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Does No-Tillage Mitigate Stover Removal in Irrigated Continuous Corn? A Multi-Location Assessment

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No-tillage (NT) may ameliorate negative effects on soil properties from corn (*Zea mays* L.) stover harvest, but few long-term irrigated continuous corn production systems have been evaluated to test this hypothesis. We evaluated three long-term no-tillage sites (4–13 yr) in Nebraska and Colorado that spanned a range of precipitation and soil organic carbon (SOC) levels. We measured SOC, $\delta^{13}\text{C}$ of SOC, soil microbial biomass (SMB) and composition (i.e., phospholipid fatty acids, PLFAs), and water stable aggregation at all sites under stover retention vs. removal (~60%). Surface SOC stocks (0–30-cm depth) increased across the gradient and were 46.4, 57.4, and 63.1 Mg C ha⁻¹ for Colorado, central Nebraska, and eastern Nebraska, respectively. Overall, residue removal decreased SOC stocks by 6% and soil aggregation by 12% in the 0- to 30-cm depth. The $\delta^{13}\text{C}$ signature of SOC indicated less new surface C storage under residue removal in Colorado, but not at the two Nebraska sites. Residue harvest did not decrease SMB or change soil microbial community structure, suggesting that high plant productivity buffered community composition from stover harvest impacts under NT but stimulated microbial activity levels that led to SOC loss. The high rates of stover removal used in this study decreased SOC stocks and aggregation at all sites compared to residue retained treatments, suggesting that no-tillage alone was not sufficient to maintain erosion protection and soil function.

Abbreviations ARDEC, Agricultural Development and Education Center in Colorado; dbRDA, distance-based redundancy analysis; ENREC, eastern Nebraska Extension and Research Center; Harv, >60% residue removal; NT, no-tillage; PLFA, phospholipid fatty acid; PLFA-C, phospholipid fatty-acid carbon; Ret, residue retained; SCAL, south central Agricultural Laboratory in Nebraska; SMB, soil microbial biomass; SOC, soil organic carbon.

The return of crop residues such as corn stover to the soil is essential to agroecosystem functioning and maintaining soil C stocks, aggregation, and soil microbial communities. Harvesting corn stover, however, provides livestock feed, is anticipated to become a large-scale feedstock for cellulosic ethanol production, and removal can enhance early season plant growth (Sindelar et al., 2013). Developing sustainable harvest recommendations that protect the soil from erosion and SOC loss has been of considerable interest (Johnson et al., 2014; Wilhelm et al., 2007). Conservation management practices, such as NT, manure application (Blanco-Canqui et al., 2014), and cover crops (Osborne et al., 2014) may mitigate the negative environmental effects of stover removal by reducing soil disturbance and providing additional soil C input (Johnson et al., 2013; Sindelar et al., 2013). However, the effects of stover removal can be site- and treatment-specific (Osborne et al., 2014), such that the overall effect remains uncertain (Qin et al., 2016). Recent meta-analyses find conflicting effects of residue removal on SOC stocks, ranging from no consistent effect even with removal rates as high as 70% (Qin et al., 2016) to overall SOC loss with removal rates of 25 to 100% (Anderson-Teixeira et al., 2009).

Core Ideas

- Long-term residue removal with irrigation may degrade soil despite no-tillage.
- Residue removal was assessed across three irrigated corn no-tillage sites.
- Stover removal decreased soil organic C stocks by 6% and soil aggregation by 12%.
- Stover removal did not affect soil microbial biomass or composition.
- No tillage alone is not adequate to reduce erosion and maintain soil function.

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Table 1. Site characteristics for the three irrigated long-term residue removal studies sampled.

Site	State	MAT†	MAP	Soil series and type	Bulk density	pH	SOC	Irrigation amount	Removal rate	Fertilizer N rate	Study duration
		°C	mm		g cm ⁻³		g kg ⁻¹	mm	%	kg ha ⁻¹	yr
ARDEC‡	Colorado	10.6	383	Fort Collins clay loam	1.49	8.3	10.5	411 ± 6	66	202	9
SCAL§	Nebraska	10.3	731	Hastings silt loam	1.25	6.8	16.2	134.9	63	200	4
ENREC¶	Nebraska	9.8	740	Filbert, Tomek silt loams	1.13	6.6	18.7	125 ± 7	70	202	13

† MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon.

‡ ARDEC, Colorado. Data from Halvorson and Stewart (2015).

§ SCAL, central Nebraska. Data from Blanco-Canqui et al. (2014).

¶ ENREC, eastern Nebraska. Data from Schmer et al. (2014).

A major challenge in determining overall stover removal effects include microsite factors that impact plant growth, rooting characteristics (Johnson et al., 2006), and soil water retention, all of which determine the direction and magnitude of SOC cycling (Hooker et al., 2005; Mann et al., 2002). Generally, NT stimulates surface rooting from increased water and nutrient availability due to residue cover (Qin et al., 2006). Long-term stover removal can reduce crop yields and root C inputs that subsequently reduce SOC storage (Halvorson and Stewart, 2015), soil aggregation (Jin et al., 2015; Osborne et al., 2014), and SMB (Stewart et al., 2014). However, other studies in rainfed NT corn systems in Nebraska, Minnesota, Canada, and other regions have not observed decreased SMB (Ahlschwede, 2013; Johnson et al., 2013; Spedding et al., 2004) or appreciable changes in microbial composition with residue removal (Lehman et al., 2014). Nitrogen fertilization has been shown to further impact stover removal effects, where limited crop C inputs due to low-N fertilization exacerbates both soil C loss and reduces SMB with residue removal under irrigated continuous corn (Stewart et al., 2018). No changes in microbial community structure with residue removal suggest that NT with moderate residue removal rates may be an effective management practice that does not significantly compromise agroecosystem function.

Soil microbial biomass comprises a small portion of soil C, yet it is critical to maintaining soil C cycling and soil function. Recent studies have found microbial processing to be the key transformation in efficient soil C stabilization (Cotrufo et al., 2015). Irrigation increases plant productivity and should enhance soil C storage (Lal et al., 1998), but many studies find that irrigated systems rapidly cycle soil C rather than store enhanced plant productivity in the form of SOC (Verma et al., 2005; Denef et al., 2008; Gillabel et al., 2007; Stewart et al., 2017). Microbial transformations are particularly important in irrigated production systems where stabilized C may be more susceptible to C losses through subsequent management changes (Stewart et al., 2018). Few studies span geographic gradients in precipitation and SOC to determine the generalizable effects of stover removal, particularly in irrigated systems.

Residue removal effects on C cycling in these irrigated systems will depend on the balance of plant productivity and microbial decomposition and are likely to be mediated by edaphic microsite effects as well as inherent SOC concentration and nutrient availability. We evaluated long-term residue removal effects on SOC

stocks, $\delta^{13}\text{C}$ of SOC, soil aggregation, and microbial biomass and composition (phospholipid fatty-acid carbon, PLFA-C) for three irrigated NT continuous corn sites spanning a SOC and precipitation gradient from western Colorado to eastern Nebraska. Our hypotheses were that: (i) long-term stover removal will decrease SOC stocks, soil aggregation, and the stabilization of new corn-derived C even under NT management; and (ii) stover removal will reduce microbial biomass and shift the microbial community toward a more bacterial-dominated community.

MATERIALS AND METHODS

Experimental Sites and Treatments

Three irrigated, NT continuous corn production systems in eastern Colorado, south central and eastern Nebraska (Table 1) were evaluated. All sites were managed with stover retention (Ret) or stover harvested (Harv; >60% removal) with N fertilizer rates of ~200 kg N ha⁻¹ representing typical producer practice.

Site Descriptions

Colorado. The study site is located near Fort Collins, CO, on the Agricultural Development and Education Center (ARDEC) (40°39'6" N, 104°59'57" W; 1535 m above sea level). Mean annual precipitation is 38.3 cm and mean annual temperature is 10.6°C (Table 1). The soil is a Fort Collins clay loam soil (fine-loamy, mixed, mesic Aridic Haplustalf) with a 1 to 2% slope. The study began in 2008 in an irrigated field that was NT since 2001, previously cropped to corn for two consecutive years prior to initiating the study. The full experimental design for this site is described in Halvorson and Stewart (2015). Briefly, it was a split-plot randomized complete block with three replications of three split-N rates and two residue treatments (0 or 66% removal). Fertilizer N rates were 0, 67, 202 kg N ha⁻¹. This study focuses only on the two residue treatments at the 202 kg N ha⁻¹ rate.

Corn was directly planted into the previous year's corn stover. Main plots (N rate) were 10.7 by 21.3 m and subplots (residue treatment) were 5.4 by 21.3 m. Triple superphosphate (0–46–0) was applied to avoid corn P deficiency in 1999 (56 kg P ha⁻¹), 2004 (28 kg P ha⁻¹), 2005 (53 kg P ha⁻¹), 2009 (20 kg P ha⁻¹), 2010 (56 kg P ha⁻¹), and 2013 (56 kg P ha⁻¹). A linear-move sprinkler irrigation system applied water as needed during the growing season, averaging 411 mm each year over the study duration. Fertilizer N was surface banded annually (202 kg N ha⁻¹) near the corn row at emergence in mid-May as polymer-coated urea (2008–2011)

or nitrification/urease inhibitor urea (SuperU, 2012–2014). Herbicides were applied after planting and used as needed to control weeds for the duration of the study.

For the Harv treatment, stover from the previous corn crop was removed using a two-row forage harvester to chop and place the stover into an attached weigh wagon. Stover for the 2008 crop was removed in fall 2007, and in January 2009 for the 2009 crop. All subsequent removals occurred 2 to 4 wk before planting instead of after grain harvest to minimize wind erosion over the fallow period. For the Ret treatment, all stover from the previous corn crop was retained with no field operations following combine harvest.

Central Nebraska. The study site is located at the University of Nebraska-Lincoln's South Central Agricultural Laboratory (SCAL) near Clay Center, NE, (40.582° N, 98.144° W; 552 m above sea level). Long-term (1983–2014) mean annual temperature is 10.3°C, and mean annual precipitation is 73 cm, with 45 cm occurring during the growing season (April–October). Soils are classified as Hastings silt loam (fine, smectitic, mesic Udic Argiustoll) with <3% slope. The site was established in 2010 following ridge-tilled soybean. The field encompassed an area of 5 ha managed under NT continuous corn with a variable rate linear-move irrigation system. The full experimental design for this site is described in Blanco-Canqui et al. (2014). Briefly, the experimental design is a randomized split-split-split block with four replications of irrigation treatments split into amendment treatments split into residue treatments with two levels of N fertilizer.

Main plots (irrigation) were 24 by 155 m for each level, where full irrigation maintained between 45 and 90% of the available water holding capacity to a depth of 1.2 m while the deficit irrigation treatment was 60% of fully irrigated treatments. Each irrigation-level plot was split into 24 by 52 m stover amelioration plots and treated with animal manure, winter rye (*Secale cereale* L.) crop, or non-ameliorated control. Each amelioration plot was then split again into 12 by 52 m stover management plots where stover was either harvested or retained. Finally, stover management plots were further divided into two 12 by 26 m N fertilizer plots, receiving either 125 or 200 kg N ha⁻¹ as urea ammonium nitrate post-emergence. Here, we report residue retention and harvest data only from the fully irrigated, non-ameliorated control plots under 200 kg N ha⁻¹.

For the stover removal treatment (Harv), stover was harvested in late October following grain harvest using a flail shredder, high-capacity hay rake, and round baler in 2010 and 2011, and with a self-propelled disk mower-conditioner and round baler in 2012 to 2014. Mean harvest rate was 6.5 dry Mg ha⁻¹ yr⁻¹, or 63% of total non-grain aboveground biomass over the course of the study.

Eastern Nebraska. The study site is located near Ithaca, NE, at the University of Nebraska-Lincoln's Eastern Nebraska Extension and Research Center (ENREC) (41°9'43.3" N, 96°24'41.4" W; 349 m above sea level). Long-term (1981–2010) mean annual precipitation is 74 cm and temperature is 9.8°C. Soils are classified as Filbert silt loam (fine, smectitic, mesic Vertic Argialboll) and Tomek silt loam (fine, smectitic, mesic Pachic Argiudoll). The site was established in 2000, and the full experimental design for

this site is described in Schmer et al. (2014) and Jin et al. (2017). Briefly, the experimental design is a randomized complete block with factorial treatment arranged in split plots with six replications of tillage treatment split into residue treatments.

Main plots (tillage) were 9 by 45.6 m per treatment, either NT or conventional disk tillage. Subplot (residue removal) treatments were initiated in 2001. Subplots within the main plots were arranged in 9 by 15.2 m blocks and split by stover removal rate: none (0%), moderate (~35%), and high (~70%). Nitrogen fertilizer was applied at 202 kg N ha⁻¹ for the last 7 yr of the study. A solid set sprinkler system was used for irrigation in 2001 and 2002, after which (2003–2014) supplemental water was applied when necessary using a linear-move irrigation system. Here, we report residue retention and harvest data only from the high removal rate plots under 202 kg N ha⁻¹.

For the stover removal treatment (Harv), stover was harvested every fall, typically late October or November using a flail chopper with an attached weigh wagon. For the high harvest treatment, stover from all corn rows was harvested at a stalk cutting height of 10 cm for minimum soil disturbance. The moderate removal treatment had 50% of the corn rows harvested, where harvested rows within each plot were alternated every other year. The 50 or 100% of rows harvested corresponded to ~70 and ~35% of standing non-grain biomass removed, respectively (Schmer et al., 2014).

Soil Sampling and Preparation

Soils were sampled at all sites in the fall of 2014 using a 4.1 cm diam. (for the Nebraska sites) and 5-cm diam. (Colorado) Giddings probe for a single core from each field replicate. Intact cores were separated into three depths (0–7.5, 7.5–15, and 15–30 cm), sieved through 8 mm mesh, and all large roots and non-soil materials removed. Soil bulk density was determined for each sampling depth using the core method. Soil bulk density was used to calculate SOC and total soil nitrogen (TSN) mass. A field-moist 20-g subsample was hand-picked to remove plant material, frozen at –22°C for microbial analysis, and the rest of the sample was air-dried for soil characterization and aggregate analyses. Frozen soil subsamples for PLFA analysis were then freeze-dried (Labconco FreeZone 77530, Kansas City, MO) and stored at room temperature until lipid extraction.

Soil Carbon and Nitrogen Analyses

Air-dried 8-mm sieved soils were finely ground and analyzed for soil total C, SOC, TSN, and δ¹³C of SOC using a Europa Scientific carbon nitrogen analyzer with a Solid/Liquid Preparation Module (Dumas combustion sample preparation system) coupled to a Europa 20-20 stable isotope analyzer continuous flow isotope ratio mass spectrometer (Europa Scientific Ltd., Crewe, England). Carbonates were removed before stable isotope analysis using a modified acid fumigation method (Harris et al., 2001). Briefly, 15 mg soil samples were weighed into open silver capsules, moistened with 30 μL DI H₂O to approximately field capacity, then fumigated for 8 h in a concen-

trated HCl vacuum atmosphere. Fumigated samples were then removed from vacuum, air dried in the fume hood overnight to remove HCl fumes, then oven dried at 55°C for 12 h. Silver capsules were folded closed and wrapped in tin capsules before analyses. All analyses are expressed as oven dry weight (55°C).

Soil Aggregation

Air-dried 8-mm sieved soils were sieved with a modified Yoder apparatus into four smaller fractions: 2 to 8 mm, 2 mm to 250 μm , 250 to 53 μm , and <53 μm (Kemper and Rosenau 1986, Follett et al., 2009). Briefly, a 50-g subsample was placed on a 2-mm sieve in 1 cm water and allowed to slake for 5 min. Stacked sieves were gently sieved in a column of water for 10 min. Fractions were transferred to pans and dried at 50°C. Ten milliliters of flocculent (0.5 M CaCl + 0.5 MgCl) was added to the <53 μm fraction to hasten silt and clay settling after which the supernatant was discarded. Aggregate fractions >53 μm were summed and expressed as gram aggregates per kilogram soil. Profile (0–30 cm) aggregation was expressed by a weighted average of depth increments.

Phospholipid Fatty Acid Extraction and Identification

Freeze-dried soil subsamples were analyzed for PLFAs (Ward Laboratories, Inc.; Kearney, NE). Methods for extraction were adapted and modified from Clapperton et al. (2005) and described in detail in Stewart et al. (2018). Briefly, 2 g soil samples were extracted for phospholipids, which were then converted fatty acid methyl esters (FAMES) for analysis by gas chromatography (GC; Agilent 7890A). Peaks were identified as specific PLFAs based on comparison of retention times to known standards with an external standard. Soil microbial biomass was estimated as the total abundance of known PLFA biomarkers (see below), and reported as $\mu\text{g PLFA-C g}^{-1}$ dry soil. Profile microbial biomass was estimated as a depth-weighted average for 0 to 30 cm.

Known PLFAs were classified into general bacteria, gram-positive bacteria, gram-negative bacteria, actinomycetes, arbuscular mycorrhizal fungi (AMF), saprotrophic fungi, and universal biomarkers. The 3-OH 12:0, 14:0, 15:0, 3-OH 14:0, 17:0, and 18:0 are used as general bacterial indicators (Frostegard et al., 2011; Zelles, 1999). The i-15:0, a-15:0, i-16:0, i-17:0, and a-17:0 are classified as Gram-positive bacteria while 2-OH 10:0, 2-OH 12:0, 2-OH 14:0, 16:1 ω 7, 17:0cy, 2-OH 16:0, c18:1 ω 7, and 19:0cy are classified as Gram-negative bacteria (Zelles, 1999). The 10ME16:0, 10ME17:0 and 10ME18:0 are classified as actinomycete biomarkers. The 16:1 ω 5, 20:4 ω 6, 20:4 ω 3, and 20:1 are biomarkers for arbuscular mycorrhizal fungi (AMF) (Graham et al., 1995), and 18:3 ω 3, c18:2 ω 9,12, and c18:1 ω 9 are biomarkers for saprotrophic fungi (Zelles, 1999). The 16:0 fatty acid is classified as a universal PLFA (Zelles, 1999). Soil microbial community structure was evaluated by individual PLFA biomarkers or by functional groups defined above and reported as relative abundance (mol%).

Data Analysis and Statistics

We used a general linear mixed effects model to analyze fixed main effects of site, residue management, and soil depth and their interactions, with replicate as a random effect (SAS v. 9.4) (SAS Institute, Cary, NC). Response variables were SOC concentration, SOC stocks, SOC $\delta^{13}\text{C}$, bulk density, microbial biomass, fungal/bacterial ratio, gram-positive/gram-negative ratio, and water stable aggregation (g kg^{-1} soil). Profile SOC stocks (Mg C ha^{-1}) and weight average microbial biomass (ng PLFA-C g^{-1} soil) were run with a general linear mixed effects model with main effects of site and residue management with block as a random effect. Where necessary, data were log transformed to meet assumptions of normality and equal variance. Significant treatment effects were evaluated with multiple comparisons, with *P* values noted in the text after Bonferroni adjustment. Differences are considered significant when *P* < 0.05, unless otherwise noted.

Microbial community structure was analyzed by calculating the relative abundance of PLFA biomarkers (mol%) by microbial functional group followed by distance-based redundancy analysis (dbRDA) using Bray–Curtis distances, resulting eigenvalues were used to evaluate whether microbial biomarkers grouped by experimental treatment. Permutation-based ANOVA was performed on all dbRDA models to determine main effects significance among group differences. Ellipsoids represent standard error around the multivariate-group centroids.

RESULTS

Soil Organic Carbon Concentration

Soil organic C concentration increased across sites from West to East (Colorado to eastern Nebraska) from 10.5, 16.2, to 18.7 g C kg^{-1} soil averaged across depths and residue treatments (data not shown, $P_{\text{Site}} < 0.0001$). Residue harvest decreased SOC by 15% in the 0- to 7.5-cm depth only ($P_{\text{Depth} \times \text{Residue}} = 0.007$). Averaged across sites and residue treatments, SOC concentration decreased with depth, from 19.3 g C kg^{-1} to 13.8 to 12.3 g C kg^{-1} in the 0- to 7.5-, 7.5- to 15-, and 15- to 30-cm depths, respectively ($P_{\text{Depth}} < 0.0001$). There were no three-way interactions.

Bulk Density

Sites and soil depths differed in bulk density, but there was no residue effect (data not shown, Table 2). Bulk density was 1.13 g cm^{-3} at ENREC, to 1.25 and 1.49 g cm^{-3} at SCAL and ARDEC, respectively ($P_{\text{Site}} < 0.0001$). Bulk density increased with depth, from 1.16 g cm^{-3} in the 0- to 7.5-cm depth to 1.36 and 1.35 g cm^{-3} for the 7.5- to 15- and 15- to 30-cm depths, respectively ($P_{\text{Depth}} < 0.0001$).

Soil Organic Carbon Stocks

Soil C stock values paralleled site and depth patterns for SOC concentrations, increasing from West to East and decreasing with soil depth, with a significant site \times depth interaction driven by the high C mass of the 7.5- to 15-cm depth at ENREC ($P_{\text{Site} \times \text{Depth}} < 0.0001$) (Fig. 1a; Tables 2, 3). Stover harvest decreased SOC stocks relative to residue retained ($P_{\text{Residue}} =$

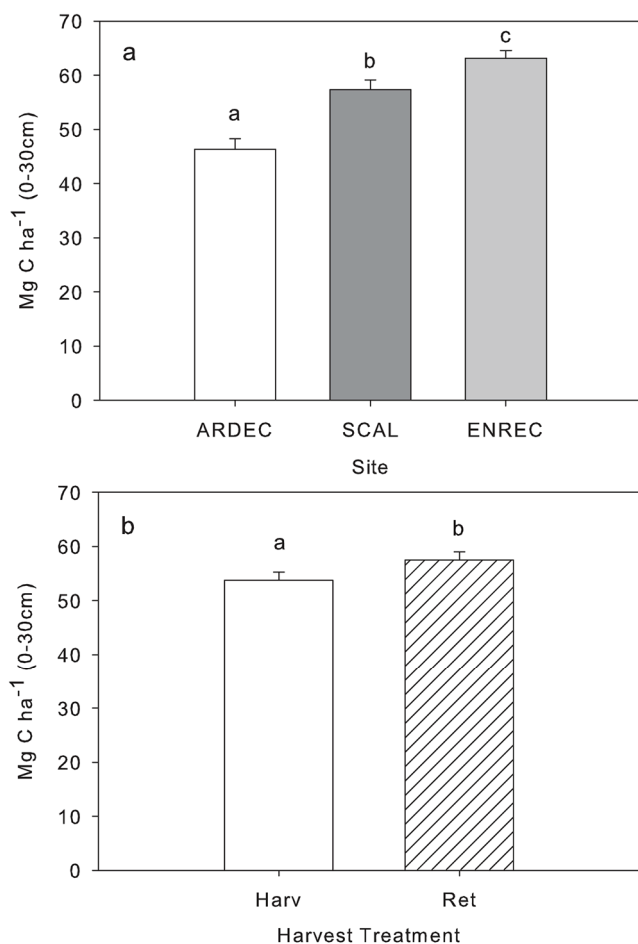


Fig. 1. (a) Soil organic C stocks (Mg C ha⁻¹) for the three sites, Colorado (ARDEC), central Nebraska (SCAL), and eastern Nebraska (ENREC) (b) for the 0- to 30-cm depth with corn stover either harvested (Harv) or retained (Ret). Error bars represent standard error of the mean.

0.016) (Fig. 1b; Tables 2, 3). Because there were no residue treatment interactions, SOC stocks were summed over all depths and reassessed for site, residue, and their interaction effects. For the 0- to 30-cm depth, SOC stocks increased from West to East, from 46.4, 57.4, to 63.1 Mg C ha⁻¹ for ARDEC, SCAL, and ENREC, respectively ($P_{\text{Site}} < 0.0001$) (Fig. 1a, Table 3). Residue harvest reduced soil C mass by 6% relative to residue retained soils, or 57.5 to 53.8 Mg C ha⁻¹ ($P_{\text{Residue}} = 0.041$) (Fig. 1b, Table 3). There was no site \times residue interaction.

Table 3. Soil organic C mass (Mg C ha⁻¹) for three sites located in Colorado (ARDEC), central Nebraska (SCAL), and eastern Nebraska (ENREC) for the three soil depths with stover either harvested (Harv) or retained (Ret). Lowercase letters indicate significant differences between sites and uppercase letters differences between residue treatments.

Site	Depth	Harv	Ret	Residue mean
	cm	Mg C ha ⁻¹		
ARDEC	0–7.5	14.2	16.9	15.6
	7.5–15	11.4	11.4	11.4
	15–30	19.1	19.4	19.3
	0–30 mean	44.8	47.9	46.4a
SCAL	0–7.5	17.4	18.1	17.8
	7.5–15	14.9	15.8	15.3
	15–30	23.2	25.3	24.3
	0–30 mean	55.5	59.2	57.4b
ENREC	0–7.5	14.6	18.2	16.4
	7.5–15	15.5	16.4	16.0
	15–30	30.8	30.7	30.8
	0–30 mean	60.9	65.3	63.1c
Site mean	0–30 mean	53.8A	57.5B	

$\delta^{13}\text{C}$ of Soil Organic Carbon

Soil organic $\delta^{13}\text{C}$ generally increased from West to East, and residue harvest further decreased $\delta^{13}\text{C}$ at ARDEC but not at SCAL or ENREC ($P_{\text{Site} \times \text{Residue}} = 0.044$) (Tables 2, 4). This residue effect was limited to near-surface soils (0–7.5 cm), but not the deeper soil depths ($P_{\text{Depth} \times \text{Residue}} = 0.019$) (Tables 2, 4). Changes in SOC $\delta^{13}\text{C}$ with soil depth was site-specific ($P_{\text{Site} \times \text{Depth}} < 0.0001$), where SOC $\delta^{13}\text{C}$ increased with depth at ENREC and SCAL, but decreased with depth at ARDEC (Tables 2, 4).

Aggregation

There was no main or interaction effects of depth on soil aggregation (Table 2), so aggregate values were pooled as means weighted by depth increment for the full 0- to 30-cm soil depth, then re-analyzed for main and interaction effects of site and residue treatment. Aggregation in the top 30 cm of soil was site-specific, with SCAL > ARDEC > ENREC. Residue harvest decreased aggregation 12% averaged over sites from 609 to 537 g kg⁻¹ ($P = 0.005$, Fig. 2b) driven primarily by decreased

Table 2. P values for main and interaction effects of variables for three sites located in Colorado (ARDEC) central Nebraska (SCAL), and eastern Nebraska (ENREC) for the three soil depths with stover either harvested (Harv) or retained (Ret).

Effect	Num df	Den df	SOC†	Bulk density	SOC stocks	SOC $\delta^{13}\text{C}$	Aggregation	PLFA
			g kg ⁻¹	g cm ⁻³	kg ha ⁻¹	‰	%	ng PLFA-C g ⁻¹
Site	2	55	0.000	0.000	0.000	0.000	0.001	0.126
Residue	1	55	0.039	0.383	0.016	0.228	0.019	0.198
Depth	2	55	0.000	0.000	0.000	0.000	0.173	0.000
Site \times Residue	2	55	0.291	0.227	0.946	0.044	0.064	0.244
Site \times Depth	4	55	0.164	0.907	0.000	0.000	0.894	0.086
Depth \times Residue	2	55	0.007	0.627	0.262	0.019	0.575	0.737
Site \times Depth \times Residue	4	55	0.968	0.282	0.422	0.851	0.509	0.548

† SOC, soil organic carbon; PLFA, phospholipid fatty acids.

aggregation at ARDEC (28%) but not at ENREC or SCAL ($P_{\text{Site} \times \text{Residue}} = 0.011$) (Fig. 2).

Soil Microbial Biomass

Soil microbial biomass (PLFA-C) was not affected by main or interaction effects of site and residue but decreased with depth, from 2369.4 to 1051.3 to 790 ng PLFA-C g⁻¹ soil in the 0- to 7.5-, 7.5- to 15-, and 15- to 30-cm depths, respectively ($P_{\text{Depth}} < 0.0001$) (Fig. 3). There were no significant interactions of site, residue and depth. Residue harvest tended to decrease microbial biomass in the 0 to 7.5 depth and through the 0- to 30-cm depth (i.e., mean weighted by depth increment) ($P_{\text{Residue}} = 0.119$), but again, there were no significant main or interaction effects of site.

Microbial Community Composition

Fungal/bacterial ratio was greater and the gram-positive/gram-negative ratio was lower in Colorado compared to the two Nebraska sites ($P_{\text{Site}} < 0.0001$) (Table 5). In addition, fungal/bacterial ratio decreased with depth and gram-positive/gram-negative ratio increased with depth ($P_{\text{Depth}} = 0.023$) (Table 5). There was no main or interaction residue effects or any other interaction effects.

Permutation-based ANOVA on soil microbial composition based on the relative abundance of functional groups (mol%) indicated differences between sites ($P < 0.001$) and soil depths ($P < 0.001$), but not residue treatments or any other treatment interactions (Fig. 4). Specifically, the dbRDA of microbial community structure separated sites

Table 4. Soil organic $\delta^{13}\text{C}$ for Colorado (ARDEC), central Nebraska (SCAL), and eastern Nebraska (ENREC) sites by soil depth with stover harvested (Harv) or retained (Ret). Lowercase letters indicate differences between harvest treatments within depths or differences between depths within sites. Uppercase letters represent average harvest differences or differences between sites.

Site	Depth cm	Harv	Ret	Residue mean
				$\delta^{13}\text{C}$ of SOC, %
ARDEC	0–7.5	-18.59	-17.60	-18.10a
	7.5–15	-19.24	-19.31	-19.28b
	15–30	-19.85	-19.48	-19.67c
	0–30 mean	-19.23a	-18.80b	-19.01c
SCAL	0–7.5	-16.60	-16.56	-16.58a
	7.5–15	-15.53	-15.83	-15.68b
	15–30	-14.16	-14.66	-14.41c
	0–30 mean	-15.43a	-15.68a	-15.56a
ENREC	0–7.5	-16.94	-16.27	-16.61a
	7.5–15	-16.62	-16.65	-16.64ab
	15–30	-15.95	-15.96	-15.96b
	0–30 mean	-16.51a	-16.29a	-16.40B
All Sites	0–7.5 mean	-17.38b	-16.81a	
	7.5–15 mean	-17.13a	-17.27a	
	15–30 mean	-16.66a	-16.70a	
	0–30 mean	-17.06A	-16.93A	

and explained 19.8% of variability along Axis 1 and 0.5% along Axis 2 (Fig. 4). Along Axis 1, ENREC separating along universal, SCAL along actinomycetes, and ARDEC separated toward AMF, fungi and gram-negative bacteria. Microbial community structure also separated by soil depth, which explained 13.3% of variability along Axis 1 and 0.3% along Axis 2. Along Axis 1, surface soils (0–7.5 cm) separat-

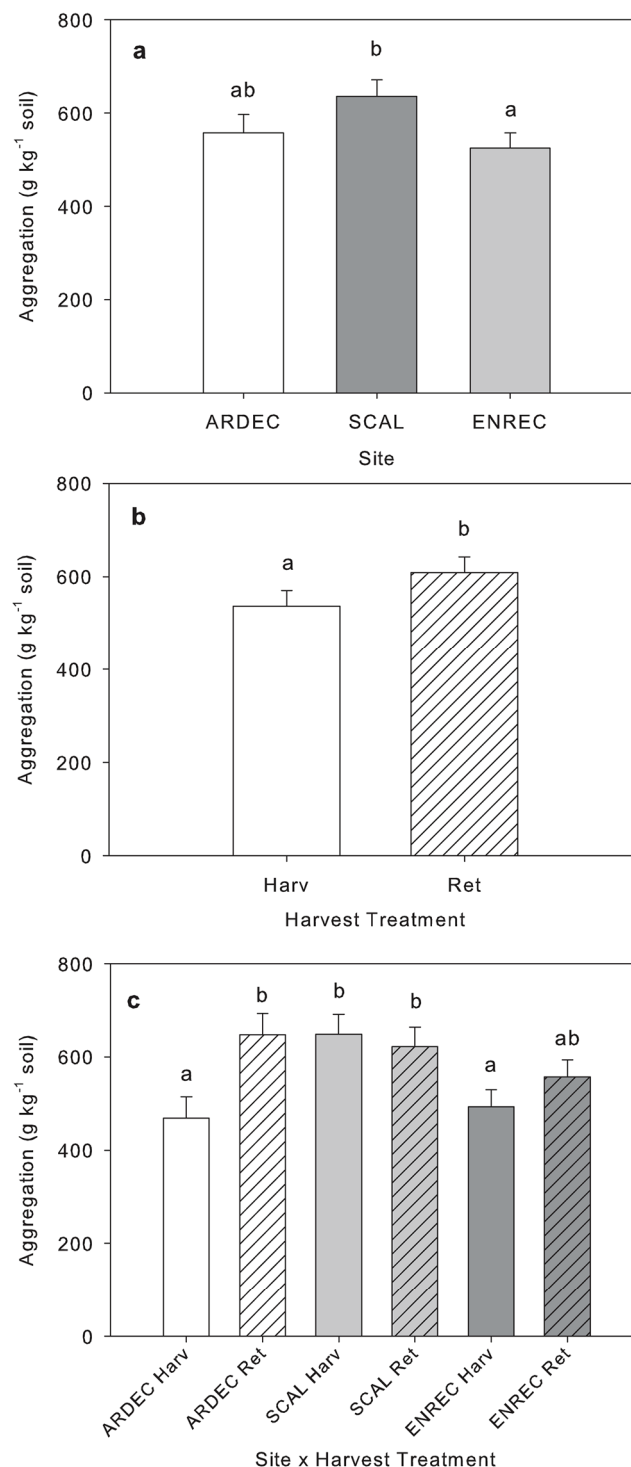


Fig. 2. (a) Soil aggregation (g kg⁻¹ soil) for the three sites, Colorado (ARDEC), central Nebraska (SCAL), and eastern Nebraska (ENREC) (b) for the 0- to 30-cm depth with corn stover either harvested (Harv) or retained (Ret) and (c) their interaction. Error bars represent standard error of the mean.

ed toward gram-negative bacteria, fungi and AMF, and the deepest depth (15–30 cm) separated toward gram-positive bacteria and universal.

DISCUSSION

Despite long-term NT management (4–13 yr), stover harvest at these three irrigated continuous corn sites resulted in lower SOC stocks and aggregation compared to residue retained treatments. This suggests that NT management alone is inadequate to reduce erosion potential and maintain soil function in mesic and semiarid irrigated corn production systems, even though these effects are not always observed in the short term. Residue harvest did not decrease SMB or change soil microbial community structure, suggesting that NT and the high plant productivity in these irrigated systems buffered microbial community properties from residue harvest impacts. In short, high crop productivity with irrigation appears to stimulate activity levels of the existing soil microbial community, leading to SOC losses as a result of enhanced microbial decomposition and aggregate turnover in these NT continuous corn systems.

Soil Organic Carbon and $\delta^{13}\text{C}$ of Soil Organic Carbon

In the top 30 cm of soils, residue removal resulted in 6% lower SOC stocks despite NT management practices. Our findings are consistent with low SOC storage potentials reported in

intensively-managed irrigated corn systems, where high productivity levels are offset by greater decomposition rates stimulated by higher water and nutrient availabilities (Verma et al., 2005; Follett et al., 2013; Schmer et al., 2014). Furthermore, similar field-based findings have been reported in rainfed systems documenting reduced C stocks with long-term residue removal compared to stover retained treatments (Anderson-Teixeira et al., 2009; Lehman and Osborne 2016) or no change in soil C stocks compared to an increase with stover retention (Clapp et al., 2000; Halvorson and Stewart, 2015). Studies reporting no change in SOC under NT with residue removal tend to be in soils with high initial soil C (Moebius-Clune et al., 2008; Reicosky et al., 1997) or with short experimental duration with relatively low removal rates (Johnson et al., 2014).

The SOC $\delta^{13}\text{C}$ signature indicated less storage of new C when residue was removed at the most arid site in Colorado (ARDEC). Other studies that have used $\delta^{13}\text{C}$ find lower incorporation of new corn-derived C with residue removal. For example, Clapp et al. (2000) reported that new C accounted for 5% of total SOC with residue removal compared to 15% without residue removal under NT on a Mollisol in Minnesota. At ARDEC, lower C storage of new C suggests rapid C cycling from enhanced decomposition (Stewart et al., 2018). No changes in SOC $\delta^{13}\text{C}$ signature at the Nebraska sites indicated that SOC losses may be due to irrigation-enhanced decomposition or erosion of existing soil C rather than reduction in new C inputs with residue removal. For all three sites, residue removal reduced C inputs from stover overall. Further, removal of the protective physical barrier of residue likely stimulated residue or SOC decomposition rates by exposing soils to greater evaporation rates and fluctuations in surface temperature (Steiner, 1994; Wilhelm et al., 2004). Stover removal also may have changed plant rooting patterns. Although NT can promote surface rooting, residue removal treatments in rainfed systems resulted in fewer, deeper roots, presumably in response to surface drying (Stewart et al., 2016).

Residue Removal Impacts on Microbial Biomass and Microbial Community Structure

Despite decreased SOC with residue removal, there was little effect of residue removal on SMB and its community composition. Other no-till continuous corn studies have also

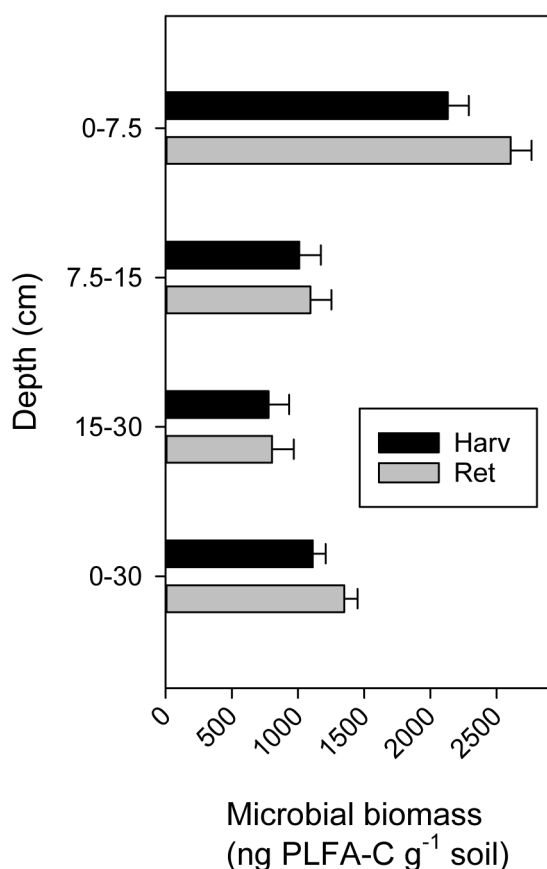


Fig. 3. Soil microbial biomass (ng PLFA-C g⁻¹ soil) averaged over the three sites, Colorado (ARDEC), central Nebraska (SCAL), and eastern Nebraska (ENREC) for the three soil depths and weight averaged for the 0 to 30 cm with residue either harvested (Harv) or retained (Ret). Errors represent standard error of the mean.

Table 5. Fungal/bacterial ratios (F/B) and gram-positive/gram-negative ratios (Gpos/Gneg) for three sites located in Colorado (ARDEC), central Nebraska (SCAL), and eastern Nebraska (ENREC) for the three soil depths.

Effect	F/B	Gpos/Gneg
ARDEC	0.179a	0.87a
ENREC	0.078b	1.79b
SCAL	0.091b	1.85b
0–7.5 cm	0.144a	1.03a
7.5–15 cm	0.106ab	1.43b
15–30 cm	0.098b	2.05c
	<i>P</i>	<i>P</i>
Site	<0.0001	<0.0001
Residue	0.444	0.505
Depth	0.023	<0.0001

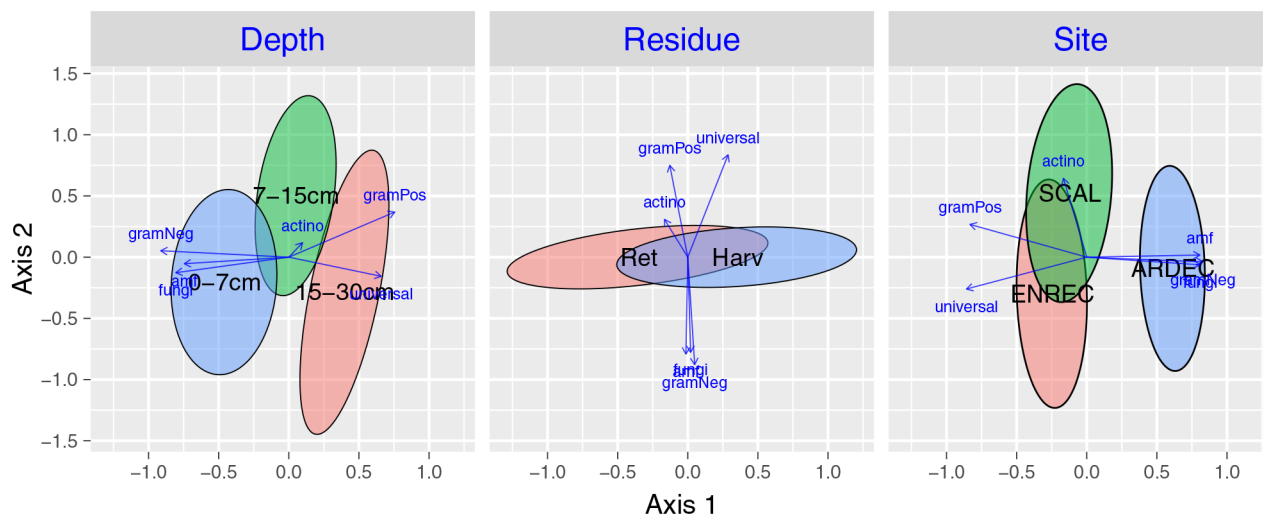


Fig. 4. Soil microbial community composition (mol%) for the three sites, Colorado (ARDEC), central Nebraska (SCAL), and eastern Nebraska (ENREC), three soil depths and residue treatments (harvested or retained). Ellipses represent standard error from the distance-based redundancy analysis (dbRDA).

reported no effect of residue removal on SMB (Ahlschwede, 2013; Johnson et al., 2013; Spedding et al., 2004). Although we expected relatively more fungal biomass with residue retention, fungal biomass may have been well equilibrated to the long-term NT practices, resulting in a comparatively small effect of residue removal. A previous study at the irrigated Colorado site (ARDEC) also found that residue removal did not alter the relative abundance of PLFAs, fungal/bacteria ratios, or gram-positive/gram-negative ratios (Stewart et al., 2018). Many other rainfed long-term NT studies have similar results. Lehman et al. (2014) found only one of four NT rainfed corn or mixed rotation sites to have greater fungal/bacteria ratios under residue retained compared to harvested (98% aboveground biomass) after 9 to 10 yr of treatment. Two other sites (Nebraska, 10 yr and Minnesota, 8 yr) showed no difference with residue harvest. One site had greater fungal abundance after only 2 yr of removal. Johnson et al. (2013) found FAME profiles to differ under CT with residue removal, but not under NT.

Both irrigation and NT management practices potentially dampen the two largest drivers of microbial response: C input and microclimatic effects. Generally, residue removal limits C inputs by reducing overall aboveground C, decreasing surface rooting, and stimulating decomposition through increased soil surface drying, destabilization of surface temperatures, and decreased water retention (Steiner, 1994; Wilhelm et al., 2004). Irrigation, however, can moderate soil microclimatic variability and increase C input and nutrient availability for microbial use by boosting crop productivity and promoting the movement of dissolved organic C and nutrients between previously isolated soil pores. Similarly, NT reduces overall soil disturbance and can provide greater belowground C inputs and higher soil connectivity by supporting more surface rooting (Stewart et al., 2016). Thus, the lack of residue removal effect on SMB and soil microbial community structure may be attributed to the ameliorating effects of irrigation and NT in these long-term continuous corn systems.

Aggregation as a Sensitive Indicator for Residue Removal

Soil aggregation was an indicator of residue removal, but only for two of three study sites (ARDEC, ENREC). In rainfed continuous corn production systems, residue removal can decrease surface aggregation (Blanco-Canqui and Lal, 2007; Jin et al., 2015) from decreased aboveground and root-C return (Stewart et al., 2016). Soil aggregates form around organic C sources like particulate organic matter, root exudates, and microbial exudates (Six et al., 2004). The stability of these aggregates is expected to decrease with residue removal by greater exposure to disruption by dry-wet and freeze-thaw cycles (Wienhold et al., 2013). Irrigation could exacerbate residue removal effects on aggregation due to more frequent dry-wet cycles and increased microbial decomposition (discussed previously), as noted for two of three sites in this study. Gillabel et al. (2007), however, found no difference in microaggregation between irrigated and dryland sites, despite two and a half times greater aboveground productivity under irrigation. We also found no aggregate response to residue removal at the SCAL site. The lack of aggregate response may be due to high C inputs, high initial SOC concentration, and/or high mineral content, all of which confer soils with greater ability to absorb reductions in C input without altering soil structure (Blanco-Canqui and Lal, 2007; Stewart et al., 2014). The use of NT could further buffer soils from residue removal impacts on soil aggregation, but several years may be required before the benefits of NT appear.

CONCLUSIONS

Residue removal in irrigated NT continuous corn systems appears to have two contrasting mechanisms that both exacerbate C losses and while buffering the microbial community. Enhanced crop productivity and root exudation increase C availability to the microbial community, buffering residue removal effects. However, the addition of irrigation water accelerates microbial decomposition and aggregate turnover, leading to a depletion of existing SOC stocks compared to residue retained. While soil microbial biomass

and community structure did not change in these NT systems, NT alone is inadequate for maintaining both soil C stocks and soil aggregation with residue removal in these irrigated systems.

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