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Changes in Water-Extractable Organic Carbon with Cover Crop Planting under Continuous Corn Silage Production

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ABSTRACT: Long-term row crop agricultural production has dramatically reduced the pool of soil organic carbon. The implementation of cover crops in Midwestern agroecosystems is primarily to reduce losses of nitrogenous fertilizers, but has also been shown to restore soil carbon stocks over time. If labile carbon within agricultural soils could be increased, it could improve soil health, and if mobilized into subsurface drainage, it may positively impact watershed biogeochemistry. We tested for potential differences in water-extractable organic carbon (WEOC) at two different soil profiles (0–5 cm and 5–20 cm) between plots planted with cereal rye/daikon radish (cover crop), corn, and zero control (no vegetation) within the Illinois State University Research and Teaching Farm. We also tested for potential differences in denitrification within the upper soil profile throughout the growing year. We modeled excitation–emission matrices from soil cores through parallel factor analysis. We found no difference in WEOC concentrations between each crop treatment ($P = 0.2850$), but concentrations of WEOC were significantly lower in the 5–20 cm profile than that in the upper (0–5 cm) profile ($P = 0.0033$). There was a significant increase in WEOC after each treatment in samples after cover crop termination. The parallel factor analysis model found humic and fulvic acids to be the dominant fractions of WEOC in all soils tested. Humic and fulvic acids accounted for ~70% and 30% of model variation. Denitrification rates did not differ across treatments ($P = 0.3520$), which is likely attributed to soil WEOC being in limiting quantities and in primarily recalcitrant fractions. After three years, cover crops do not appear to alter soil WEOC quantity and type. Restoring the availability of carbon within agricultural soils will not be a short-term fix, and fields will likely be a net carbon sink, contributing minimal labile carbon to receiving waterways.

KEYWORDS: water-extractable organic carbon, cover crops, denitrification, PARAFAC

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Introduction

The conversion of natural wetland and prairie habitats to row crop agriculture lands has led to long-term reductions in organic carbon (DOC) in soil.[1,2] The availability of soil carbon is an essential component of soil health that influences soil microbial activity, nutrient availability, water holding capacity, and water filtration. Thus, practices to restore soil carbon and organic matter are being investigated globally to increase the sustainability of row crop agriculture. In many Midwestern agroecosystems, soil DOC is low, recalcitrant, and relatively immobile because of farming practices, such as annual tillage and overapplication of nitrogen (N).[3,4] Bioavailable carbon (ie, sugars and amino acids) is rapidly leached from crop residues and can infiltrate to lower levels in the soil profiles, but is often assimilated by soil bacteria before doing so.[5,6] Agricultural practices that reduce soil carbon results in a commensurate decrease in carbon lost as DOC in drainage tiles and receiving waterways altering biogeochemical processes.[7] Winter cover crops have the potential to reduce soil and N loss, but may also increase soil DOC.[8] In particular, increased carbon levels can not only lead to increased soil denitrification rates, but also increased denitrification rates in receiving waterways.[9]

Tillage practices coupled with continual corn and soybean production have led to an overall decrease in soil DOC.[10] Long-term conventional crop residue management, through the overapplication of N, is conducted to increase plant biomass decomposition and reduce the labile fractions of C. Excess labile DOC can be a detriment to soil N by promoting elevated bacterial activity, and subsequent loss of nitrates. As a result, efforts are now in place to restore the available pool of carbon to improve soil health for the benefit of increased grain yields. The decomposition of plant biomass is determined by the C:N ratio of the residues (roots, leaves, and stalk). Common cover crops, such as cereal rye and tillage radish, have low C:N ratios, 26:1 and 20:1, respectively, relative to corn (>60:1) and soybeans (30:1).[11,12] The low C:N ratio promotes rapid decomposition due to low lignin content and less energy required for microbial degradation and increasing carbon and N content in upper soil horizons.[13–15]

The primary goal of winter cover crops is to serve as an adaptive management technique to absorb residual N from the previous growing season, mineralized N from crop residue, and applied N fertilizer during the winter and spring under climatic conditions conducive for leaching and denitrification. Then, stored N within the plant biomass is released to the soil solution upon termination of the cover crop due to mineralization, ammonification, and nitrification to become available for the subsequent cash crop.[16]

Terminated cover crops rapidly leach labile fractions of DOC and water-extractable organic carbon (WEOC); however, the amount and bioavailability of WEOC that accumulate within agricultural soils is largely unknown.[17,18] In some instances, the DOC level has not increased with cover crop implementation, but this is likely attributed to soil leaching.[19] WEOC is characterized as the fraction of DOC present that is easily transferred into an aqueous environment and consists of a largely bioavailable and mobile portion of DOC.[20] If WEOC is present in elevated concentrations, as a result of cover crop implementation, it will serve as a means to restore soil health.[21] Due to this increased mobility, this fraction of DOC may account for a large proportion entering aquatic ecosystems and may control many of the biogeochemical processes in those systems.

Within corn and soybean agroecosystems, WEOC is present in limited quantities due to the recalcitrant components of residues; however, winter cropping systems hold the potential to increase soil organic matter, along with WEOC, due to more rapid decomposition.[22] In perennial grasses, such as *Arundo donax* (giant reed) used for biofuel production, soil DOC levels significantly increased within the upper soil profile after long-term incorporation (40 years).[23] Similar to biofuel production, implementing winter cover crops within corn and soybean rotation will increase the plant biomass in a field and subsequent residue incorporation into field soils. Focusing on the potential contribution winter cover crops may have on the fractions of WEOC is an important management question, because the availability of WEOC relative to water-extractable organic N in soils was a better predictor of soil respiration and fosters higher rates of bacterial respiration than that of DOC.[24] If the labile fractions of WEOC leach into receiving aquatic ecosystems, higher rates of denitrification may result.[25]

A limited number of studies have shown that DOC levels significantly increase within two to three years of cover crop implementation, but can take considerably longer depending on the cash crop used and rotation.[26,27] An increase in soil DOC levels has been observed within the vineyard and corn/soybean agroecosystems; however, the types of DOC present have not been investigated.[26] The increase in soil organic matter associated with incorporating cover crop residues is a benefit to soil health, but the contribution to labile forms may be minimal due to the high lignin and cellulose content or rapid assimilation prior to leaching through soil.[8] An accumulation of humic substances would benefit soil water retention and N holding capacity of soils, but may result in a lessened response by heterotrophic bacteria in receiving waterways relative to more labile fractions.[28] Cover crops have the ability to increase soil porosity by potentially allowing labile fractions of WEOC to more rapidly enter the subsurface drainage.[29] If mobile fractions of DOC are increased with the incorporation of winter cover crops, identifying any bioavailable fractions within the soil profile would show the potential for increased input of labile WEOC under periods of soil saturation.

An increase in labile organic carbon levels within agricultural soils could foster elevated rates of denitrification under moist soil conditions.[30] Terrestrial denitrification must be considered when studying allochthonous inputs of WEOC. The activity of soil bacteria mediates the quantity of WEOC that may enter tile drainage, in addition to its bioavailability. The incorporation of crop residues into agricultural soils through tillage puts plant biomass in direct contact with soil microbiota, promoting decomposition and rapid assimilation of labile WEOC fractions that are leached from plants. The residues of soybeans and vetch within an experimental field plot result in a spike in denitrification, when field collected soils were incubated in a controlled laboratory environment, but these spikes were brief (<5 days), and rates did not differ irrespective of the residue type after this initial spike.[31] Bacterial uptake limits WEOC inputs, but the mobility of WEOC is further reduced in soils with increased iron and clay content.[32] These soil factors, more specifically the Fe/Al content of native clay, limit the desorption of WEOC and subsequent leaching into subsurface drainage.[33] This is an important consideration as the loam soils typical of central Illinois are often high in clay content.[34] If an increase in terrestrial denitrification after long-term cover crop planting was to occur, allochthonous WEOC inputs would continue to be minimal and have a little positive impact on nitrate reduction in receiving aquatic environments.

The availability of DOC plays an important role in the dynamics of heterotrophic N processing, in aquatic systems, primarily via denitrification.[32] As in terrestrial systems, the presence of elevated N concentrations can shift denitrification to become C-limited.[35] Denitrification is an energetically costly process and requires a continuous DOC supply in the presence of nitrate saturation often seen in agricultural watersheds. Increasing the soil DOC pool over time may lead to larger inputs of labile WEOC during critically important seasonal periods. If bacterial demand for DOC could be met, denitrification may be able to adequately increase during seasonally intense nitrate inputs.[36] The limited size of mitigation wetlands relative to watershed areas would potentially benefit from an increase in allochthonous DOC delivery, rather than wetland plant management as suggested in other studies.[37] Forested headwater streams experience increases in heterotrophic activity, as a result of leaf litter decay and an associated pulse of nutrients.[38] Agroecosystems experience similar seasonal fluxes of nutrients postharvest from the decomposition of corn and soybean residues, but the DOC inputs low relative nitrate levels.

The primary objective of the proposed study was to test how the implementation of cover crops (cereal rye/tillage radish) may improve the quantity and quality of WEOC in agricultural field soils under a long-term corn/soybean rotation. We specifically tested for differences in amounts of water-extractable carbon from soils under different crop treatments (winter cover crops and corn, corn only, and no crops). The additional residue accumulation from cover crops may cause a shift to more bioavailable carbon. To test for this potential change in carbon bioavailability, we modeled excitation–emission matrices (EEMs) from soil extractions from each treatment plot with the aid of a parallel factor analysis (PARAFAC). Terrestrial denitrification is limited by both soil N and carbon availability and often serves as a sink for both nutrients, thus potentially impacting the biogeochemistry of receiving waterways. We also subjected field soils from each plot to denitrification assays to examine possible crop treatment and seasonal effects on bacterial activity.

Materials and Methods

Site description. The study of WEOC levels within soils under different crop types was examined in an experimental system within the Illinois State University Teaching and Agriculture Research Farm near Lexington, IL, USA. The experimental cover crop system began in 2011 within an experimental field comprising nine plots ($N = 9$; 2,023 m^2, half-acre), arranged in a randomized complete block design replicated three times. The crop treatments used in this study were a zero control (no corn, no cover crop, and no N), control (corn and N), and cereal rye/tillage radish (corn, N, and cover crop). All treatment plots were tilled and planted within poorly drained Drummer and Elpaso silt clay loams. Background soil and chemical parameters collected from the study site along with monthly precipitation data are listed in Tables 1 and 2, respectively.

Table 1. Nutrient and chemical properties of soils from the Illinois State University Agricultural Teaching and Research Farm test plots.

SOIL MEASURES	SOIL TEST mg kg^{-1}
Mg	150.5
Ca	221.9
K	4285.2
P	636.8
Inorganic N	
NO_3-N	18.5
NH_4-N	7.7
Soil chemical properties	
pH	6.3
TOC (%)	2.4
CEC ($cmol_c kg^{-1}$)	31.8

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Table 2. Average air temperatures throughout the implementation of the agricultural test plots.

MONTH	2011	2012	2013
	MEAN AIR TEMPERATURE (°C)		
January	−6.78	−0.78	−2.5
February	−3.22	0.78	−2.72
March	3.28	12.61	0.11
April	9.2	11.89	9.28
May	15.26	19.61	17.61
June	21.5	21.78	21.78
July	26.39	27.61	22.72
August	22.89	22.72	22.39
September	17.28	18.11	20.39
October	13	11	N/A
November	6.89	4.22	N/A
December	1.61	2.22	N/A

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Anhydrous ammonia (200 kg ha^{-1}) was directly knifed into all plots in November 2012. During the fall of 2011, N was added in the form of $(NH_4)SO_4$, at a rate of 50 kg ha^{-1} to promote cover crop growth. This was not necessary for the 2012 planting, due to the available soil N. In 2012, the daikon radish/cereal rye plots were terminated in March by applying glycophosphate to allow for mineralization of N prior to planting. Soils were slightly tilled to incorporate residues into the upper soil profile and represent practices common in the region.

Soil collection. To test for potential differences in WEOC levels under different cropping systems, soil cores were collected within the same plots throughout 2013 (April–October). The spring sampling dates were April 5 and April 15, which encompassed the week before cover crop termination, along with an additional sample prior to corn planting in May. A single sample, July 15, was used during the summer growing season, during a period of rapid nutrient uptake by corn plants. Two fall samples were also incorporated into the analysis, which were prior to harvest (February 9) and postharvest before soil freeze-up (February 10).

WEOC analysis. To estimate WEOC levels, soil cores were randomly collected per treatment within each plot up to a depth of 80 cm, but two subdivided depth fractions were analyzed 0–5 cm and 5–20 cm. The two subdivided cores were combined into a composite sample, dried at 105°C for 24 hours, and sieved through a 1 mm mesh prior to being analyzed. To measure NH_3, NO_3, and WEOC levels, a 5 g subsample was taken from each treatment and depth profile.[39] Soil samples were suspended in 50 mL of 0.01 M $CaCl_2$, placed on a rotary shaker for 15 minutes, and followed by 10 minutes of centrifugation at 4,000 rpm. The supernatant was filtered through Whatman 42 filter paper and acidified to a pH of 2 using 2 M HCl.[40]

The concentrations of DOC from field soil cores were calculated from optical data using a PerkinElmer Lambda 35 UV-Vis Spectrophotometer. From each soil extraction, 4 mL of extractant was transferred into a 1 cm quartz cuvette and the absorbance was measured at 360 nm.[41,42] The specific ultraviolet absorbance at 254 nm was also tested. This is a measure of recalcitrance of WEOC, the higher the specific ultra violet absorbance, the more recalcitrant the WEOC is within the extractant.

To test for potential differences in the dominant fractions of C between crop treatments, fluorescence EEMs of WEOC were measured using a PerkinElmer LS-55 Spectrofluorometer. Synchronous scans were done across excitation wavelengths from 240 to 480 nm at 5 nm intervals and emission wavelengths of 300 to 600 nm at 0.5 nm intervals at a scan speed of 1,200 nm/second. By analyzing EEMs using a multivariate modeling technique, PARAFAC, the composition of WEOC (humic-like, fulvic-like, and protein-like) can be assessed using the fluorescent characteristics of each soil extraction.[43] After creating EEMs for each sample, the data were exported into a Microsoft Excel (2010) worksheet with the aid of Spekwin32 spectroscopy software.[44]

Prior to the EEM scans, a 290 nm cutoff filter was applied to all samples in order to reduce second-order Rayleigh scattering. Spectral corrections were performed with factory-supplied data from previous instrument calibrations. Inner filter effects were accounted for with absorption corrections that were applied to the blank and sample EEMs. A sample blank (Milli-Q water) was scanned on each day of EEM analysis to subtract the fluorescence intensity from each sample to limit scatter bands.[45] Raw machine units were normalized into Raman units with the aid of the drEEM Toolbox 0.2.0.[45] WEOC characterization was performed via PARAFAC modeling with MATLAB R2014a.[46] The drEEM Toolbox 0.2.0 (http://www.models.life.ku.dk) was imported to MATLAB to perform PARAFAC.[45]

PARAFAC modeling allows for multiple fluorescence spectra to be overlain and decompose the data to allow for relative estimates of different components or *types* of WEOC. By validating an appropriate number of components from the overall model, the percentage of contributions can be calculated from the F_{Max} values provided in the model output. The relative contributions of each component can be applied to the total WEOC content within each sample to calculate what percentage of total WEOC is accounted for by each fraction of C (referred to as components) that was modeled. By accounting for the proportions of different C fractions, it was possible to test how cropping systems alter the relative composition of WEOC throughout the upper soil profile.

Using PARAFAC modeling, we attempted to validate a range of 2- to 6-component models with the fluorescent EEMs measured. Only two unique components were identified across all soil extractions. A 3-component model was validated; however, the core consistency values were considerably lower (37.8%). The core consistency values were compared for the 2- and 3-component models, both of which were validated via split-half analysis.[47] The core consistency diagnostics provides a measure of how well the spectral loadings account for variation in the dataset.[48] Core consistency values should be ~100%; the 2-component model had a much higher value (98.7%) than that of the 3-component model, and therefore, the 2-component model was selected. The percentage of relative contributions (from F_{Max}) of each of the two components to the overall model was then calculated to test for different relative quantities of WEOC types between crop treatments.

Soil denitrification. The soil samples from the 0–5 cm profile were subjected to the acetylene inhibition technique to measure potential denitrification rates.[49] Denitrification was only measured in the 0–5 cm soil profile, as this is the zone of the highest bacterial activity within terrestrial and aquatic environments. Since the soils were previously dried prior analyses, a rewetting pretreatment was required. The length of rewetting and degree of saturation play an important role in measuring bacterial activity. To appropriately rewet soils, all samples were rewet to the appropriate water-filled pore space (WFPS). Using WFPS as the metric to reach the desired soil saturation allowed a quantifiable way to accurately rewet soils and standardize conditions across samples. Field soils were rewetted to 70% WFPS, as this degree of saturation has been shown to maximize bacterial activity in terrestrial soils.[50] Soils were rewetted for 24 hours prior to being assayed for denitrification.[51]

To measure potential differences in denitrification rates between crop treatments over time, 10 g of dried soils were allocated to 150 mL glass media bottles in triplicates. After the 24-hour rewetting period, soils were assayed via the acetylene inhibition technique. Each replicate received 50 mL of Milli-Q water and was amended with 5 mL of 0.1 M chloramphenicol solution. The headspace of each media bottle was then purged with N_2 gas for five minutes to create anaerobic conditions. Fifteen milliliters of acetylene (C_2H_2) gas was injected into the headspace and shaken to be incorporated into the soil. The addition of C_2H_2 blocks the conversion of NO_3 to N_2 gas, and therefore, the end product for this assay is N_2O. A 10 mL headspace gas sample was taken 15 minutes after the initial C_2H_2 injection, and once an hour for the following 4 hours. Denitrification rates were calculated from the production of N_2O over time per gram of dried soil. Headspace gas samples collected throughout the assay were analyzed using a Shimadzu GC-2014 gas chromatograph (Porapak Q packed column; detector temperature 300°C; oven temperature, 100°C; flow rate of carrier gas [ultrapure N gas], 10 mL/minute).

Data analysis. Potential changes in WEOC concentrations in the shallow and deep soil profiles over the course of the study were tested. A fixed-effects analysis of variance (fixed-effects ANOVA) was used to test the effect of crop treatment, time, and soil depth on WEOC concentrations in agricultural soils.[47] A planned contrast was used to test for differences in

WEOC compositions before and after the cover crops were terminated within both soil profiles. To test for differences in soil denitrification rates between soil depths and crop type, a fixed-effects ANOVA was used. Appropriate follow-up tests were performed by using Tukey's multiple comparisons. From the PARAFAC model, the percentage of variation that each model component accounted for was calculated. A one-way ANOVA test was employed to discern potential differences in the relative proportion of each component (F_{Max1} and F_{Max2}) between crop treatments.

Results

Total WEOC. Cover crops did not have significantly higher WEOC content, when compared with the corn and zero control plots ($F_{2,35} = 1.37$, $P = 0.2850$). In all treatments, a significantly higher concentration of WEOC is present in the 0–5 cm depth than that of the 5–20 cm depth profile ($F_{1,35} = 12.14$, $P = 0.0033$, Fig. 1). WEOC concentrations varied throughout the course of the study ($F_{5,35} = 3.11$, $P = 0.0399$), but there was no significant interaction between crop treatment and time ($F_{10,35} = 0.4$, $P = 0.9236$). Since no difference in WEOC levels was observed between treatments over time, a planned contrast was performed on all mean WEOC concentrations, irrespective of treatment, in the upper soil profile (0–5 cm). It was found that WEOC concentrations were significantly greater at each sampling date following cover crop termination ($F_{5,53} = 7.59$, $P < 0.0001$, Fig. 2), relative to the sampling date prior to cover crop termination. Though temperature (Table 2) and precipitation (Table 3) exhibited considerable variation throughout the study, these parameters did not lead to any significant variation in WEOC concentrations. At the lower soil profile, no differences in WEOC concentrations were observed over time (Fig. 3). When comparing all nutrient treatments at both depth profiles, there was no statistically significant difference in WEOC levels ($F_{2,35} = 0.04$, $P = 0.9563$).

PARAFAC WEOC. A 2-component model was validated with a high core consistency value (98.7%) and split-half analysis. Each component was characterized by single sharp peaks. Component 1 produced a maximum fluorescence peak at the excitation/emission wavelength of 335/421 nm. This component exhibited spectral characteristics similar to that of ubiquitous humic acids that contribute to agricultural soils from decomposing vegetation.[45] Component 2 was characterized by a fluorescence peak at the excitation/emission wavelength of 385/470 nm (Fig. 4). Component 2 closely resembled fulvic acids typical of terrestrially derived compounds previously identified in agricultural headwater streams.[46]

The relative amounts of humic-like versus fulvic-like compounds significantly differed from one another. The humic-like compounds accounted for 68% of model variation and fulvic-like compounds contribute to 31% of total variation (Fig. 5). Components 1 and 2 had similar percentage of contributions (F_{Max1} and F_{Max2}) to the overall model for each crop treatment ($F_{2,101} = 0$, $P = 0.9961$). The similarity in the relative percentage of each component suggested that soils have

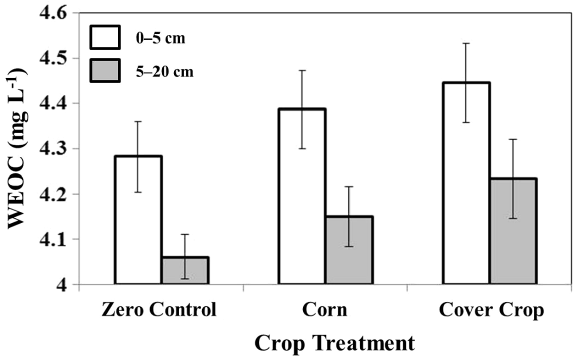

Figure 1. Mean WEOC concentrations (±SE) of all soil cores collected throughout the sampling period. Bars are separated by crop treatment and soil core depth: hollow (0–5 cm) and gray (5–20 cm).

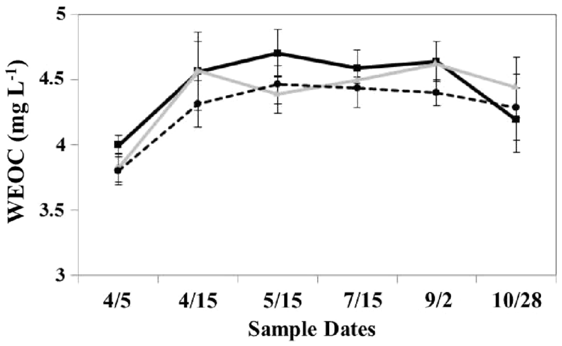

Figure 2. Change in WEOC concentration within the upper soil profile (0–5 cm) throughout the study period. Crop treatments denoted by lines as follows: zero control (dashed black), corn (gray), and cover crop (black).

Table 3. Average monthly precipitation for the experimental site since the advent of the cover crop planting.

MONTH	2011	2012	2013
	MEAN PRECIPITATION (mm)		
January	61.21	36.07	89.66
February	74.17	33.27	58.67
March	75.95	21.08	45.97
April	139.45	70.36	126.49
May	120.65	54.1	171.2
June	124.46	42.93	66.29
July	107.95	37.08	32
August	58.42	114.1	74.93
September	73.41	148.08	30.48
October	60.71	125.98	N/A
November	78.99	25.4	N/A
December	92.71	51.31	N/A

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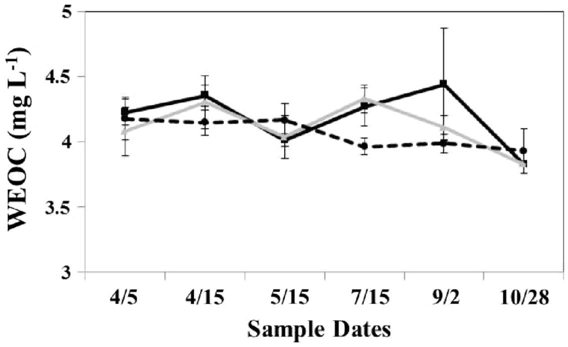

Figure 3. Change in WEOC concentration within the lower soil profile (5–20 cm) throughout the study period. Crop treatments denoted by lines as follows: zero control (dashed black), corn (gray), and cover crop (black).

similar amounts of humic and fulvic acids, irrespective of the type of crops planted.

Denitrification. Only soil cores collected in April and May were incorporated into the denitrification potential analysis. The soil samples assayed from the summer and fall collections did not produce measurable rates of denitrification, because N_2O concentrations were below the detection limit. The crop treatments did not have a significant effect on soil denitrification rates ($F_{2,26} = 1.11$, $P = 0.3520$, Fig. 6). Denitrification rates exhibited no significant variation throughout the 2013 sampling period ($F_{2,26} = 0.79$, $P = 0.4708$; Fig. 7). The interaction of sampling date and crop treatment was also not significant ($F_{4,26} = 2.36$, $P = 0.0921$).

Discussion

Within three years of cover crop implementation, cereal rye and tillage radish residues have not increased the WEOC content in soils relative to the other treatments (Fig. 1). The lack of difference may be attributed to the relatively short time period that cover crops were planted within this site. Restoring DOC within agricultural soils is often on a decadal scale, even within restored sites, where agricultural production has been halted.[50] Long-term studies have not focused on WEOC levels, but WEOC levels have been correlated with total soil DOC and it may be fair to assume that WEOC follows similar trends. The implementation of rye grass into an annual rotation has been observed to increase soil DOC (and carbohydrates) by small, but significant quantities over a five-year period.[51] Interestingly, similar results have been observed after an eight-year period of corn and sorghum residues into agricultural soils.[52] Application rates of N were lower in both these studies, and it was unclear whether test plots were

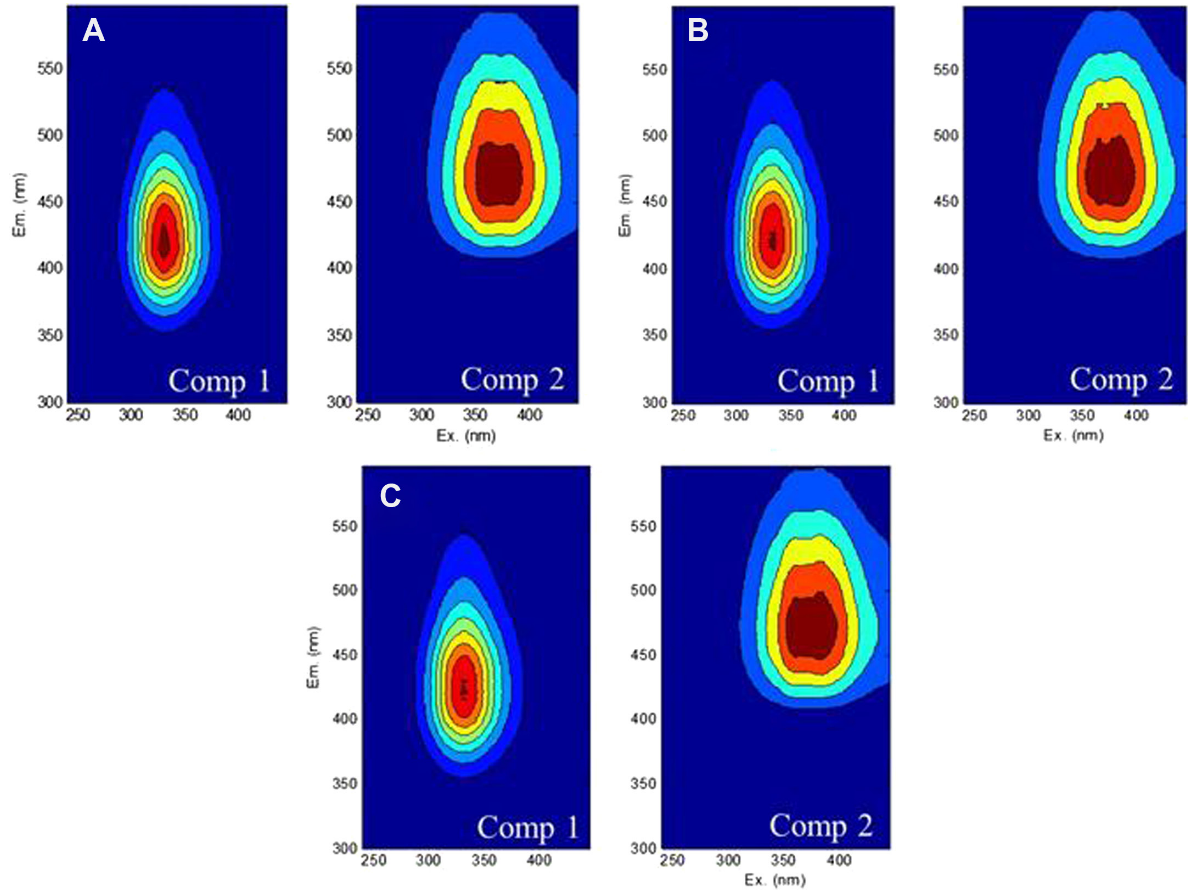

Figure 4. EEMs of the two components identified from the PARAFAC model. Components are separated by letters to denote the different crop treatments as follows: **A** (zero control), **B** (corn), and **C** (cereal rye/tillage radish).

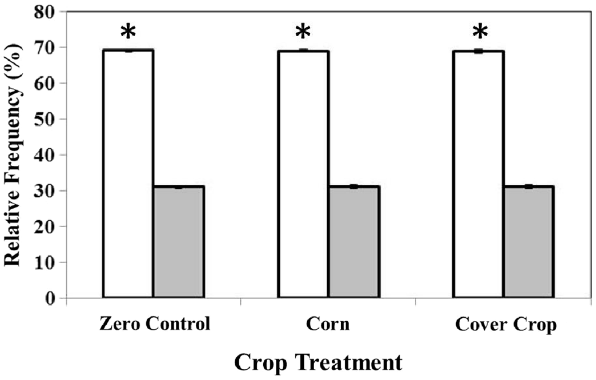

Figure 5. Mean average percentage of contribution (±SE) of each component by the PARAFAC model from soils of each crop treatment. Bars are separated by crop treatment and soil core depth: hollow (0–5 cm) and gray (5–20 cm). Significant differences are denoted by an asterisk ($P < 0.05$).

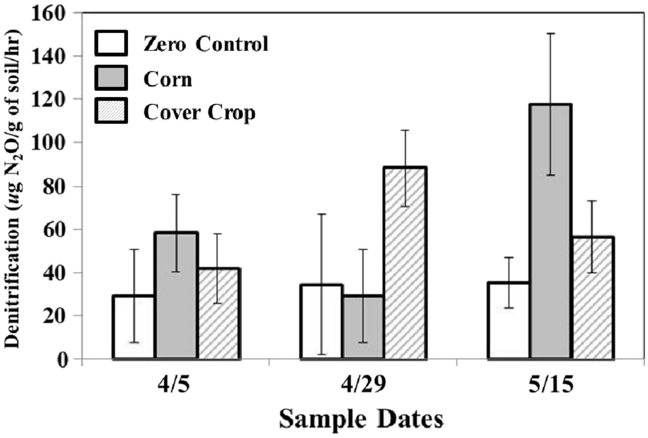

Figure 7. Mean denitrification rates (±SE) by crop treatment over time. Crop treatments are denoted as follows: zero control (no fill), corn (gray), and cover crop (diagonal).

artificially drained, each of which would have direct impacts on soil carbon restoration. The relative percentage of WEOC to total soil carbon is low, and likely remains in the soil for short periods of time, making it difficult to be observed in a field setting. Noticeable changes in allochthonous contributions of WEOC from cover crops may constitute a long-term benefit, and changes in stream and wetland biogeochemistry will not exhibit immediate changes.

The labile fractions of carbon are often leached within days of plant death and incorporation into soil or aquatic environments.[53,54] Within agricultural soils, labile fractions of DOC, such as amino acids and sugars, can be assimilated in <24 hours of release.[55] If soil sampling for this study took place soon after the termination date, a pulse in WEOC may have been observed within the upper soil profile (0–5 cm). The contribution of the rapidly leach labile fractions can be minimal in that they comprise 5%–10% of total DOC composition in terrestrial and aquatic systems.[56] Although brief, the limited availability of labile fractions of WEOC can foster dramatic changes in bacterial activity, if leached into aquatic systems.[57]

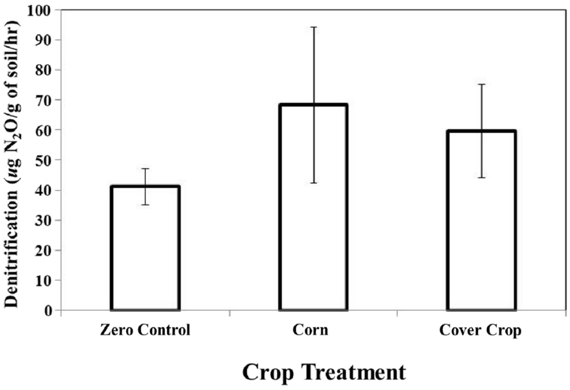

Figure 6. Overall mean denitrification rates (±SE) of all sampling dates. No significant differences were observed between crop treatments.

Concentrations of WEOC significantly decreased from the upper (0–5 cm) to the lower (5–20 cm) soil profile (Fig. 1). Although the concentrations of WEOC did not differ significantly between treatments, cover crop and corn plots maintained elevated WEOC levels relative to the zero control plots at both soil depths. Soil bacteria can rapidly respire and decompose labile fractions of carbon when available, which may account for the observed decrease in WEOC levels across the soil profile.[58] Increases in soil organic carbon are necessary to balance cellular stoichiometry in the presence of high soil N content.[59] Terrestrial soils can foster high rates of denitrification when soils become saturated, removing both carbon and N from fine particulate soils.[60] The decrease in soil WEOC suggests that a large proportion of WEOC is lost as water infiltrates deeper soil layers and enters subsurface drainage.

It was initially predicted that soils within the cover crop plots would have a higher proportion of labile fractions, due to the ease of residue decomposition relative to lignin-rich corn residues. Minimal differences in fluorescence spectra were observed between WEOC characterizations between each plot (Fig. 4). The 2-component PARAFAC model found that WEOC comprises humic-like and fulvic-like substances. For all treatments, humic-like substances (component 1) accounted for significantly more variation (~70%) in the PARAFAC model than that of the fulvic-like (component 2) fraction (30%; Fig. 4). Crop residues contribute a pulse of labile carbon (proteins, polysaccharides, and organic acids) after harvest or termination, but as previously stated, this brief period was likely missed in the sampling regime.[61]

The elevated C:N ratio and high lignin content of corn residues contribute to the slow decomposition and serve as a starting material for the formation of humic substances in agricultural soils.[62] Humic acids are formed from the combination of polyphenolic and carboxylic groups and largely resistant to bacterial degradation due to their complex aromatic structure.[63,64] Relative to corn residues, cereal rye and tillage radish have lower C:N ratios, but did not affect the relative percentage of humic acids within the associated test plot.[65] The prevalence of humic acids within the experimental plots may have been due to previous agricultural practices rather than the recent incorporation of cover crops into the annual rotation. Continual corn production prior to this study appeared to reduce the relative quantity of WEOC within the study soils, as observed by the zero control. The complexity of humic acids reduces their usage by soil bacteria; however, humic acid-oxidizing bacteria can be prevalent within soils and may account for the significant reduction observed within the upper soil profile.[66] Although humic acids occupy a greater percentage of the total WEOC composition than fulvic acids, the relative increase in humic acid concentration in cover crop plots was minimal.

Under periods of DOC limitation, bacteria that are members of *Acidobacteria, Firmicutes, Betaproteobacteria*, and *Caulobacterales* (along with other phyla) can oxidize humic acids into more readily usable compounds.[66] Component 2 of the PARAFAC model represented fulvic-like compounds, which were likely formed from the bacterial degradation of humic acids into the less aromatically complex compounds. Although fulvic acids did not comprise the dominant fraction of WEOC, they are highly soluble in water and may be exported from agricultural soils to a greater degree than humic acids.[67] The ease of transport coupled with increased bioavailability holds the potential that the contribution of fulvic acids from crop residues may have some benefit to aquatic heterotrophic bacteria. Unfortunately, the role of fulvic acids as an electron donor for denitrifying bacteria is largely unknown; the increased availability of a degradable DOC source may be an effective energy source under prolonged carbon limitation.

The availability of WEOC may also be limiting the occurrence of denitrification within the agricultural soils. Throughout the study period, there are considerable fluxes in available nitrate sources, but minimal changes in denitrification potential over time. In field soils, elevated soil nitrate levels often translate into increased denitrification rates under moist soil conditions.[68] The availability of applied N within each plot appeared to shift DOC to the rate-limiting nutrient. Throughout the study period, each treatment plot maintained similar WEOC concentrations, and denitrification rates appeared to reflect the lack of change in WEOC availability. The availability of cover crops was originally hypothesized to facilitate increased soil denitrification, but a slight decrease (not significant) was observed (Fig. 6). Although the WEOC level was slightly elevated, the immobilization of N within the cover crop biomass may have limited the availability of N to denitrifying bacteria prior to cover crop termination. The immobilization of soil N may have indirect benefits by potentially reducing the evolution of N_2O from field soils.[69] Agricultural soils have been suggested to serve as a net carbon sink, which is attributed to the high demand by soil microbiota due to artificially high soil N availability after cover crop termination or fertilizer application.

There was sufficient WEOC to facilitate the occurrence of denitrification within the experimental plots and may account for the reduction of WEOC concentrations within the top 5 cm of soil. A small percentage of the observed WEOC loss is likely due to bacterial assimilation, but bacterial activity is responsible for a large proportion DOC reduction within soils.[70] The similarity in denitrification rates across each crop treatment may also be explained by the fractions of WEOC available.[71] Humic acids can be used as an energy source to facilitate bacterial respiration, but the structural diversity of terrestrially derived humic acids can lead to low or variable activity.[72] Denitrification would likely spike immediately after cover crop termination due to the pulse amino and fatty acids leached from cover crop residues, but this increase would be brief and not accounted for with the sample frequency.

Within the initial years of cover crop implementation, the availability of WEOC and dominant fractions suggested that there will a minimal change in allochthonous carbon inputs. The lack of difference in denitrification between treatments may offer potential insight as to how allochthonous DOC from early cover crops would potentially alter the agroecosystem biogeochemistry. The mobile fractions of carbon are primarily in recalcitrant forms, minimizing the usage by heterotrophic bacteria within receiving waterways.[73] Although recalcitrant fractions of WEOC can be degraded into more usable forms within aquatic environments, the change in concentrations are insufficient to drive significant changes in aquatic denitrification (Grebliunas and Perry, submitted). The allochthonous contribution of recalcitrant DOC may be of some benefit where bacteria have used the recalcitrant fractions for growth under strong carbon limitation.[74] In some instances, photochemical degradation can also convert humic acids into more bioavailable fractions in aquatic environments.[75] Wetlands within agricultural landscapes have minimal canopy coverage, potentially allowing recalcitrant fractions of DOC to be reduced via this photochemical pathway and fostering elevated rates of denitrification if the pool of terrestrial DOC in soils were increased over time. An increase in labile DOC would be ideal to create conditions for a rapid bacterial response to seasonal pulses of nitrate with elevated rates of denitrification (Grebliunas and Perry, in preparation). Although cover crops are capable of increasing soil DOC and improving soil health, the impact on the biogeochemistry within intensely farmed watersheds may be minimal until fields assimilate a sufficient pool of DOC and begin to export appreciable amounts of DOC.

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Author Contributions

Conceived and designed the experiments: BDG, SDA, and WLP. Analyzed the data: BDG and WLP. Wrote the first draft of the manuscript: BDG. Contributed to the writing of the manuscript: BDG, SDA, and WLP. Agree with manuscript results and conclusions: BDG, SDA, and WLP. Jointly developed the structure and arguments for the paper: BDG, SDA, and WLP. Made critical revisions and approved final version: BDG, SDA, and WLP. All authors reviewed and approved of the final manuscript.

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