Soil Biology and Biochemistry 148 (2020) 107899

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: http://www.elsevier.com/locate/soilbio

Winter rye does not increase microbial necromass contributions to soil organic carbon in continuous corn silage in North Central US

Jaimie R. West [a,*], Anna M. Cates [b], Matthew D. Ruark [a], Leonardo Deiss [c], Thea Whitman [a], Yichao Rui [d]

[a] Department of Soil Science, University of Wisconsin-Madison, USA
[b] Department of Soil, Water and Climate, University of Minnesota, USA
[c] School of Environment and Natural Resources, The Ohio State University, USA
[d] Rodale Institute, USA

ARTICLE INFO

Keywords:
Soil organic carbon
Microbial necromass
Amino sugars
Mid–DRIFTS
Corn silage
Cover cropping

ABSTRACT

There is insufficient evidence for how cover crop-driven changes to the soil microbial community affect soil organic carbon (SOC) accumulation. One mechanism by which enhanced microbial activity can contribute to SOC accrual is through the conversion of plant inputs to microbial biomass and ultimately necromass that may form organo–mineral associations with soil particles. Here we investigated the effects of winter rye as a cover crop and winter rye harvested as a forage double crop on SOC, chemically labile and complex C fractions, microbial necromass biomarkers (amino sugars), and potential extracellular enzyme activities following a seven–year continuous corn silage trial in southern Wisconsin, North Central US. Whereas SOC increased when winter rye was used as a cover crop compared to no cover, there were no changes to SOC when winter rye was harvested as a forage crop. A positive relationship between chemically labile aliphatic soil–C and total SOC indicates that higher SOC stocks may result from persistence of simple compounds rather than chemically complex, aromatic materials. However, the accumulation of microbial necromass, as inferred from amino sugar biomarker concentrations, was largely unaffected by winter rye use, despite a positive relationship between SOC and amino sugar residue concentrations. Greater potential extracellular enzyme activities indicate increased microbial activity with winter rye. Together, these results suggest that despite some microbial stimulation and potential soil health benefits, winter rye did not increase the contribution of microbial necromass to SOC accrual in this seven–year continuous corn silage field trial.

1. Introduction

Intensive agricultural production is essential to feed the global population (Amundson et al., 2015), with the tradeoff of soil organic carbon (SOC) loss attributed to conversion of perennial root systems to those of annual row-crops, high rates of biomass removal in crop harvest, and soil disturbance (Guo and Gifford, 2002; King and Blesh, 2018; Lal, 2004; McDaniel et al., 2014b). Preventing further C loss from agricultural land and replenishing diminished soil C stocks are important steps towards mitigating deleterious effects of climate change by stopping the feedback systems that accelerate SOC loss; this may be accomplished through long-term cover cropping (King and Blesh, 2018; Paustian et al., 2016). Cover cropping can improve soil health and water quality, notably by reducing erosion and increasing the proportion of the year with living roots in the ground (García-González et al., 2018; Kladivko et al., 2014; Stott and Moebius-Clune, 2017). Root C inputs comprise a substantial portion of total C inputs from cover crops (Austin et al., 2017). These rhizodeposits have been shown to preferentially contribute to stable SOC as compared to inputs from aboveground residues (Kong and Six, 2012), presumably by generating and sustaining the rhizosphere and associated microbial activity that promotes protection of SOC (Kuzyakov and Blagodatskaya, 2015; Vidal et al., 2018; Zhang et al., 2019). Therefore,

Abbreviations: NoCC, no cover crop treatment; RyeCC, winter rye cover crop treatment; RyeHarv, winter rye harvested as a forage treatment; SOM, soil organic matter; SOC, soil organic carbon; MurA, muramic acid; GluN, glucosamine; GalN, galactosamine; CUE, carbon use efficiency; mid–DRIFTS, diffuse reflectance infrared Fourier transform spectroscopy in the mid–infrared region; AGB, aboveground biomass; BGB, belowground biomass.

* Corresponding author.
E-mail address: jrwest@wisc.edu (J.R. West).

https://doi.org/10.1016/j.soilbio.2020.107899
Received 30 June 2019; Received in revised form 9 June 2020; Accepted 10 June 2020
Available online 15 June 2020
0038-0717/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

long–term cover cropping is a possible means to increase quantity and quality of C inputs, and, in turn, increase microbial activity and total SOC (Kallenbach et al., 2015; McDaniel et al., 2014b).

There is mounting evidence that SOC stability and persistence is not a simple function of the chemical complexity or "recalcitrance" of soil organic matter (SOM) (Lehmann and Kleber, 2015; Schmidt et al., 2011). Instead, chemically labile organic compounds can be associated with high carbon use efficiency (CUE), which is the rate at which soil microbial communities convert organic substrates into microbial biomass. Labile substrates are preferentially used by microbes, subsequently becoming microbial necromass as the microbes die (Cotrufo et al., 2013). This microbial necromass may comprise over half of total SOC (Liang et al., 2019), and contains amino sugar residues, which are microbial cell wall components that persist and accumulate in soil by forming strong associations with mineral surfaces, thus fostering aggregation of clay particles and resulting in physically protected SOC (Cotrufo et al., 2013; Hernandez-Soriano et al., 2018; Lehmann et al., 2007). These persistent, mineral-associated biomarkers can be partitioned into bacterial and fungal origin, thus suggesting legacy microbial populations (Glaser et al., 2004; Joergensen, 2018; Liang and Balser, 2008). Increasing CUE and therefore increasing SOC accumulation necessitates optimal organic input quality and quantity for a given soil and agricultural system (Geyer et al., 2016; Kallenbach et al., 2019). The C:N ratio is a commonly-used proxy for organic input quality because this ratio can indicate the likelihood of net nitrogen mineralization vs immobilization during microbial decomposition of SOM (Vigil and Kissel, 1991; Wagger, 1989). An inadequately high C:N ratio may be caused by an inundation of C from plant biomass or by a limitation of N availability due to plant uptake. These factors may accelerate SOM turnover due to nitrogen scavenging, and thus result in decreased SOC (Cotrufo et al., 2019) and CUE (Geyer et al., 2016).

Understanding mechanisms that potentially support SOC building in an intensive corn silage (*Zea mays* L.) system could help mitigate substantial SOC loss. This cropping system is often characterized by wholesale net C loss because of the early harvest of the full corn plant (Cates and Jackson, 2018; Johnson et al., 2014), and covers about 1.3 million hectares in North Central US, over 20% of which are in Wisconsin (USDA NASS, 2019). Regional research that demonstrates long-term environmental and economic benefits over a wide range of seasonal weather patterns is necessary for adoption and optimal implementation of cover cropping as a SOC building and soil health--promoting practice (Arbuckle and Roesch-McNally, 2015; Myers and Watts, 2015; Roesch-McNally et al., 2017). Extending the cover crop growing season and harvesting the cover as a forage crop (rather than terminating the cover crop and leaving the biomass on the field) can improve the profitability of cover cropping (Plastina et al., 2018), but the net effect on SOC is unclear given the tradeoff between increased root production following a longer growing season, and removal of the aboveground biomass as a harvested forage crop (Johnson et al., 2014). Integrating a forage crop into corn silage has increased microbial biomass (Faé et al., 2009), but the specific mechanisms of SOC building in this type of system are unknown and likely contextual as the plant traits of grains and grasses, including developmental stage, have variable influences on root exudates and therefore on how microbial communities process these inputs (Finney et al., 2017; Herz et al., 2018; Zhalnina et al., 2018). For example, extracellular enzyme activities are affected by root exudate composition (Zhang et al., 2019), and generally increase with decomposition-related microbial activity (Bach and Hofmockel, 2016; Geisseler et al., 2011).

To address these challenges, we took advantage of an existing field trial, for which the agronomic results were reported in West et al. (2020). The main findings indicated that winter rye (*Secale cereal* L.) used as a cover crop did not substantially affect corn silage yield, whereas winter rye harvested as a forage double crop decreased corn silage yield in three of the seven study years. These deficits in corn yields were met by harvested rye forage yields (Supplementary Section 1.1, Supplementary Table S1). The research presented here reflects analysis of soil samples collected in 2018, following the seventh study season. A longer-term study such as this is valuable because changes in SOC and microbial processes likely take several years to become apparent and measurable.

We wanted to know: What was the effect on SOC following seven years of winter rye either as a cover crop or harvested as a forage double crop in continuous corn silage? Our objectives were to characterize changes to soil carbon and to determine the microbial role in slow-to-change soil characteristics. Integrating rye as a forage decreased plant-available N, to an extent that likely resulted in nitrogen immobilization, as evidenced by decreased corn silage yields (West et al., 2020; also seen in Clark et al., 2007). We therefore hypothesized that this double-crop rotation would stimulate SOM turnover via nitrogen scavenging, and thus decrease SOC relative to no cover crop (Cotrufo et al., 2019). In turn, we predicted no change in SOC concentration with rye as a cover because there was less of an effect on plant-available N, and no yield loss in this treatment relative to no cover. Further, we hypothesized that winter rye used both as a cover and harvested for forage would increase labile SOM inputs and stimulate microbial community activity. This hypothesized increase in microbial activity and shift in the community structure would extend to lower sampling depths when rye is harvested as a forage due to increased root production associated with the extended growing season as compared to rye as a cover crop.

We estimated mean annual aboveground and belowground C inputs for each treatment and measured SOC concentration. We characterized SOM by quantifying chemically labile aliphatic C and chemically recalcitrant aromatic C fractions in soil using diffuse reflectance infrared Fourier transform spectroscopy in the mid–infrared region (mid–DRIFTS) (Deiss et al., 2020b; Demyan et al., 2012; Margenot et al., 2015). To estimate microbial necromass accumulation and the legacy effect of soil microbial activity, we quantified the concentration of amino sugar residues (Joergensen, 2018). To assess broad changes in microbial activity, we quantified potential extracellular enzyme activities, and to assess broad changes in the microbial community, we used quantitative PCR to estimate bacterial and fungal ribosomal gene abundances. This breadth of measurements was intended to capture some aspects of the complex processes that might support SOC building in this system.

2. Materials and methods

2.1. Field site description and study design

A seven–year field experiment was conducted from 2011 to 2018 at the University of Wisconsin Arlington Agricultural Research Station (43°17′48″N, 89°23′10″W, 307 m a.s.l.) on a Plano silt loam soil (fine-silty, mixed, superactive, mesic Typic Argiudoll; Supplementary Table S3 for soil properties). Corn silage was grown annually following a fall liquid dairy manure application of approximately 100,000 L ha^{-1} and fall–established winter rye cover crop treatments. The study design was a randomized complete block design with three replicates. The whole plot factor was cover crop, including winter rye as a cover crop (RyeCC), winter rye harvested as a forage crop (RyeHarv), and no cover crop (NoCC). Winter rye was planted in early fall, immediately following the previous corn silage harvest and manure application. In the RyeCC treatment, rye was terminated with glyphosate between 11 April and 9 May the following spring, prior to corn planting. In the RyeHarv treatment, the winter rye growth period was extended to mid-to-late May, at which time the rye biomass was chopped, harvested, and stubble sprayed with glyphosate. The NoCC treatment was sprayed concurrently with RyeCC. Corn was drill seeded on the same date in all treatments following rye forage harvest in 2012, 2013, 2017 and 2018, and split-planted following rye burndown (RyeCC and NoCC treatments) or following rye forage harvest (RyeHarv treatment; 10–14 day delay in corn planting) in 2014, 2015 and 2016. Ammonium nitrate was surface

applied to corn at a rate of 112 kg N ha^{-1} approximately at the V3 growth stage of corn. The full experiment, detailed in West et al. (2020), included additional N rates, but the data described in this paper were only collected from the 112 kg N ha^{-1} treatment plots.

Winter rye shoot aboveground biomass (AGB) was sampled from two random 0.37 m^2 quadrats within each RyeCC and RyeHarv plot just prior to termination or harvest, respectively, and weighed and dried (N = 6 per year; rye growth is detailed in Supplementary Section 1.2). Corn silage was harvested by hand at approximately 65% whole plant moisture (yield is detailed in Supplementary Section 1.1, Supplementary Table S1). Soil samples were collected from each plot immediately after corn silage harvest in the seventh study season (14 Sept., 2018). Four soil cores (8 cm diameter) per plot were collected using a Giddings hydraulic probe and stored at 4 °C for up to 10 days. Soil cores were subsampled at four depths: 0–2, 14–16, 26–28, 36–38 cm, referred to as A1, A2, B1, and B2, respectively, representing the top and approximate middle of A and B horizons. Soil subsamples were combined and homogenized by plot and depth and passed through a 2 mm sieve. A portion of the composite sample was frozen at −12 °C, and the remainder was oven dried at 40 °C and ground to a powder using a coffee grinder. Frozen soil was used for quantitative PCR (Supplementary Section 4.1) and extracellular enzyme analysis. Dried soil was used for amino sugar analysis, mid–DRIFTS, and total C and N. Soil and aboveground biomass samples (corn and rye) were analyzed for total C and N content on a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy). Total C is used as a proxy for SOC because this soil is non-calcareous. Soil C:N ratio and other edaphic properties are detailed in Supplementary Section 1.3.

2.2. Estimated carbon input

Mean annual total C input (Table 1) is comprised of measured C content of AGB left on the field (corn silage stubble, winter rye forage stubble, winter rye cover crop residue), estimated belowground biomass (BGB)–C, and manure–C. The BGB–C was approximated using total AGB–C (Supplementary Section 1.2) and the allometric root C:shoot C ratio for both winter rye and corn from a neighboring study in which these responses were measured (Cates and Jackson, 2018) and corrected for depth of measurement (Jackson et al., 1996). This value for BGB–C was adjusted to account for root exudation and turnover using literature values: root exudation as 11% of AGB–C + BGB–C in corn (Jones et al., 2009), root exudation as 43% of AGB–C in cover crops (Austin et al., 2017), and root turnover rate of 53% of BGB–C in cover crops (Gill and Jackson, 2000). The C input from fall-applied manure was estimated using annual application rates, respective dry matter content, and dry matter–C content of 33.8%, measured in 2017 with a C:N ratio of 12.7 (G.R. Sanford, personal communication).

2.3. Soil assays

2.3.1. Diffuse reflectance infrared Fourier transform spectroscopy in the mid–infrared region (mid–DRIFTS)

The mid–DRIFTS analysis characterizes SOM composition and allows for inference regarding carbon persistence in soil by measuring absorbance peak areas corresponding to specific functional groups in soil samples (Supplementary Fig. S3). Spectra absorbance peaks were integrated using the local baseline technique as described by Demyan et al. (2012) and Deiss et al. (2020b), and local peak areas were determined using the triangle method (geometry package; Habel et al., 2019). We evaluated relative peak areas, or proportion of a specific peak area relative to the total peak areas for the following organic functional groups: aliphatic C–H functional group of methyl and methylene groups (peak 2930 cm^{-1}, wavenumber 3010–2800 cm^{-1}; Orlov and Schulman, 1986), aromatic C=C stretch and/or asymmetric -COO- stretch (peak 1620 cm^{-1}, wavenumber 1660–1580 cm^{-1}; Baes and Bloom, 1989), aromatic C=C stretch (peak 1530 cm^{-1}, wavenumber 1546–1520 cm^{-1}; Baes and Bloom, 1989), C–O in both polyalcoholic and ether functional groups (peak 1159 cm^{-1}, wavenumber 1170–1148 cm^{-1}; Spaccini and Piccolo, 2007), and C–O of polysaccharides or similar substances (peak 1022 cm^{-1}, wavenumber 1080 to 970 cm^{-1}; Senesi et al., 2003). Analysis focuses on the aliphatic functional groups (peak 2930 cm^{-1}; chemically labile sugars from microbial cell components and plant wax layers) and aromatic functional groups (peaks 1530 and 1620 cm^{-1}; chemically recalcitrant compounds).

Spectra were obtained using an X,Y Autosampler (Pike Technologies Inc., Madison, WI) coupled with a Nicolet iS50 spectrometer equipped with a diffuse reflectance accessory (Thermo Fisher Scientific Inc., Waltham, MA). Four spectral readings (Deiss et al., 2020a) were taken for each subsample using the random oversampling motion function of the X,Y Autosampler (within a 3 mm diameter of the sample cup's centroid) configured in AutoProTM software (Pike Technologies Inc., Madison, WI). We used anodized aluminum plates that fit 24 polystyrene sample cups (5.5 mL volume and 10 mm top opening diameter), each loaded with a soil subsample, dried for >48 h at 40 °C and 12–14% relative humidity. All measurements were recorded from 4000 to 400 cm^{-1} at 4 cm^{-1} wavenumber resolution using 24 co-added scans (Deiss et al., 2020b).

2.3.2. Amino sugar residue analysis

We determined the concentration of several amino sugars in soil samples, including: Muramic acid (MurA), a specific biomarker for bacteria in soil that appears exclusively in murein layers of bacterial cell walls (Parsons, 1981); Glucosamine (GluN), which predominantly represents chitin of fungal cell walls, though is also present in bacterial cell wall murein and some exudates (Parsons, 1981); and Galactosamine

Table 1
Estimated mean annual carbon inputs. Inputs include unharvested aboveground biomass (AGB) residue–C from corn silage and winter rye, estimates of belowground biomass (BGB)–C from corn silage and winter rye, and manure–C, annually applied at a uniform rate across treatments (*with standard error*). Cover crop treatment effect was significant ($p < 0.001$, repeated measures analysis).

	Total C input	Corn silage		Winter rye		Manure
		AGB residue–C	BGB–C	AGB residue–C	BGB–C	C
Cover Crop†	Mg ha^{-1} yr^{-1}	kg ha^{-1} yr^{-1}				
NoCC	4.69 (*0.15*) b	710 (*34*)	2434 (*117*)	*na*	*na*	1546 (*18*)
RyeCC	5.88 (*0.14*) a	721 (*33*)	2469 (*114*)	637 (*116*)	506 (*92*)	1546 (*18*)
RyeHarv	5.82 (*0.12*) a	591 (*34*)	2026 (*117*)	186 (*11*)	1475 (*85*)	1546 (*18*)

† NoCC = No cover crop; RyeCC = winter rye as a cover crop; RyeHarv = winter rye harvested as a forage crop prior to corn planting.

(GalN), which is present in large concentrations and thought to contribute to extracellular polymeric substances (Sradnick et al., 2013). Some studies have also included mannosamine, though recent works cite low overall concentration and unclear specificity as reasons to exclude this amino sugar from analysis (Engelking et al., 2007; Joergensen, 2018; Sradnick et al., 2013). Bacterial–GluN was estimated by assuming a 1:2 M ratio of MurA:GluN in soil bacteria, with fungal–GluN constituting the remainder of total GluN (Engelking et al., 2007; Joergensen, 2018).

Amino sugar concentration in soil samples was determined by conversion to aldonitrile acetates followed by gas chromatography (Guerrant and Moss, 1984; Zhang and Amelung, 1996) as presented by Liang et al. (2012). Briefly, oven-dry soil was hydrolyzed with 6 M HCl at 105 °C for 8 h to release the amino sugar monomers, which were then purified and derivatized. Internal standards myo-inositol and N-methylglucamine were added to each sample prior to purification and derivatization, respectively, to control for sample loss at each step. Extracts were analyzed with an Agilent 6890 GC (Agilent Technologies, Wilmington. DE, US) with a DB5-UI fused silica capillary column (30 m long, 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Inc.) and a flame ionization detector (300 °C), using hydrogen as the carrier gas. The injector was operated in split mode (90:1) at 250 °C; sample injection volume was 1 μL. The individual amino sugar derivatives were identified by comparison of retention time with those of standards.

2.3.3. Estimating legacy microbial biomass–C from amino sugar residues

The legacy microbial biomass–C (both fungal–C and bacterial–C) can be estimated using the measured quantity of amino sugar residues, which accumulate over time. To clarify, these legacy microbial biomass–C estimates reflect the bygone microbial biomass from which microbial residues accumulate, and thus do not serve as a snapshot of contemporary soil microbial biomass–C. Legacy fungal–C was estimated by multiplying fungal–GluN by a conversion factor of 9 (Appuhn and Joergensen, 2006), which assumes that fungal biomass is 46% C (Jenkinson, 1988). Estimating legacy bacterial–C from MurA concentration is more complicated due to the marked differences between MurA concentrations in Gram–negative (Gr–) and Gram–positive (Gr+) bacterial cell walls, which range from 2.0 to 7.2 mg MurA g dry weight^{-1} (Gr–) and 1.7–42.2 mg MurA g^{-1} (Gr+) in laboratory cultures (Appuhn and Joergensen, 2006). In this study, we used the weighted average values of 3.7 and 13.9 mg MurA g^{-1}, respectively (Appuhn and Joergensen, 2006), combined with a ratio of Gr– to Gr+ bacteria in soil of 43:57. The Gr– to Gr+ ratio was determined using lipid biomarker measurements from an adjacent study field of the same soil type and similar management history (see Supplementary Section 2). Finally, we assumed that bacterial biomass is 46% (Jenkinson, 1988) to arrive at a conversion factor of 48.3 to estimate legacy bacterial biomass–C from measured MurA concentrations.

Appuhn and Joergensen (2006) established a conversion factor of 45 for estimation of legacy bacterial biomass–C, using a ratio of 35:65 Gr– to Gr+ bacteria in soil, based on a lab incubation using one soil (Joergensen and Potthoff, 2005); this conversion factor has been uniformly applied to various agricultural, grassland, and forest soils (e.g., Khan et al., 2016). We used local lipid biomarker data in order to more accurately represent the soil community in our study, though our field-based conversion factor (48.3) and the commonly used conversion factor (45) result in similar legacy bacterial–C estimates. However, Gr– to Gr+ ratios can vary widely across management systems (Liang et al., 2016; Oates et al., 2016), and therefore we underscore the opportunity to improve the precision of legacy microbial biomass–C estimates using system– and soil–specific bacterial–C conversion factors, where lipid biomarker data are available.

2.3.4. Extracellular enzyme analysis

Selected potential extracellular enzyme activities were measured using a fluorescence enzyme assay (Bell et al., 2013). Enzymes included cellobiohydrolase, β–xylosidase, β–glucosidase, phosphatase, N–acetylglucosaminidase, and leucine aminopeptidase. Briefly, 1.0 g of thawed soil was blended for 1 min in 100 mL of 50 mM sodium acetate buffer (pH 6.0). Soil slurries were subsampled while in suspension using a stir plate. In black 96-well plates, 200 μL of soil slurry was reacted with 50 μL of either 200 μM enzyme substrate or 0–100 μM fluorescence standards. A standard curve was developed for each sample to correct for soil-specific background fluorescence quenching (Freeman et al., 1995). Plates were incubated in the dark at room temperature and fluorescence was measured at 3, 4, and 5 h, at which point the reaction was stable. Results are presented as potential activity per hour using the data collected at 5 h of reaction time. Potential enzyme activity measured on soil that has been frozen may not reflect the activity of fresh soil, particularly for phosphatase and N–acetylglucosaminidase (DeForest, 2009), but this assay was not feasible at the time when soil was freshly sampled. Thus, we will only use these data for cross-treatment comparison within this study, and not as absolute values of the in-situ soil conditions. Further, potential extracellular enzyme activity measurements must be interpreted with caveats because legacy enzymes stabilized on clay or organic matter surfaces may mask treatment-driven differences in the potential enzyme activities of active microbial communities (Burns, 1982; Nannipieri et al., 2017).

2.4. Statistical analysis

All statistical analysis was done in R version 3.5.2 (R Core Team, 2018). Soil response data were subjected to the Quade test (Quade, 1979; Conover, 1999; function quade.test in the stats package in R; R Core Team, 2018), which is an extension of the Wilcoxon signed ranks test and is a non-parametric test specifically for complete block data that is unreplicated within plot (i.e., N = 1 per plot; 4 soil cores per plot were composited by depth). There were 3 replicates. Significant treatment effects ($p < 0.1$) were subjected to the Quade pairwise post-hoc test for multiple comparisons (function posthoc.quade.test in the PMCMR package in R; Pohlert, 2014). Corn silage yield, total forage yield, and total carbon inputs were each analyzed across all study years, using repeated measures analysis with compound symmetry correlation structure, $\alpha = 0.05$ (lme function in the nlme package for R; Pinheiro et al., 2018). Yields were also analyzed within study years due to a significant effect of year on total forage yield. Relationships were determined between selected response variables using Pearson correlation analysis (stat_cor function of ggpubr package for R and using ggplot2; Kassambara, 2018; Wickham, 2016).

3. Results

3.1. Estimated carbon inputs and soil organic carbon

The estimated mean annual C input (unharvested AGB–C + estimated BGB–C + manure–C; detailed in section 2.2) was 4.7, 5.9, and 5.8 Mg C ha^{-1} yr^{-1} in NoCC, RyeCC, and RyeHarv, respectively (Table 1). Mean annual C inputs in RyeCC and RyeHarv treatments were >23% greater than that of NoCC ($p < 0.001$). The mean annual C:N ratio of rye AGB at termination in RyeCC treatment was 12.8 (SD = 2.1). The mean annual C:N ratio of harvested rye in RyeHarv treatment was 20.7 (SD = 6.6). The C:N ratios of rye residue in RyeCC vs harvested rye in RyeHarv were statistically different ($p < 0.001$, Wilcoxon rank sum test; Supplementary Section 1.2).

Soil organic C decreased with depth and was significantly affected by cover crop treatment at depths A1, B1, and B2 ($p < 0.05$; Fig. 1). Pairwise comparison of data at these depths demonstrated significantly greater concentration of SOC in RyeCC relative to NoCC at depth A1 (22.5 and 20.5 mg g^{-1}, respectively; 10% greater) and B2 (6.9 and 6.1 mg g^{-1}, respectively; 13% greater), and significantly greater SOC in RyeCC relative to RyeHarv at B1 (9.4 and 8.3 mg g^{-1}, respectively; 13% greater); $p < 0.05$ for each significant pairwise comparison. SOC

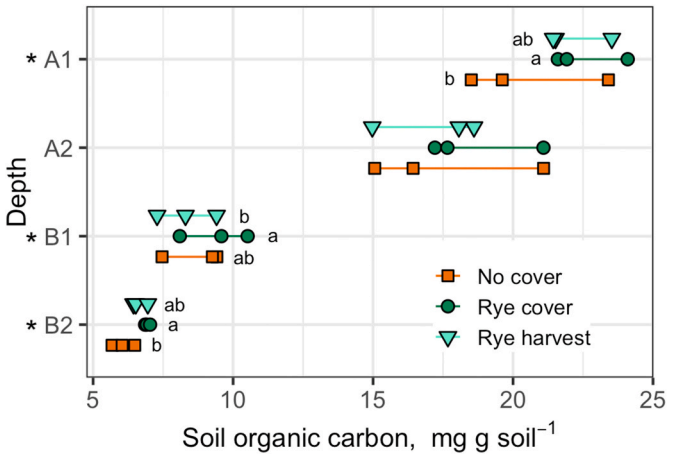


Fig. 1. *Soil organic carbon (SOC) by depth and cover crop treatment.* Each point represents a single measurement, and lines connect each treatment and depth combination for clarity. Cover crop treatment effect was significant at depths A1, B1, and B2 ($p < 0.05$; indicated by asterisks). Letters within depth indicate statistically significant pairwise treatment differences ($\alpha = 0.1$, Quade post–hoc test). Soil depths represent the top and middle of A and B horizons: A1 = 0–2 cm; A2 = 14–16 cm; B1 = 26–28 cm; B2 = 36–38 cm. (N = 3).

measurements at A2 represented the widest range of any depth with no significant differences between cover treatments.

3.2. SOM characterization with Mid–DRIFTS

There was a significant cover treatment effect at A1 in aliphatic C ($p = 0.020$). Pairwise comparison demonstrated a higher concentration in RyeHarv compared to NoCC ($p = 0.024$), with no differentiation between RyeCC and either other treatment (Table 2, Supplemental Fig. S1). There was no effect of cover crop treatment on aliphatic C at any other depth, nor any effect on aromatic C (Fig. 2) or any other functional group at any depth (Table 2). The relative abundances of aromatic C and aliphatic C were similar across treatments at A1. With increasing depth, aromatic C increased and aliphatic C decreased (Fig. 2). There was a positive relationship between SOC and aliphatic C ($R^2 = 0.52, 0.65, 0.68$ for depths A1, A2, and B2, $p < 0.05$; Fig. 3a), coupled with an inverse relationship between SOC and aromatic C across cover treatments ($R^2 = 0.63, 0.48$ for depths A2, and B2, $p < 0.05$; Fig. 3b). Slopes were steepest at B2. SOC was also strongly correlated to the ratio of aliphatic C:aromatic C ($R^2 \geq 0.39$ for all depths, $p < 0.1$ at A1 and B1, and $p < 0.01$ at A2 and B2; Fig. 3c).

3.3. Amino sugar residues

There were no significant cover crop treatment differences in the individual amino sugar residue concentrations (GluN, MurA, GalN) or total amino sugar concentration at any measured depth. Total and individual amino sugar concentrations decreased with depth, with the exception of MurA, which was highest at depth A2, followed by A1, B1, and B2 (Table 3). Total GluN (both bacterial and fungal fractions) comprised 72% of total amino sugars at each depth, with fungal–GluN making up the majority (63–65% of total amino sugars across depths). There was a positive relationship between total amino sugar concentration and SOC, with R^2 of 0.57, 0.63, and 0.39 ($p = 0.019, 0.011, 0.070$) at A2, B1, and B2, respectively (Fig. 4).

Legacy microbial biomass–C, which is an estimate of microbial biomass–C that turned over in the soil to leave behind the measured concentration of amino sugar residues, demonstrated a positive relationship with SOC, with R^2 of 0.36, 0.59, and 0.82 ($p = 0.086, 0.016, 0.0008$) at

Table 2
Soil organic matter functional groups by depth and cover crop treatment. Aliphatic, aromatic, and C–O functional groups were determined using diffuse reflectance infrared Fourier transform spectroscopy in the mid–infrared region (mid–DRIFTS), and are presented as relative peak area. The effect of cover crop on aliphatic C at depth A1 was significant ($p = 0.02$); means followed by the same letter are not significantly different. There were no other statistically significant treatment effects ($\alpha = 0.1$).

	Soil organic matter functional groups			
	Aliphatic C-H (2930 cm^{-1})	**Aromatic C=C (1620 cm^{-1} + 1530 cm^{-1})**	**C-O (1159 cm^{-1})**	**C-O (1022 cm^{-1})**
	relative peak area, %			
Cover Crop †	Depth A1 ‡			
NoCC	22.0 b	32.3	4.8	40.9
RyeCC	25.6 ab	29.7	4.5	40.3
RyeHarv	26.3 a	31.3	4.6	37.7
	Depth A2			
NoCC	17.0	31.9	4.6	46.4
RyeCC	19.4	32.1	5.0	43.5
RyeHarv	17.7	33.6	5.1	43.6
	Depth B1			
NoCC	8.7	43.8	5.7	41.7
RyeCC	8.5	41.4	5.6	44.5
RyeHarv	9.1	41.3	5.5	44.1
	Depth B2			
NoCC	4.0	57.5	6.8	31.7
RyeCC	7.9	47.4	6.4	38.4
RyeHarv	6.7	56.1	6.7	30.4

† NoCC = No cover crop; RyeCC = winter rye as a cover crop; RyeHarv = winter rye harvested as a forage crop prior to corn planting.
‡ Soil depths represent the top and middle of A and B horizons: A1 = 0–2 cm; A2 = 14–16 cm; B1 = 26–28 cm; B2 = 36–38 cm. (N=3).

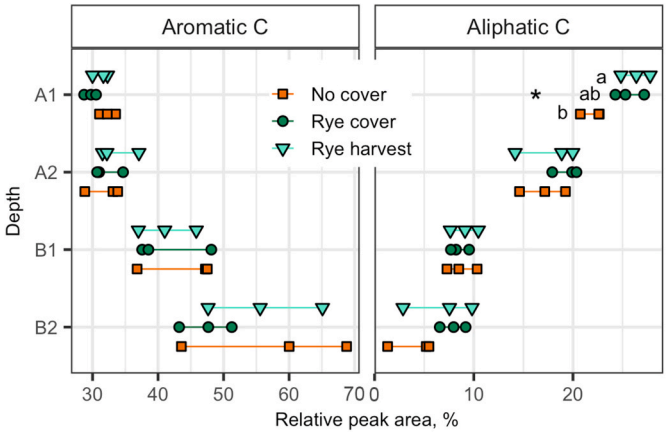


Fig. 2. *Soil organic matter functional groups by depth and cover crop treatment.* Measured using diffuse reflectance infrared Fourier transform spectroscopy in the mid–infrared region (mid–DRIFTS). Results are presented as relative peak area of SOM functional groups: aliphatic C = 2930 cm^{-1}; aromatic C = 1530 + 1620 cm^{-1}. Each point represents a single measurement, and lines connect each treatment and depth combination for clarity. Cover crop treatment effect was significant for aliphatic C at depth A1 ($p < 0.05$; indicated by an asterisk). Letters within depth indicate statistically significant pairwise treatment differences ($\alpha = 0.1$, Quade post–hoc test). Soil depths represent the top and middle of A and B horizons: A1 = 0–2 cm; A2 = 14–16 cm; B1 = 26–28 cm; B2 = 36–38 cm. (N = 3).

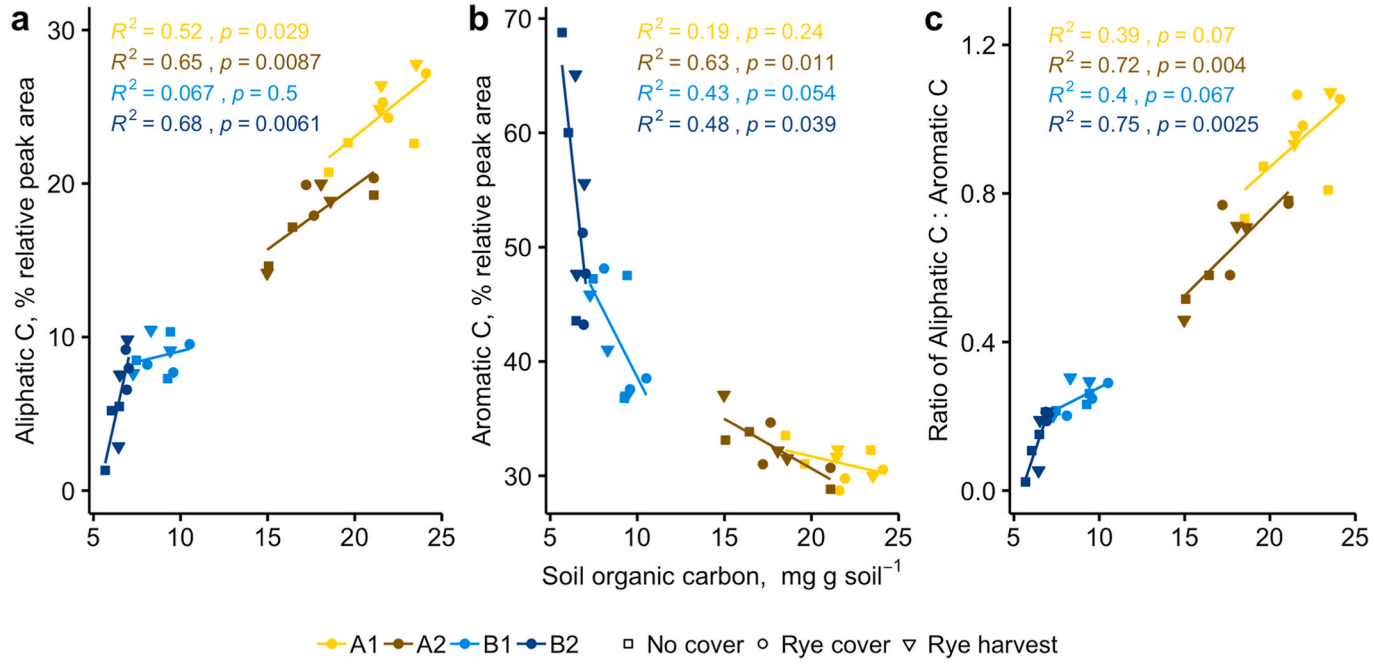


Fig. 3. *Relationships between soil organic matter functional groups and soil organic carbon (SOC) by depth.* (a) Aliphatic C relative peak area vs SOC; (b) Aromatic C relative peak area vs SOC; (c) Ratio of aliphatic C:aromatic C vs SOC. Soil depths represent the top and middle of A and B horizons: A1 = 0–2 cm; A2 = 14–16 cm; B1 = 26–28 cm; B2 = 36–38 cm. (N = 3).

Table 3
Amino sugar residue concentrations. Concentrations of total and individual amino sugars and estimates of legacy microbial biomass–C by depth and cover crop treatment. There were no statistically significant treatment effects (α = 0.1).

	Amino sugar						Legacy microbial–C	
	Total amino sugars†	GluN‡	GalN‡	MurA‡	Fungal–GluN§	Fungal–GluN: MurA	Fungal ¶	Bacterial
	μg g^{-1} soil						mg g^{-1} soil	
Cover Crop ††	Depth A1 ‡‡							
NoCC	1855	1330	442	83	1212	14.7	10.9	4.0
RyeCC	2017	1427	502	88	1302	15.4	11.7	4.2
RyeHarv	2028	1467	468	92	1337	14.6	12.0	4.4
	Depth A2							
NoCC	1909	1383	428	98	1244	12.7	11.2	4.7
RyeCC	1860	1339	417	104	1193	11.9	10.7	5.0
RyeHarv	1867	1356	421	89	1230	14.2	11.1	4.3
	Depth B1							
NoCC	1245	903	258	84	783	9.6	7.0	4.1
RyeCC	1280	928	274	77	819	11.6	7.4	3.7
RyeHarv	1185	860	249	77	751	10.1	6.8	3.7
	Depth B2							
NoCC	960	694	206	59	610	10.8	5.5	2.8
RyeCC	1087	786	246	55	709	13.2	6.4	2.6
RyeHarv	1003	724	213	66	631	9.8	5.7	3.2

† Total amino sugars = GluN + GalN + MurA.
‡ GluN = glucosamine; GalN = galactosamine; MurA = muramic acid.
§ Fungal–GluN = estimated glucosamine attributable to fungi.
¶ Legacy microbial biomass–C from which the accumulated amino sugars originated was estimated using MurA (for bacterial–C) and fungal–GluN (for fungal–C).
†† NoCC = No cover crop; RyeCC = winter rye as a cover crop; RyeHarv = winter rye harvested as a forage crop prior to corn planting.
‡‡ Soil depths represent the top and middle of A and B horizons: A1 = 0–2 cm; A2 = 14–16 cm; B1 = 26–28 cm; B2 = 36–38 cm. (N=3).

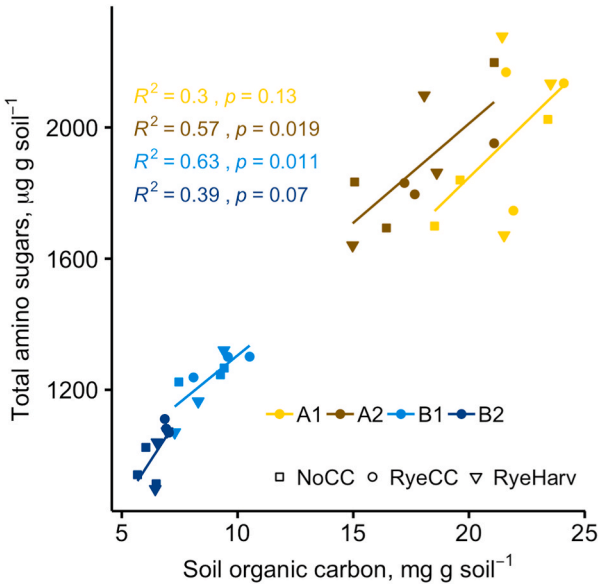


Fig. 4. *Total amino sugar concentration* vs *soil organic carbon (SOC) by depth*. Total amino sugar concentration is the sum of glucosamine, galactosamine, and muramic acid residue concentrations in soil. Soil depths represent the top and middle of A and B horizons: A1 = 0–2 cm; A2 = 14–16 cm; B1 = 26–28 cm; B2 = 36–38 cm. (N = 3). NoCC = No cover crop; RyeCC = winter rye as a cover crop; RyeHarv = winter rye harvested as a forage crop prior to corn planting.

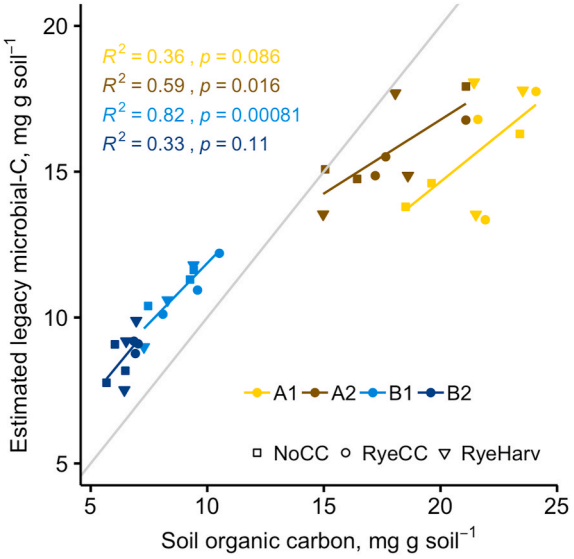


Fig. 5. *Estimated legacy microbial biomass–C* vs *soil organic carbon (SOC)*. Fungal–GluN and MurA were used to estimate the amount of legacy microbial biomass–C attributable to microbial residues from fungi and bacteria, respectively. The gray 1:1 slope line represents a theoretical condition in which estimated legacy microbial biomass–C is equal to SOC, and how the relationship between these parameters shifts with depth. Soil depths represent the top and middle of A and B horizons: A1 = 0–2 cm; A2 = 14–16 cm; B1 = 26–28 cm; B2 = 36–38 cm. (N = 3). NoCC = No cover crop; RyeCC = winter rye as a cover crop; RyeHarv = winter rye harvested as a forage crop prior to corn planting.

A1, A2, and B1, respectively (Fig. 5).

Fungal–GluN:MurA generally decreased with depth (Table 3, Supplementary Fig. S4) and ratios of estimated legacy fungal–C:bacterial–C were 2.8, 2.4, 2.0, and 2.1 at A1, A2, B1, and B2, respectively. However, 18S:16S gene copy ratios determined via quantitative PCR (see Supplementary Section 4, Supplementary Fig. S5) demonstrated a wide range without a clear trend with depth.

3.4. Potential extracellular enzyme activity

At depth A1, there was a significant effect of cover crop treatment on the potential activity for the following extracellular enzymes: β–glucosidase (BG), cellobiohydrolase (CELL), β–xylosidase (XYL), phosphatase (PHOS), N–acetylglucosaminidase (NAG; p = 0.02, 0.02, 0.07, 0.02, 0.02, respectively; Fig. 6). For each of these enzymes, RyeCC exhibited higher potential activity relative to NoCC (p = 0.02, 0.02, 0.09, 0.02, 0.02, respectively). However, potential activity in RyeHarv at depth A1 did not significantly differ from NoCC or RyeCC. There was no significant cover treatment effect on potential activity of leucine aminopeptidase (LAP) at A1, and no treatment effect for any potential enzyme activities at any other depths measured. Broadly, potential enzyme activity declined with depth for all enzymes except PHOS, but this decline was more pronounced with RyeCC, where potential enzyme activity was greater at A1.

4. Discussion

4.1. Evidence for SOC accrual mechanisms

Following seven years of cover crop use, SOC concentrations increased by up to 13% (Fig. 1). However, SOC increases were inconsistent throughout our sampling depth and across treatments. Specific measurements of SOM fractions and microbial biomarkers failed to identify the mechanisms that increased SOC, which demonstrates that these parameters are well-buffered to the range of environmental and management conditions in this study. For example, total and individual amino sugar residue concentrations did not respond to either of the cover crop treatments (Table 3), and aliphatic C was the only significant SOM treatment response, which increased in the RyeHarv treatment at the soil surface (Table 2 and Fig. 2). Despite slight or statistically insignificant cover crop treatment-driven changes in carbon and microbial parameters, the data do indicate positive relationships between SOC and easily degradable aliphatic C compounds (Fig. 3a) and between SOC and microbial amino sugar residues (Fig. 4). These relationships support the theory that chemically labile compounds contribute meaningfully to SOC stabilization and persistence (Cotrufo et al., 2013; Lehmann and Kleber, 2015; Liang et al., 2017; Schmidt et al., 2011), potentially via organo-mineral associations and microaggregate formation attributable to anabolic microbial activities (Hernandez-Soriano et al., 2018; Lehmann et al., 2007; Six et al., 2006).

Slight changes in concentrations of amino sugars are fundamentally difficult to detect in soil because this necromass persists over numerous "generations" of microbial communities (Khan et al., 2016); this accumulation may mute signals indicative of management or treatment differences. For example, amino sugar concentrations measure about 2 orders of magnitude higher in soil than in microbial biomass extracts (Glaser et al., 2004). Furthermore, the substantial rate of annual manure application in this study, which comprised 26–33% of total C inputs yr^{-1} (Table 1) and was applied uniformly across all treatments, could have increased total amino sugar accumulation (Faust et al., 2017; Peltre et al., 2017) and muted potential cover crop-driven signals in amino sugar concentration between treatments. As further evidence for amino sugar residue accumulation, we can note the positive relationship between SOC and legacy microbial biomass–C, which is estimated from amino sugar residue concentrations (Fig. 5).

4.2. Effects of winter rye on soil carbon

Winter rye as a cover crop or winter rye harvested as a forage increased estimated mean annual C inputs by >23% compared to NoCC over seven years of continuous corn silage (Table 1). Estimates of C inputs help delineate differences in aboveground vs belowground rye

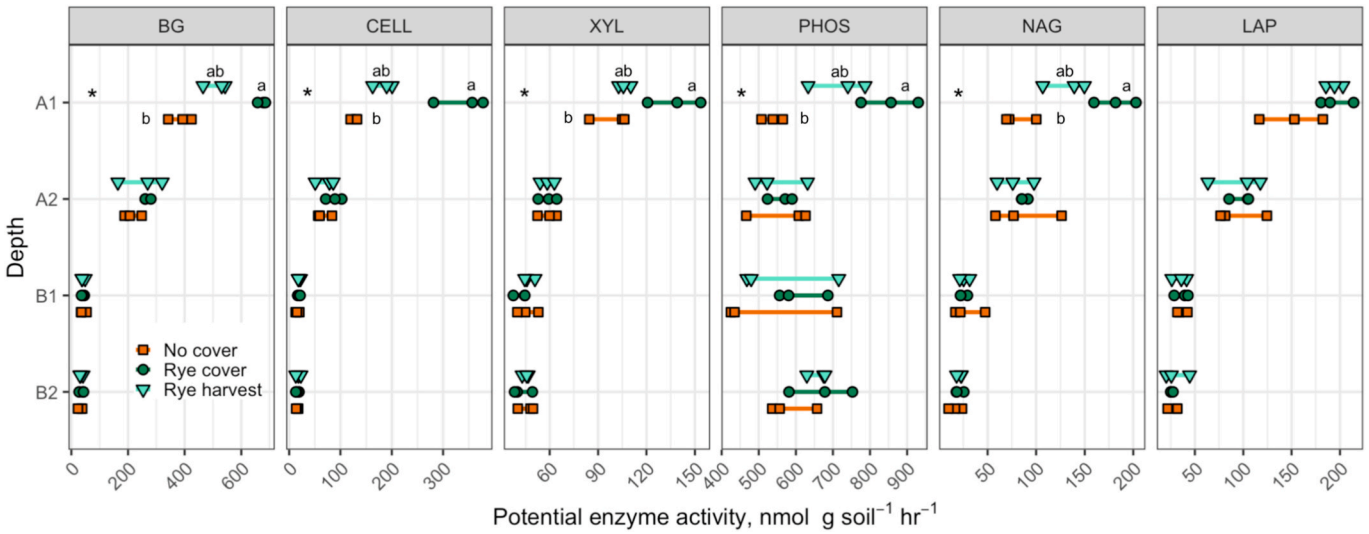


Fig. 6. *Potential extracellular enzyme activity by depth and cover crop treatment.* Each point represents a single measurement, and lines connect each treatment and depth combination for clarity. Cover crop treatment effect was significant at depth A1 for each enzyme except LAP. ($p < 0.05$; indicated by asterisks). Letters within depth indicate statistically significant pairwise treatment differences ($\alpha = 0.1$, Quade post–hoc test). BG = β–glucosidase; CELL = cellobiohydrolase; XYL = β–xylosidase; PHOS = phosphatase; NAG = N–acetylglucosaminidase; LAP = leucine aminopeptidase. Soil depths represent the top and middle of A and B horizons: A1 = 0–2 cm; A2 = 14–16 cm; B1 = 26–28 cm; B2 = 36–38 cm. (N = 3).

inputs as affected by management. For example, whereas the rye biomass in the RyeCC treatment was terminated and left on the soil surface, the aboveground rye biomass in the RyeHarv treatment was mostly harvested and removed from the field. However, the RyeHarv treatment had contributed substantially greater estimated belowground inputs due to the extended rye growth period (Table 1). Similarly, Cates and Jackson (2018) found that rye cover significantly increased total C inputs when integrated into a corn silage system, mitigating a portion of SOC loss that characterizes corn silage production (Johnson et al., 2014).

Though important, the quantity of biomass and C input is only one aspect of SOC building, and we must also consider the traits or general quality of that biomass (Finney et al., 2016). The relatively low (i.e., favorable) rye C:N ratio in the RyeCC treatment (mean C:N = 12.8) likely made this substrate more conducive to SOC building relative to the rye in the RyeHarv treatment (mean C:N = 20.7). This may be evident in the significantly greater SOC concentration in RyeCC compared to NoCC, which was not apparent in RyeHarv (Fig. 1), despite similar total C inputs in both rye treatments and greater estimated BGB–C in RyeHarv (Table 1). Though previous work may suggest that the greater root BGB–C inputs in RyeHarv would have increased SOC (Lavallee et al., 2018; Rasse et al., 2005), SOC storage also depends on N availability (Cotrufo et al., 2019), which was likely a limiting factor in the RyeHarv treatment given that soil nitrate-N concentrations 0–30 cm measured at preplant and in-season were significantly lower compared to both NoCC and RyeCC (years 2012–2016; reported in West et al., 2020). Nitrogen scavenging to balance an inundation of C would be expected to lower CUE (Geyer et al., 2016), and thus the "top-down" regulators of CUE (here, rye residue quality and quantity) were not likely optimal for SOC accrual. In short, the microbial community failed to translate substantial C inputs into stored SOC, particularly in the RyeHarv treatment. Furthermore, the significantly higher C:N ratio of the more mature rye biomass in the RyeHarv treatment compared to that of the less mature rye biomass in the RyeCC treatment, through microbial nitrogen immobilization, may have contributed to the lower corn silage yields that were observed in the RyeHarv treatment compared to NoCC in three of the seven study years (Supplementary Table S1).

Though the C inputs in the RyeHarv treatment did not increase SOC, this management practice demonstrates potential for future accumulation given the trend towards greater SOC at A1 and B2 ($p = 0.141$ at both depths, posthoc Quade test for RyeHarv vs NoCC; Fig. 1). Furthermore, there was a significantly higher concentration of aliphatic C at A1 relative to NoCC (Fig. 2), which can be viewed as an indicator of potential SOC accrual given that we saw a positive relationship between the relative aliphatic C and SOC concentrations in this study (Fig. 3a; $R^2 > 0.5$ and $p < 0.1$ for depths A1, A2, and B2). The relationship between total C inputs and SOC evidently varies with input quality, cover crop type, weather, and soil C storage capacity (Castellano et al., 2015; Kallenbach et al., 2015; Ladoni et al., 2016; McDaniel et al., 2014a).

4.3. Effects of winter rye on potential extracellular enzyme activity

Only the RyeCC treatment showed evidence for increased microbial activity, as indicated by increased potential extracellular enzyme activities at A1 (Fig. 6; also seen in Baldrian, 2014; Nevins et al., 2018). Conversely, potential enzyme activities in the RyeHarv treatment were not significantly higher than NoCC. This difference between RyeCC and RyeHarv is notable because RyeHarv showed evidence for nutrient immobilization (e.g., low soil nitrate and occasional corn silage yield decreases), and also had higher estimated BGB–C inputs (Table 1); these factors led us to hypothesize that RyeHarv treatment would demonstrate increased potential extracellular enzyme activity, particularly at lower depths. It is possible that this hypothesis was not supported due to sub-optimal rye input quality in the RyeHarv system (i.e., high C:N ratio of rye forage). Insufficient N availability may hamper extracellular enzyme production and enzyme activities (Moorhead et al., 2012). The rapid drop-off of potential extracellular enzyme activities that we saw at lower depths across all treatments was also observed by Freeman et al. (1995), and likely reflects depth-associated decreases in both high-quality inputs and active microbial biomass (Eilers et al., 2012).

4.4. Amino sugar-based estimates of legacy microbial biomass–C

Legacy bacterial– and fungal–C can be estimated using amino sugar residue concentrations and literature-based conversion factors (Appuhn and Joergensen, 2006; Engelking et al., 2007). These estimates help to interpret the accumulation of amino sugar residues over time. Higher concentrations of amino sugar residues in soil translate to higher estimates of legacy microbial biomass–C, and both concentrations and estimates were positively correlated to SOC concentration in this study

(Figs. 4 and 5). These relationships underscore the persistence of amino sugar residues in soil, and this trend becomes more prominent at depth: The increased proportion of estimated legacy microbial–C relative to SOC at depths B1 and B2 indicates an increasing accumulation, or lower turnover, of microbial residues with increasing depth, as visualized relative to the 1:1 slope line in Fig. 5. Alternatively, the apparent increase in amino sugar accumulation at depth may be explained in part by a shift in bacterial community composition towards Gr+ bacteria (Fierer et al., 2003; Xiang et al., 2017). Taking this shift into account, the conversion factor used to estimate legacy bacterial–C would decrease and therefore these C estimates would also decrease, bringing the B horizon points in Fig. 5 closer to or possibly below the 1:1 slope line. Depth–specific lipid biomarker analysis would help to clarify the relationships between community composition, amino sugar residues, and depth. Without this information, interpretation of these types of estimates should be restricted to within-study comparison due to inherent limitations of using broad literature-based assumptions to estimate legacy microbial–C.

4.5. Effect of winter rye on soil microbial community

There was no treatment-driven shift in fungal to bacterial ratios as measured by fungal–GluN:MurA or 18S:16S gene copy number ratios within any soil depth (Supplementary Figs. S4 and S5, respectively). This finding is consistent with some previous work that found fungal to bacterial ratios to be unaffected by winter rye use (Barel et al., 2019). That said, this metric is dynamic, and other work indicates that the microbial community responds variably to cover crop use. For example, winter rye increased fungal to bacterial ratios determined by PLFA analysis, but results were sensitive to sample timing (Finney et al., 2017).

5. Conclusions

Winter rye demonstrated potential to increase total C input and SOC when used for seven years in continuous corn silage, a high-production cropping system often characterized by net C loss. However, the SOC gains were only observed at some soil depths, and only when rye was used as a cover crop. Gains in SOC were not observed when rye was harvested as a forage double crop, despite some changes to SOM characteristics compared to no rye. When comparing the rye treatments, there were no differences in SOM characteristics (e.g., aliphatic and aromatic C), amino sugar residue concentrations, or potential enzyme activities. Overall, winter rye use appears to be a viable option for mitigating C loss in continuous corn silage, though an inefficient means of C building in this system.

The strong relationships observed between SOC and both relative aliphatic C concentration and total amino sugar residue concentration underscore the importance of microbial pathways to SOC accumulation, and support the use of these evidently stable and slow-to-change responses as indicators of potential SOC accrual. Our results suggest that SOC can be increased in high-removal agronomic systems by focusing on C inputs that specifically promote aliphatic C and microbial residue accumulation. Annual manure applications may have forestalled winter rye-driven increases in SOC, or otherwise masked detection of possible treatment effects on the measured parameters. Future research could explore the potential for SOC building with cover crops of various residue qualities, or in agronomic systems with lower rates of biomass removal. Additionally, isotopic tracing studies would also be valuable for conclusively determining the fate of biomass–C as it moves through the system from plants to living microbial biomass, and, ultimately, to microbial necromass.

Acknowledgements

The soil analyses presented in this paper were made possible by the University of Wisconsin–Madison O.N. Allen Soil & Environmental Small Grants Program and the Foundation for Food and Agricultural Research and the School of Environment and Natural Resources at Ohio State University. The field project was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture (award number 201368002-20525) and the University of Wisconsin–Madison Nutrient and Pest Management Program. The authors would also like to thank Kevin Shelley, Dr. Harry Read, Andrew Larson, Peter Wakeman, Jamie Woolet, Dr. Gregg Sanford, Dr. Steve Culman, Dr. Gary Oates, the field crew at the UW Arlington Agricultural Research Station, and the anonymous manuscript reviewers.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2020.107899.

References

Amundson, R., Berhe, A.A., Hopmans, J.W., Olson, C., Sztein, A.E., Sparks, D.L., 2015. Soil and human security in the 21st century. Science 348. https://doi.org/10.1126/science.1261071, 1261071–1261071.

Appuhn, A., Joergensen, R., 2006. Microbial colonisation of roots as a function of plant species. Soil Biology and Biochemistry 38, 1040–1051. https://doi.org/10.1016/j.soilbio.2005.09.002.

Arbuckle, J.G., Roesch-McNally, G.E., 2015. Cover crop adoption in Iowa: the role of perceived practice characteristics. Journal of Soil and Water Conservation 70, 418–429. https://doi.org/10.2489/jswc.70.6.418.

Austin, E.E., Wickings, K., McDaniel, M.D., Robertson, G.P., Grandy, A.S., 2017. Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. GCB Bioenergy 9, 1252–1263. https://doi.org/10.1111/gcbb.12428.

Bach, E.M., Hofmockel, K.S., 2016. A time for every season: soil aggregate turnover stimulates decomposition and reduces carbon loss in grasslands managed for bioenergy. GCB Bioenergy 8, 588–599. https://doi.org/10.1111/gcbb.12267.

Baes, A.U., Bloom, P.R., 1989. Diffuse reflectance and transmission fourier transform infrared (DRIFT) spectroscopy of Humic and Fulvic acids. Soil Science Society of America Journal 53, 695–700. https://doi.org/10.2136/sssaj1989.03615995005300030008x.

Baldrian, P., 2014. Distribution of extracellular enzymes in soils: spatial heterogeneity and determining factors at various scales. Soil Science Society of America Journal 78, 11–18. https://doi.org/10.2136/sssaj2013.04.0155dgs.

Barel, J.M., Kuyper, T.W., Paul, J., de Boer, W., Cornelissen, J.H.C., De Deyn, G.B., 2019. Winter cover crop legacy effects on litter decomposition act through litter quality and microbial community changes. Journal of Applied Ecology 56, 132–143. https://doi.org/10.1111/1365-2664.13261.

Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. Journal of Visualized Experiments. https://doi.org/10.3791/50961.

Burns, R.G., 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biology and Biochemistry 14, 423–427. https://doi.org/10.1016/0038-0717(82)90099-2.

Castellano, M.J., Mueller, K.E., Olk, D.C., Sawyer, J.E., Six, J., 2015. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Global Change Biology 21, 3200–3209. https://doi.org/10.1111/gcb.12982.

Cates, A.M., Jackson, R.D., 2018. Cover crop effects on net ecosystem carbon balance in grain and silage maize. Agronomy Journal. https://doi.org/10.2134/agronj2018.01.0045, 0, 0.

Clark, A.J., Meisinger, J.J., Decker, A.M., Mulford, F.R., 2007. Effects of a grass-selective herbicide in a vetch–rye cover crop system on nitrogen management. Agronomy Journal 99, 36–42. https://doi.org/10.2134/agronj2005.0361.

Conover, W.J., 1999. Practical Nonparametric Statistics. John Wiley & Sons, New York, NY.

Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience 12, 989–994. https://doi.org/10.1038/s41561-019-0484-6.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988–995. https://doi.org/10.1111/gcb.12113.

DeForest, J.L., 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. Soil Biology and Biochemistry 41, 1180–1186. https://doi.org/10.1016/j.soilbio.2009.02.029.

Deiss, L., Culman, S.W., Demyan, M.S., 2020a. Grinding and sample replication often improves Mid-DRIFTS predictions of soil properties. Soil Science Society of America Journal. https://doi.org/10.1002/saj2.20021.

Deiss, L., Margenot, A.J., Culman, S.W., Demyan, M.S., 2020b. Optimizing acquisition parameters in diffuse reflectance infrared fourier transform spectroscopy of soils. Soil Science Society of America Journal. https://doi.org/10.1002/saj2.20028.
Demyan, M.S., Rasche, F., Schulz, E., Breulmann, M., Müller, T., Cadisch, G., 2012. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem. European Journal of Soil Science 63, 189–199. https://doi.org/10.1111/j.1365-2389.2011.01420.x.
Eilers, K.G., Debenport, S., Anderson, S., Fierer, N., 2012. Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biology and Biochemistry 50, 58–65. https://doi.org/10.1016/J.SOILBIO.2012.03.011.
Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biology and Biochemistry 39, 2111–2118. https://doi.org/10.1016/j.soilbio.2007.03.020.
Faé, G.S., Sulc, R.M., Barker, D.J., Dick, R.P., Eastridge, M.L., Lorenz, N., 2009. Integrating winter annual forages into a No-till corn silage system. Agronomy Journal 101, 1286. https://doi.org/10.2134/agronj2009.0144.
Faust, S., Heinze, S., Ngosong, C., Sradnick, A., Oltmanns, M., Raupp, J., Geisseler, D., Joergensen, R.G., 2017. Effect of biodynamic soil amendments on microbial communities in comparison with inorganic fertilization. Applied Soil Ecology 114, 82–89. https://doi.org/10.1016/j.apsoil.2017.03.006.
Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry 35, 167–176. https://doi.org/10.1016/S0038-0717(02)00251-1.
Finney, D.M., Buyer, J.S., Kaye, J.P., 2017. Living cover crops have immediate impacts on soil microbial community structure and function. Journal of Soil and Water Conservation 72, 361–373. https://doi.org/10.2489/jswc.72.4.361.
Finney, D.M., White, C.M., Kaye, J.P., 2016. Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. Agronomy Journal 108, 39–52. https://doi.org/10.2134/agronj15.0182.
Freeman, C., Liska, G., Ostle, N.J., Jones, S.E., Lock, M.A., 1995. The use of fluorogenic substrates for measuring enzyme activity in peatlands. Plant and Soil 175, 147–152. https://doi.org/10.1007/BF02413020.
García-González, I., Hontoria, C., Gabriel, J.L., Alonso-Ayuso, M., Quemada, M., 2018. Cover crops to mitigate soil degradation and enhance soil functionality in irrigated land. Geoderma 322, 81–88. https://doi.org/10.1016/j.geoderma.2018.02.024.
Geisseler, D., Horwath, W.R., Scow, K.M., 2011. Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. Pedobiologia 54, 71–78. https://doi.org/10.1016/j.pedobi.2010.10.001.
Geyer, K.M., Kyker-Snowman, E., Grandy, A.S., Frey, S.D., 2016. Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. Biogeochemistry 127, 173–188. https://doi.org/10.1007/s10533-016-0191-y.
Gill, R.A., Jackson, R.B., 2000. Global patterns of root turnover for terrestrial ecosystems. New Phytologist 147, 13–31.
Glaser, B., Turrión, M.-B., Alef, K., 2004. Amino sugars and muramic acid—biomarkers for soil microbial community structure analysis. Soil Biology and Biochemistry 36, 399–407. https://doi.org/10.1016/J.SOILBIO.2003.10.013.
Guerrant, G.O., Moss, C.W., 1984. Determination of monosaccharides as aldononitrile, O-methyloxime, alditol, and cyclitol acetate derivatives by gas-chromatography. Analytical Chemistry 56, 633–638. https://doi.org/10.1021/ac00268a010.
Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345–360. https://doi.org/10.1046/j.1354-1013.2002.00486.x.
Habel, K., Grasman, R., Gramacy, R.B., Mozharovskyi, P., Sterratt, D.C., 2019. Geometry: Mesh Generation and Surface Tessellation.
Hernandez-Soriano, M.C., Dalal, R.C., Warren, F.J., Wang, P., Green, K., Tobin, M.J., Menzies, N.W., Kopittke, P.M., 2018. Soil organic carbon stabilization: mapping carbon speciation from intact microaggregates. Environmental Science & Technology 52, 12275–12284. https://doi.org/10.1021/acs.est.8b03095.
Herz, K., Dietz, S., Gorzolka, K., Haider, S., Jandt, U., Scheel, D., Bruelheide, H., 2018. Linking root exudates to functional plant traits. PloS One 13, e0204128.
Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E., Schulze, E.D., 1996. A global analysis of root distributions for terrestrial biomes. Oecologia 108, 389–411. https://doi.org/10.1007/BF00333714.
Jenkinson, D.S., 1988. The determination of microbial biomass carbon and nitrogen in soil. In: Wilson, J.R. (Ed.), Advances in Nitrogen Cycling in Agricultural Ecosystems. CAB International, Wallingford, UK, pp. 368–386. Wallingford.
Joergensen, R.G., 2018. Amino sugars as specific indices for fungal and bacterial residues in soil. Biology and Fertility of Soils 54, 559–568. https://doi.org/10.1007/s00374-018-1288-3.
Joergensen, R.G., Potthoff, M., 2005. Microbial reaction in activity, biomass, and community structure after long-term continuous mixing of a grassland soil. Soil Biology and Biochemistry 37, 1249–1258. https://doi.org/10.1016/j.soilbio.2004.11.021.
Johnson, J.M.F., Novak, J.M., Varvel, G.E., Stott, D.E., Osborne, S.L., Karlen, D.L., Lamb, J.A., Baker, J., Adler, P.R., 2014. Crop residue mass needed to maintain soil organic carbon levels: can it Be determined? BioEnergy Research 7, 481–490. https://doi.org/10.1007/s12155-013-9402-8.
Jones, D.L., Nguyen, C., Finlay, R.D., 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant and Soil 321, 5–33. https://doi.org/10.1007/s11104-009-9925-0.
Kallenbach, C.M., Grandy, A.S., Frey, S.D., Diefendorf, A.F., 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biology and Biochemistry 91, 279–290. https://doi.org/10.1016/j.soilbio.2015.09.005.
Kallenbach, C.M., Wallenstein, M.D., Schipanksi, M.E., Grandy, A.S., 2019. Managing agroecosystems for soil microbial carbon use efficiency: ecological unknowns, potential outcomes, and a path forward. Frontiers in Microbiology 10, 51. https://doi.org/10.3389/fmicb.2019.01146.
Kassambara, A., 2018. Ggpubr: "Ggplot2". Based Publication Ready Plots.
Khan, K.S., Mack, R., Castillo, X., Kaiser, M., Joergensen, R.G., 2016. Microbial biomass, fungal and bacterial residues, and their relationships to the soil organic matter C/N/P/S ratios. Geoderma 271, 115–123. https://doi.org/10.1016/j.geoderma.2016.02.019.
King, A.E., Blesh, J., 2018. Crop rotations for increased soil carbon: perenniality as a guiding principle. Ecological Applications 28, 249–261. https://doi.org/10.1002/eap.1648.
Kladivko, E.J., Kaspar, T.C., Jaynes, D.B., Malone, R.W., Singer, J., Morin, X.K., Searchinger, T., 2014. Cover crops in the upper midwestern United States: potential adoption and reduction of nitrate leaching in the Mississippi River Basin. Journal of Soil and Water Conservation. https://doi.org/10.2489/jswc.69.4.279.
Kong, A.Y.Y., Six, J., 2012. Microbial community assimilation of cover crop rhizodeposition within soil microenvironments in alternative and conventional cropping systems. Plant and Soil 356, 315–330. https://doi.org/10.1007/s11104-011-1120-4.
Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: concept & review. Soil Biology and Biochemistry 83, 184–199. https://doi.org/10.1016/j.soilbio.2015.01.025.
Ladoni, M., Basir, A., Robertson, P.G., Kravchenko, A.N., 2016. Scaling-up: cover crops differentially influence soil carbon in agricultural fields with diverse topography. Agriculture, Ecosystems & Environment 225, 93–103. https://doi.org/10.1016/j.agee.2016.03.021.
Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304, 1623–1627. https://doi.org/10.1126/science.1097396.
Lavallee, J.M., Conant, R.T., Paul, E.A., Cotrufo, M.F., 2018. Incorporation of shoot versus root-derived 13C and 15N into mineral-associated organic matter fractions: results of a soil slurry incubation with dual-labelled plant material. Biogeochemistry 137, 379–393. https://doi.org/10.1007/s10533-018-0428-z.
Lehmann, J., Kinyangi, J., Solomon, D., 2007. Organic matter stabilization in soil microaggregates: implications from spatial heterogeneity of organic carbon contents and carbon forms. Biogeochemistry 85, 45–57. https://doi.org/10.1007/s10533-007-9105-3.
Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68. https://doi.org/10.1038/nature16069.
Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology 25, 3578–3590. https://doi.org/10.1111/gcb.14781.
Liang, C., Balser, T.C., 2008. Preferential sequestration of microbial carbon in subsoils of a glacial-landscape toposequence, Dane County, WI, USA. Geoderma 148, 113–119. https://doi.org/10.1016/J.GEODERMA.2008.09.012.
Liang, C., Kao-Kniffin, J., Sanford, G.R., Wickings, K., Balser, T.C., Jackson, R.D., 2016. Microorganisms and their residues under restored perennial grassland communities of varying diversity. Soil Biology and Biochemistry 103, 192–200. https://doi.org/10.1016/J.SOILBIO.2016.08.002.
Liang, C., Read, H.W., Balser, T.C., 2012. GC-based detection of aldononitrile acetate derivatized glucosamine and muramic acid for microbial residue determination in soil. Journal of Visualized Experiments. https://doi.org/10.3791/3767 e3767–e3767.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 17105. https://doi.org/10.1038/nmicrobiol.2017.105.
Margenot, A.J., Calderón, F.J., Bowles, T.M., Parikh, S.J., Jackson, L.E., 2015. Soil organic matter functional group composition in relation to organic carbon, nitrogen, and phosphorus fractions in organically managed tomato fields. Soil Science Society of America Journal 79, 772–782. https://doi.org/10.2136/sssaj2015.02.0070.
McDaniel, M.D., Grandy, A.S., Tiemann, L.K., Weintraub, M.N., 2014a. Crop rotation complexity regulates the decomposition of high and low quality residues. Soil Biology and Biochemistry 78, 243–254. https://doi.org/10.1016/j.soilbio.2014.07.027.
McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014b. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecological Applications 24, 560–570. https://doi.org/10.1890/13-0616.1.
Moorhead, D.L., Lashermes, G., Sinsabaugh, R.L., 2012. A theoretical model of C- and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. Soil Biology and Biochemistry 53, 133–141. https://doi.org/10.1016/j.soilbio.2012.05.011.
Myers, R., Watts, C., 2015. Progress and perspectives with cover crops: interpreting three years of farmer surveys on cover crops. Journal of Soil and Water Conservation 70, 125A–129A. https://doi.org/10.2489/jswc.70.6.125A.
Nannipieri, P., Trasar-Cepeda, C., Dick, R.P., 2017. Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. Biology and Fertility of Soils 54, 11–19. https://doi.org/10.1007/s00374-017-1245-6.
Nevins, C.J., Nakatsu, C., Armstrong, S., 2018. Characterization of microbial community response to cover crop residue decomposition. Soil Biology and Biochemistry 127, 39–49. https://doi.org/10.1016/j.soilbio.2018.09.015.
Oates, L.G., Duncan, D.S., Sanford, G.R., Liang, C., Jackson, R.D., 2016. Bioenergy cropping systems that incorporate native grasses stimulate growth of plant-associated soil microbes in the absence of nitrogen fertilization. Agriculture, Ecosystems & Environment 233, 396–403. https://doi.org/10.1016/j.agee.2016.09.008.

Orlov, A.Y., Schulman, E.I., 1986. Additional symmetries for integrable equations and conformal algebra representation. Letters in Mathematical Physics 12, 171–179. https://doi.org/10.1007/BF00416506.
Parsons, J.W., 1981. Chemistry and distribution of amino sugars in soils and soil organisms. In: Paul, E.A., Ladd, J.N. (Eds.), Soil Biochemistry, vol. 5. Marcel Dekker, New York, pp. 197–227.
Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart soils. Nature 532, 49–57. https://doi.org/10.1038/nature17174.
Peltre, C., Gregorich, E.G., Bruun, S., Jensen, L.S., Magid, J., 2017. Repeated application of organic waste affects soil organic matter composition: evidence from thermal analysis, FTIR-PAS, amino sugars and lignin biomarkers. Soil Biology and Biochemistry 104, 117–127. https://doi.org/10.1016/j.soilbio.2016.10.016.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2018. Nlme: Linear and Nonlinear Mixed Effects Models. R Core Team.
Plastina, A., Liu, F., Miguez, F., Carlson, S., 2018. Cover crops use in Midwestern US agriculture: perceived benefits and net returns. Renewable Agriculture and Food Systems. https://doi.org/10.1017/S1742170518000194.
Pohlert, T., 2014. The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR).
Quade, D., 1979. Using weighted rankings in the analysis of complete blocks with additive block effects. Journal of the American Statistical Association 74, 680–683. https://doi.org/10.1080/01621459.1979.10481670.
R Core Team, 2018. R: A Language and Environment for Statistical Computing.
Rasse, D.P., Rumpel, C., Dignac, M.F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant and Soil 269, 341–356. https://doi.org/10.1007/s11104-004-0907-y.
Roesch-McNally, G.E., Basche, A.D., Arbuckle, J.G., Tyndall, J.C., Miguez, F.E., Bowman, T., Clay, R., 2017. The trouble with cover crops: farmers' experiences with overcoming barriers to adoption. Renewable Agriculture and Food Systems 33, 322–333. https://doi.org/10.1017/S1742170517000096.
Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56. https://doi.org/10.1038/nature10386.
Senesi, N., D'Orazio, V., Ricca, G., 2003. Humic acids in the first generation of EUROSOILS. Geoderma 116, 325–344. https://doi.org/10.1016/S0016-7061(03)00107-1.
Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555. https://doi.org/10.2136/sssaj2004.0347.
Spaccini, R., Piccolo, A., 2007. Molecular characterization of compost at increasing stages of maturity. 1. Chemical fractionation and infrared spectroscopy. Journal of Agricultural and Food Chemistry 55, 2293–2302. https://doi.org/10.1021/jf0625398.
Sradnick, A., Ingold, M., Marold, J., Murugan, R., Buerkert, A., Joergensen, R.G., 2013. Impact of activated charcoal and tannin amendments on microbial biomass and residues in an irrigated sandy soil under arid subtropical conditions. Biology and Fertility of Soils 50, 95–103. https://doi.org/10.1007/s00374-013-0837-z.
Stott, D.E., Moebius-Clune, B.N., 2017. Soil health: challenges and opportunities. In: Field, D.J., Morgan, C.L.S., McBratney, A.B. (Eds.), Global Soil Security, Progress in Soil Science. Springer International Publishing, Cham, pp. 109–121. https://doi.org/10.1007/978-3-319-43394-3_10.
USDA NASS, 2019. Crop Production 2018 Summary (No. ISSN: 1057-7823). United States Department of Agriculture.
Vidal, A., Hirte, J., Bender, S.F., Mayer, J., Gattinger, A., Höschen, C., Schädler, S., Iqbal, T.M., Mueller, C.W., 2018. Linking 3D soil structure and plant-microbe-soil carbon transfer in the rhizosphere. Frontiers in Environmental Science 6, 36. https://doi.org/10.3389/fenvs.2018.00009.
Vigil, M.F., Kissel, D.E., 1991. Equations for estimating the amount of nitrogen mineralized from crop residues. Soil Science Society of America Journal 55, 757–761. https://doi.org/10.2136/sssaj1991.03615995005500030020x.
Wagger, M.G., 1989. Time of desiccation effects on plant composition and subsequent nitrogen release from several winter annual cover crops. Agronomy Journal 81, 236–241. https://doi.org/10.2134/agronj1989.00021962008100020020x.
West, J.R., Ruark, M.D., Shelley, K.B., 2020. Sustainable intensification of corn silage cropping systems with winter rye. Agronomy for Sustainable Development 40. https://doi.org/10.1007/s13593-020-00615-6.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis.
Xiang, Y., Cheng, M., Huang, Y., An, S., Darboux, F., 2017. Changes in soil microbial community and its effect on carbon sequestration following afforestation on the loess plateau, China. International Journal of Environmental Research and Public Health 14, 948. https://doi.org/10.3390/ijerph14080948.
Zhalnina, K., Louie, K.B., Hao, Z., Mansoori, N., da Rocha, U.N., Shi, S., Cho, H., Karaoz, U., Loqué, D., Bowen, B.P., Firestone, M.K., Northen, T.R., Brodie, E.L., 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nature Microbiology 3, 470–480. https://doi.org/10.1038/s41564-018-0129-3.
Zhang, Xudong, Amelung, W., 1996. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biology and Biochemistry 28, 1201–1206. https://doi.org/10.1016/0038-0717(96)00117-4.
Zhang, Xuechen, Dippold, M.A., Kuzyakov, Y., Razavi, B.S., 2019. Spatial pattern of enzyme activities depends on root exudate composition. Soil Biology and Biochemistry 133, 83–93. https://doi.org/10.1016/j.soilbio.2019.02.010.