. Significant relationships were found for dextrose and trehalose while mannitol and cellulose were not significantly related to the NIRS predicted concentrations. Lignin concentrations were found to be below detection limit and, thus, no external validation of the NIRS method was possible.

Carbon compounds showed significant land-use effects for lignin, significant sample date effects for cellulose, lignin, mannitol and trehalose, and significant interactions for dextrose (Figure 3; Table 2). Dextrose concentrations were greater in agricultural and residential sites compared to forested sites. Soil dextrose in agricultural and residential land use showed no sample date effect while for forested systems the greatest concentrations were found in May 2013 compared to March 2013 and November 2012 ($F_{3,53} = 10.4$, $P < .001$). In November 2012 forested sites had lower dextrose concentrations compared to residential and agricultural land use ($F_{7,31} = 4.6$, $P < .001$) while in May 2013 dextrose concentrations were greater in forested sites than agricultural land use ($F_{7,32} = 9.5$, $P < .001$). Mannitol ($F = 0.6$, $P = .58$) and trehalose ($F = 0.2$, $P = .81$) concentrations showed no significant land-use effects. Mannitol concentrations were greatest in July 2012 compared to November 2012, March and May 2013 ($P < .001$) while trehalose concentrations were greatest in May 2013 compared to July 2012 and March 2013 ($P < .05$). May 2013 was greater in cellulose concentrations than July 2012, November 2012, and March 2013 ($P < .001$). Lignin concentrations

Table 1. Near infrared reflectance spectroscopy (NIRS) prediction calibration and validation statistics on 0–15 cm mineral soils. External validation was based on comparison with other analytical approaches.

	Calibration dataset		Validation dataset		External validation	
	p	r^2 (N)	p	r^2 (N)	p	r^2 (N)
Dextrose	<.001	0.95 (31)	.008	0.66 (9)	.0565	0.38 (10)
Mannitol	<.001	1.00 (31)	.038	0.48 (9)	.12	0.49 (6)
Trehalose	<.001	0.53 (31)	<.001	0.82 (9)	.0298	0.51 (9)
Cellulose	<.001	1.00 (31)	.002	0.75 (9)	.68	0.03 (9)
Lignin	<.001	0.69 (31)	.015	0.60 (9)	bdl	bdl
Total C-mix†	<.001	0.90 (31)	<.001	0.88 (9)	-	-

†determined on combusted soil mixed with C-compounds
‡Saturation after 0.35 μg C g_{soil}^{-1}. Samples over threshold not included in validation
bdl = below detection limit

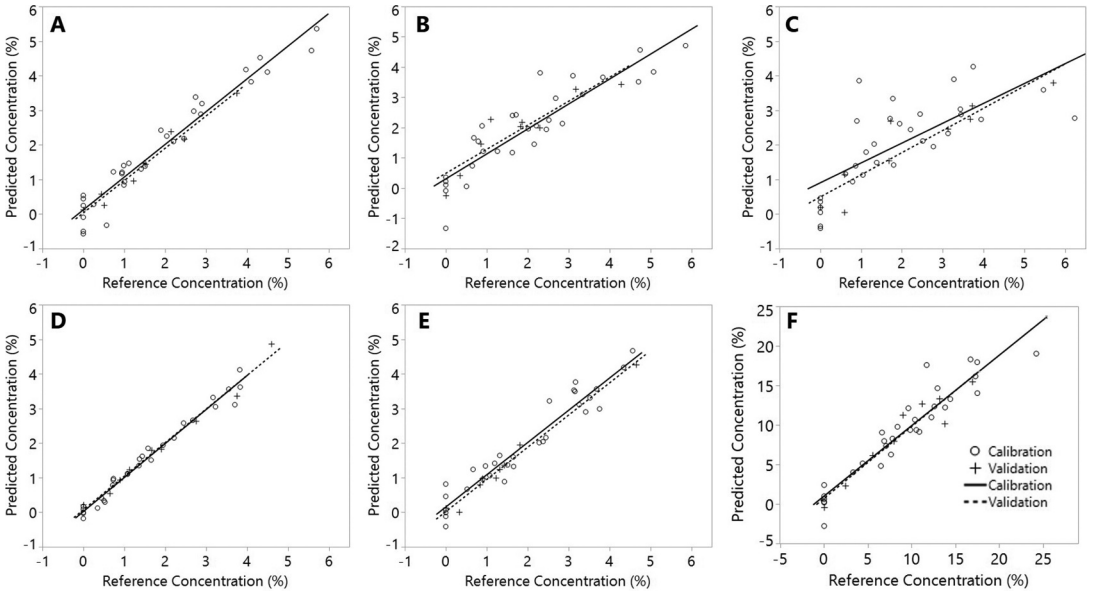

Figure 1. Scatterplots showing the regressions for the calibration (red circles) and validation (blue crosses) for soil dextrose (a), mannitol (b), trehalose (c), cellulose (d), lignin (e) and total carbon concentrations (f). Calibration statistics are presented in Table 1.

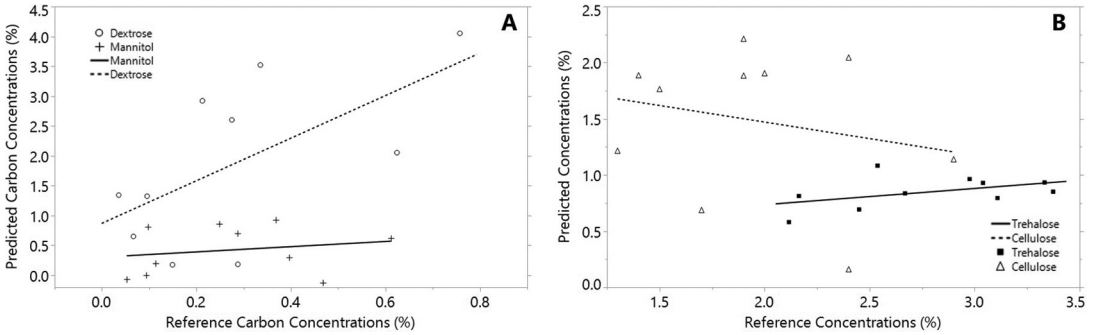

Figure 2. Scatterplots showing the regressions for the soil dextrose (a), mannitol (a), trehalose (b) and cellulose (b) against an external validation method. Calibration statistics are presented in Table 1.

were greater in residential sites than agricultural sites (P = .053) and May 2013 was greater than July 2012, November 2012 and March 2013 (P < .001).

Drivers of fluxes and processes

Overall, Spearman correlation analysis (Table 3) showed that both HMWCs (lignin and cellulose) and LMWCs (dextrose, mannitol, and trehalose) correlated with greenhouse gas fluxes but were highly land-use and compound dependent. Soil dextrose concentrations showed a trend of correlating negatively with CO_2 fluxes, particularly in residential sites. Inversely, soil mannitol concentrations showed a positive trend with CO_2 fluxes in agricultural sites. Trehalose concentrations were significantly correlated with CH_4 flux in residential ecosystems. Cellulose concentrations proved to be negatively correlated with CO_2 flux in both the forested and residential sites. N_2O flux showed a trend for a positive relationship with cellulose concentrations. Lignin concentrations showed no significant correlations with N_2O or CH_4 flux but did show a positive trend with CO_2 flux in agricultural land use.

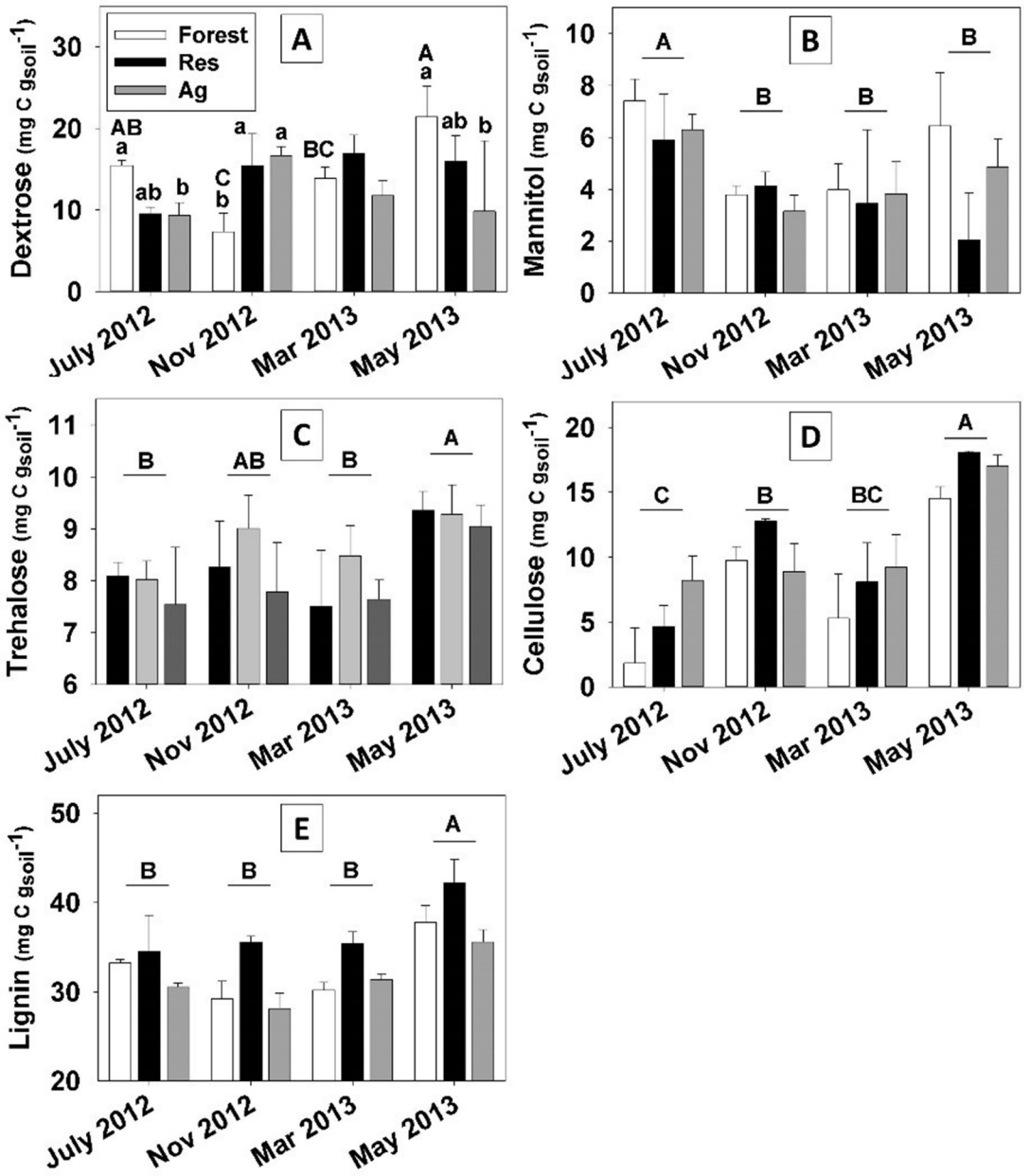

Figure 3. Concentration of dextrose (a), mannitol (b), trehalose (c), cellulose (d) and lignin (e) in the southern Appalachian Mountains based on near infrared reflectance spectroscopy (NIRS) models. The bars indicate the mean and the error bars indicate the standard error of the mean. Different lower-case letters indicate significant differences between land uses and different capitalized letters indicate differences between sample dates ($p < .05$). Forest = forested riparian zones; Res = residential development; Ag = pasture management.

Discussion

The main goal of the current study was to investigate the potential for NIRS to advance understanding of LMWCs and assess their role in microbial driven greenhouse gas fluxes. Our results indicate that NIRS can be calibrated and validated for the five compounds tested. However, only mannitol and dextrose concentrations were able to be validated by traditional extraction-based methods. This result

Table 2. Mixed model statistic comparing effect of land use and time of sampling on low molecular weight compounds reflecting carbon quality.

	Variable	Land-use			Time			Land-use x Time		
		df,df_{error}	F	p	df,df_{error}	F	p	df,df_{error}	F	p
Carbon Quality	Cellulose	2,5	2.5	.18	3,140	30.2	<.001	6,140	1.4	.22
	Dextrose	2,5	0.4	.68	3,140	2.5	.06	6,140	6.4	<.001
	Lignin	2,5	5.4	.05	3,140	17.3	<.001	6,140	0.9	.48
	Mannitol	2,5	0.6	.58	3,140	8.1	<.001	6,140	1.7	.12
	Trehalose	2,5	0.2	.81	3,140	4.1	.008	6,140	0.5	.81

does not necessarily invalidate this approach because these extraction methods are known to create a variety of artifacts which is why many recent studies have focused on FTIR and NMR approaches (Calderón et al. 2011; He et al. 2007).

The second objective was to determine the relationships between carbon composition and greenhouse gas fluxes which are dependent on a variety of LMWCs. Stoichiometric principles would inform us that carbon use efficiency would be greater under nutrient limiting conditions while nitrogen use efficiency would be greater under low carbon conditions. LMWCs and HMWCs were found to have differential relationships with greenhouse gas fluxes.

Temporal dynamics were found to be more important for LMWCs and HMWCs than land-use type. However, dextrose concentrations were greater in the July and May sampling time point than the agricultural land-use type. It is likely that tree roots in the forested sites are exuding greater amounts of dextrose during the growing season as found for the July and May sampling times. In general, our data showed that the May sampling time was greater in all carbon compound concentrations. This is similar to findings by Guggenberger and Zech (1994) showing the summer to be the season with the greatest carbon inputs of both carbohydrates and lignin. Seasonal differences in root exudation have been observed before and a likely explanation for the carbon concentrations we observed (Kaiser et al. 2010).

CO_2 fluxes were negatively correlated with dextrose. This is contradictory to other studies finding increased emission with greater dextrose concentrations (Liang et al. 2015) and is also a critical assumption of the Substrate-Induced Respiration (SIR) assay (West and Sparling 1986). Liang et al. (2015) found that at N saturated conditions CO_2 fluxes were reduced with dextrose additions and fluxes did not increase unless dextrose was added to relieve microbial C-limitation. They stated that this result supports a dynamic carbon use efficiency (CUE). Perhaps the dextrose concentrations in our study should be interpreted inversely with greater dextrose concentrations indicating a lack of utilization. Further, this would suggest that the magnitude of the response is associated with the level of N limitation. Trehalose concentrations, on the other hand, showed no significant effects on CO_2 fluxes. Trehalose concentrations, specifically under residential land-use, showed significant correlations with CH_4 flux. Increased CH_4 flux rates with the greater availability of labile carbon has been previously found (Hedin et al. 1998) but we are not aware of any other studies suggesting trehalose to be a preferred substrate for methanogenesis. It is also possible that microsites higher in trehalose were able to sustain higher respiration rates and thus, correlated with the creation of anoxic microsites that are conducive to methanogenesis (Von Fischer and Hedin 2007). N_2O fluxes did not yield strong relationships with any of the carbon compounds except for cellulose concentrations. This is suggestive that nitrification, not denitrification, is the dominant process for N_2O production, which is in line with the dominance of nitrifier denitrification found previously for soils in this region (Baas, Knoepp, and Mohan 2019). Cellulose concentrations might just be indicating a lower bulk density due to greater organic carbon concentrations (Heuscher, Brandt, and Jardine 2005) resulting in a more oxygenated environment that is conducive for nitrification (Knoepp and Vose 2007). It is not uncommon that soils high in cattle-based manure inputs are characterized by high nitrification-based N_2O fluxes (Dendooven et al. 1998; Paul, Beauchamp, and Zhang 1993).

Table 3. Spearman rank correlations for greenhouse gas efflux rates and soil and environmental variables. The values indicate the correlation coefficient ρ (rho). Coefficients with $p < .1$ are shown in bold.

Correlation	CO_2				CH_4				N_2O			
Variable	All	For	Res	Ag	All	For	Res	Ag	All	For	Res	Ag
Dextrose	**−0.16**†	0.16	**−0.31**†	−0.20	0.09	0.02	0.00	**0.31**†	−0.07	−0.09	−0.17	0.04
Mannitol	0.13	0.15	0.20	**0.23**†	0.07	−0.02	0.29	0.25	−0.02	−0.19	0.10	0.16
Trehalose	−0.06	−0.04	−0.06	0.01	0.14	0.10	**0.47***	0.00	0.04	0.11	0.21	−0.08
Cellulose	−0.05	**−0.32***	**−0.36***	0.19	−0.18	−0.04	−0.28	−0.15	**0.16**†	0.17	0.11	0.04
Lignin	0.06	0.18	0.06	**0.22**†	0.05	0.09	0.27	−0.09	−0.05	−0.08	0.15	−0.11

In conclusion, calibrating a variety of LMWC and HMWC concentrations to NIRS spectra is possible and a potentially affordable and powerful tool to unravel relationships between carbon composition and ecosystem function. This is particularly the case if NIRS is used in the field and can capture fine-scale spatial variance.

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