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ARTICLE

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Diversifying and perennializing plants in agroecosystems alters retention of new C and N from crop residues

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Abstract

Managing soils to retain new plant inputs is key to moving toward a sustainable and regenerative agriculture. Management practices, like diversifying and perennializing agroecosystems, may affect the decomposer organisms that regulate how new residue is converted to persistent soil organic matter. Here we tested whether 12 years of diversifying/perennializing plants in agroecosystems through extended rotations or grassland restoration would decrease losses of new plant residue inputs and, thus, increase retention of carbon (C) and nitrogen (N) in soil. We tracked dual-labeled (^{13}C and ^{15}N), isotopically enriched wheat (*Triticum aestivum*) residue in situ for 2 years as it decomposed in three agroecosystems: maize–soybean (CS) rotation, maize–soybean–wheat plus red clover and cereal rye cover crops (CSW2), and spring fallow management with regeneration of natural grassland species (seven to 10 species; SF). We measured losses of wheat residue (C_{wheat} and N_{wheat}) in leached soil solution and greenhouse gas fluxes, as well as how much was recovered in microbial biomass and bulk soil at 5-cm increments down to 20 cm. CSW2 and SF both had unique, significant effects on residue decomposition and retention dynamics that were clear only when using nuanced metrics that able to tease apart subtle differences. For example, SF retained a greater portion of C_{wheat} in 0–5 cm surface soils (155%, $p = 0.035$) and narrowed the C_{wheat} to N_{wheat} ratio ($p < 0.030$) compared to CS. CSW2 increased an index of carbon-retention efficiency, C_{wheat} retained in the mesocosm divided by total measured, from 0.18 to 0.27 (49%, $p = 0.001$), compared to CS. Overall, we found that diversifying and extending the duration of living plants in agroecosystems can lead to greater retention of new residue inputs in subtle ways that require further investigation to fully understand.

KEYWORDS
biodiversity, decomposition, dual-labeled litter, microbial biomass, regenerative agriculture, soil organic matter, stable isotope, sustainability

INTRODUCTION

Sustainable agroecosystem management minimizes the losses of plant inputs to soil for both environmental and economic reasons (Paustian et al., 2016). Converting natural ecosystems to agriculture has caused a nearly 50% loss of soil organic matter (SOM) in surface soils and increased CO_2 concentrations in the atmosphere (De et al., 2020; Guo & Gifford, 2002). Furthermore, nitrogen (N) losses from these same agroecosystems have contributed to both water quality impairment and climate change (Boesch et al., 2009; Rabalais et al., 2001). Since aboveground crop litter (or residue) is important to soil organic carbon (SOC) and N pools, managing agroecosystems to retain these elements is key to mitigating both climate change and water quality issues across the globe.

Soils harbor a diverse "dis-assembly line" of biota that decompose litter, recycling nutrients and setting a portion of plant-derived organic materials on a trajectory toward persistent SOM. Although belowground plant inputs have received much attention recently, in agroecosystems, aboveground crop residues serve as a major plant input to SOM (Turmel et al., 2015; Veen et al., 2019). The many management decisions producers make in agroecosystems have a crucial, yet unclear, role in regulating how crop residues become nutrients and persistent SOM (Angst et al., 2021). Across the residue-to-SOM continuum, past management practices (like crop choice) alter contemporary "upstream" processes early in decomposition that can affect how persistent SOM is formed "downstream" (Grandy & Neff, 2008). Over many growing seasons, this cumulative effect of crop management on soil biota and resources available to them is likely to alter the trajectory of new plant residue to SOM.

Prescott (2010) used a railroad analogy to describe the potential for management practices to serve as sidings, or side tracks, that slow residue decomposition compared to the primary pathway of rapid decomposition. Using management practices like N addition (through fertilizer or leguminous crop), minimizing disturbance to physically protect SOM, using perennial and deep-rooted crops, or encouraging enhanced soil biological activity through crop diversification, it may be possible to divert more residue C and N into more persistent SOM pools. In one example in Michigan, USA, Alfisol soils, two decades of no-tillage and reestablishment of grassland plant communities altered soil biota and their activities, and this affected the chemical composition of residue and its mass loss (Wickings et al., 2011, 2012). Another example, in a nearby Michigan experiment, showed organic management increased the retention of new plant inputs, simulated with chemical compounds (e.g., glucose, phenol, cellulose; Kallenbach et al., 2015). Finally, in California, USA study, 15 years of low-input and organic management increased retention of residue-derived C in Entisols and Alfisols, but especially C derived from roots, compared to conventional agroecosystems (Kong & Six, 2010).

Other studies, however, have reported no difference in the retention of newly added plant residues due to historical, long-term management practices (Jenkinson, 1965; Kong & Six, 2010). For example, in a long-term manure addition experiment at Rothamsted, UK (>100 years), adding manure resulted in substantial increases in soil fertility and 127% greater total organic C compared to no-manure control; but despite these differences, there was no difference in the retention of fresh plant residue (Jenkinson, 1965). These mixed findings indicate that agroecosystem management effects on residue-to-SOM dynamics likely depend on complex interactions involving past management, soil type, climate, and residue characteristics. However, the role of management and effects on decomposers typically plays a subordinate role to residue quality and climate (Bradford et al., 2016; Swift et al., 1979).

Diversifying and extending crop rotations has been widely shown to enhance soil biological activity and alter microbial community structure (Kim et al., 2020; McDaniel, Tiemann, & Grandy, 2014; Tiemann et al., 2015; Venter et al., 2016) and is a likely management candidate for driving greater residue retention. The effects of plant diversification on the soil microbial community are especially notable, with meta-analyses reporting average increases in microbial biomass C by 20%, richness by 15%, and diversity by 3% (McDaniel, Tiemann, & Grandy, 2014; Venter et al., 2016). Diverse crop rotations also tend to accumulate soil carbon C and N by 3%–6% over 5- to 10-year periods, the typical length of experiments in meta-analyses (McDaniel, Tiemann, & Grandy, 2014; West & Post, 2002). Furthermore, these soil benefits are concomitant with decreased losses of C and N from the field (Drinkwater et al., 1998) and increased crop yields (Zhao et al., 2020), especially during years with unfavorable weather (Bowles et al., 2020). Although the mechanisms through which this positive rotation effect occurs are not entirely clear, it appears that greater residue retention in mineral soils might be part of the rotation effect, driven by a legacy of quantity, quality, or diversity of prior plant inputs (Hooper et al., 2000; McDaniel, Tiemann, & Grandy, 2014).

These benefits from plant diversity and perenniality that increase SOM, soil biological activity, and even plant growth may be the result of positive feedback between soils and plants, a feedback that includes greater residue retention (Wardle et al., 2004). One method to test this is to keep the quantity and quality of residue constant but

change the history of plant or crop diversity. For example, McDaniel, Grandy et al. (2014) showed in a laboratory setting that after 12 years, crop rotations altered how new residues were decomposed. They also found that soils with a history of diverse and longer crop rotations underwent more rapid and complete decomposition, particularly of low-quality residues (i.e., C-to-N ratios >38). This begs the question of whether a history of greater agricultural plant diversity and perennialization could increase retention of new residue C and N in the field.

We designed a field experiment to test whether the history of crop (or plant) diversification and perennialization affected soil retention of new residue C and N. We incubated dual-labeled (^{13}C and ^{15}N), isotopically enriched wheat (*Triticum aestivum* L.) residue in situ for 2 years under three management practices with a prior 12 years of different crop or plant diversity. We hypothesized that more diverse and perennialized agroecosystems would retain more of the new wheat C and N and, accordingly, lose less to leaching or greenhouse gas fluxes over the 2 years. Diversified/perennialized agroecosystems would retain more residue C in microbial biomass and more residue N relative to C in this N-limited agroecosystem experiment (McDaniel & Grandy, 2016).

MATERIALS AND METHODS

Site and experiment description

This experiment was conducted at the W.K. Kellogg Biological Station Long-term Ecological Research (KBS-LTER) site near Hickory Corners, MI, in the United States. Mean annual temperature and precipitation at the site are 9.7°C and 902 mm, respectively. The experimental plots were located within the Biodiversity Gradient Experiment (BGE) (KBS, 2019). The BGE is a crop rotation experiment initiated in 2000. Cropping biodiversity and perenniality in the BGE is increased through the systematic addition of crops in rotations ranging from single-crop monocultures of maize, soybean, and wheat to a spring fallow with up to 10 naturally occurring grassland species. The experimental setup was a randomized block design consisting of 9.1 × 27.4-m plots, with each crop rotation replicated across four blocks. The two main soil series found at the site are Kalamazoo, a fine-loamy, mixed, mesic Typic Hapludalf, and Oshtemo, a coarse-loamy, mixed, mesic Typic Hapludalf (KBS, 2019). Both soils have highly variable chemical characteristics. Across the treatments, soil pH (1:1 volume DI H_2O) ranges from 5.2 to 6.9, soil C from 1.4 to 4.6 g C kg^{-1}, and bulk densities from 0.99–1.65 g cm^{-3} (Table 1). Further details on the soils or experimental design and agronomic management practices may be found in McDaniel and Grandy (2016) and Smith et al. (2008).

We focused on three treatments from this long-term experiment (Table 1). First, we chose the maize–soybean (*Zea maize-Glycine max*, CS) rotation because it is the most common rotation used in the Midwestern United States—as much as 94% of cropland in some Midwest US states like Iowa (USDA, 2020). Second, the maize–soy–wheat (*T. aestivum*) + two cover crops (red clover and cereal rye, *Trifolium pratense* and *Secale cereale*) rotation (CSW2) is the managed cropping system with the greatest crop diversity and the most aspirational cropping system in the experiment. The third treatment, spring fallow (SF), is a regeneration of natural grassland species after tillage. Total biodiversity in the SF plots can be up to 10 species of plants, with the three most abundant plants

TABLE 1 Treatment and soil characteristics for 0–10 cm depth (means ± SE*, $n = 4$).

Crop rotation or plant diversity treatment	Abbreviation	No. crop (or plant) species	pH†	Bulk density (g cm^{-3})	Carbon (g kg^{-1})	Nitrogen (g kg^{-1})	Carbon-to-nitrogen ratio	Microbial biomass carbon (mg C kg^{-1})‡
Maize–soy	CS	2	6.66 ± 0.12	1.38 ± 0.13	7.70 ± 2.11	0.73 ± 0.26	10.91 ± 1.97	257 ± 116b
Maize–soy–wheat + red clover and cereal rye cover crops	CSW2	5	6.37 ± 0.25	1.39 ± 0.13	9.63 ± 1.29	0.91 ± 0.04	10.58 ± 1.00	427 ± 122ab
Spring fallow (spring tilled and natural regeneration of seedbank)	SF	7–10	6.70 ± 0.28	1.28 ± 0.10	8.74 ± 2.41	0.77 ± 0.16	11.29 ± 0.80	682 ± 181a

*Significant difference among treatments indicated by lowercase letters ($p < 0.05$).
†pH in 1:1 DI H_2O (w:w).
‡Chloroform-fumigation extraction collected in autumn 2012.

being pigweed (*Chenopodium album*), foxtails (*Setaria pumila* and *Setaria faberi* Herrm.), and crabgrass (*Digitaria sanguinalis*) (Smith & Gross, 2006). All treatments are chisel-plowed in the spring to a depth of 15 cm. Unlike many commercial farms, however, no treatments receive external chemical amendments (i.e., fertilizer or pesticides).

Dual-labeled (^{13}C and ^{15}N) wheat residue and mesocosm setup

We used dual-labeled (^{13}C and ^{15}N) wheat straw to track the fate of the residue's C and N as it decomposed over 2 years. Wheat straw better reflects the chemical composition and complexity of plant inputs to SOM than isotopically enriched compounds like glucose. The wheat was grown in enriched $^{13}CO_2$ (10 atom percent, At%) in plexiglass growth chambers under conditions similar to those in Bird et al. (2003). The wheat was fertilized weekly with $K^{15}NO_3$ (30 At%). When mature, the wheat was harvested, and shoots (i.e., straw) and roots were separated, dried, and stored until use. We only used the shoots for this experiment. The shoots had an average total C content of 35.5% (6.44 At% ^{13}C) and total N content of 1.8% (10.9 At% ^{15}N). Before adding to the soil mesocosms the wheat straw was cut to ~2 mm fragments.

This experiment used in situ mesocosms to contain the wheat residue as it decomposed on the soil surface (Appendix S1: Figure S1). These mesocosms combine the benefits of natural variation in weather conditions in the field with the ability to monitor and contain the decomposition products, similar to a greenhouse or laboratory incubation. Each mesocosm consisted of a polyvinyl chloride (PVC) tube (30 cm long, 10 cm diameter) with 10 equally spaced, 2-mm-diameter holes drilled into the side for soil fauna movement. This tube was beveled at one end and inserted into the soil until 5 cm remained above the soil surface. The PVC tubes and soil therein were gently withdrawn from the surrounding soil in order to prevent any further disturbance. Once excavated, a 1-mm nylon mesh was placed over the soil at the bottom of the tube, and then a cap filled with combusted (500°C overnight) and DI-washed sand and fit over the mesh to provide contact with soil and prevent blockage of soil water by air pockets. At the bottom of each cap, a nylon Swagelok elbow fitting attached to clear tubing connected the base of each soil mesocosm to a 250-ml lysimeter bottle. This bottle was housed in a nearby hollow PVC tube and designed to collect all soil water leached through the soil mesocosm profile (Appendix S1: Figure S1). The soil mesocosms were then placed vertically back into the excavated pit, and soil was carefully placed back around them.

The soil mesocosms were installed on 4 August 2011 in the three agricultural management systems (Table 1). After installation, mesocosms were left for nearly 2 months to allow for recovery from the installation disturbance. Then, on 1 October 2011, we added 7.29 g of the dual-labeled wheat straw to duplicate mesocosms in each plot—two out of three mesocosms in each plot (one left as control with no residue). After addition of dual-labeled wheat, a 2-mm mesh was placed over the mesocosm to prevent residue movement. One mesocosm from each plot was retrieved at 1 year and a second retrieved 2 years after application of the wheat straw (along with the control mesocosms). At collection, the mesocosms were split in half vertically within 2 to 3 days of excavation. Then soils from within the open mesocosm were carefully extracted from 0–5, 5–10, 10–15, and 15–20 cm depths to prevent contamination between soil depths. At 1 year some visually observable wheat residue remained in the surface soils, but by 2 years most of the visually observed wheat residue was absent. Soils were placed at 4°C for 1 or 2 days while being sieved to <2 mm, and rocks were removed. The weights of each depth increment were used to calculate bulk densities. Fresh samples were used to measure gravimetric water content and soil microbial biomass. The remainder of the soil was air-dried for 1 month, and a subsample of this was dried to 105°C and then ground.

Soil, gas, and water sample collection and analyses

Emissions of $^{13}CO_2$ were measured 10 times throughout the 2-year experiment. $^{13}CO_2$ was measured using a non-steady-state, static chamber placed on top of the mesocosms. The chamber height was 15 cm with a volume of 1.3 L. A $^{13}CO_2$ measuring event began with the placement of a chamber on the soil mesocosm. A syringe was used to mix the chamber air, then a sample was extracted and transferred into a pre-evacuated 12-ml Exetainer vial (Labco, Ceredigion, UK). Four gas samples were taken at 15-min intervals. Gas samples were analyzed for CO_2 concentrations and δ ^{13}C-CO_2 on a ThermoScientific PreCon-GasBench system interfaced to a ThermoScientific Delta V Plus isotope ratio mass spectrometer (IRMS; ThermoScientific, Bremen, DE) located at the University of California, Davis Stable Isotope Facility. During one event in early summer of 2012, we measured $^{13}CO_2$ fluxes before and then 1 day after a manipulated 2.5-cm rainfall event to look at how wetting–drying affected wheat C dynamics (15 and 16 June 2012). This was intended to simulate a wetting event during a warm and dry period when we would expect a strong pulse of CO_2. We wanted to determine whether a strong drying–wetting event changed the source of $^{13}CO_2$ equally among treatments.

Lysimeter water samples were checked regularly during the growing season (approximately one per month) and only collected when bottles appeared to have water samples. At the end of the experiment, lysimeter water had been collected 12 times—4, 9, 23, 41, 54, 75, 236, 385, 399, 564, and 707 days after adding the wheat residue. Lysimeter samples were transported in coolers and kept frozen until analysis.

At each microcosm harvest (1 and 2 years after wheat addition), soil microbial biomass C was determined using a chloroform fumigation and extraction method (Vance et al., 1987), modified for direct extraction in individual test tubes (McDaniel, Grandy, et al., 2014). Briefly, two sets of fresh, sieved soil (5 g) were weighed in 50-ml test tubes, and 1 ml of chloroform was added to one set of tubes and capped. The tubes sat overnight (24 h) and were then uncapped and exposed to open air in a fume hood to allow chloroform to evaporate for 1 h. Soils were then extracted in the tubes with 25 ml of 0.5 M K_2SO_4. The chloroform fumigated and nonfumigated extracts were analyzed on a TOC-TN analyzer (TOC-V-CPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). We used 0.45 for the fumigation extraction efficiency (Joergensen, 1996). The nonchloroformed subsample was also used for salt-extractable organic C (SEOC), a pool of labile and moderately labile organic C.

All solid and liquid samples (microbial biomass carbon [MBC] extracts and lysimeter samples) were analyzed at the University of California, Davis Stable Isotope Facility. Bulk soil isotope signatures (^{13}C and ^{15}N) were measured on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 IRMS (Sercon Ltd., Cheshire, UK). A subsample of each soil was also analyzed for total organic C and N on a Costech ECS 4010 CHNSO analyzer at the University of New Hampshire for quality assurance. Dissolved organic C (DOC) was analyzed for ^{13}C using an OI Analytical Model 1030 TOC Analyzer (OI Analytical, College Station, TX) interfaced to the PDZ Europa 20-20 IRMS using a GD-100 Gas Trap Interface (Graden Instruments).

Ancillary soil properties measured to explain residue-to-soil-organic-matter dynamics

We analyzed surface soils outside the mesocosms to characterize their physical, chemical, and biological properties on 1 November 2012. These soils were composites of three 0- to 10-cm-deep cores collected with a 5-cm-diameter PVC tube. Soil was emptied into a plastic bag and placed in a cooler until arriving at the lab. An abbreviated list of these soil properties includes texture (including multiple sand fractions), bulk density, total organic C, total N, total phosphorus, SEOC (N), 0.5 M K_2SO_4-extractable ammonium, 0.5 M K_2SO_4-extractable nitrate, pH, particulate organic matter, basal respiration, potential mineralizable C (N), and several extracellular enzyme activities according to the microplate method. All biological measurements and nutrient extractions were carried out first on fresh soil. The remaining measurements were carried out on air- or oven-dried soil. The treatment effects on these properties and specific methods for these analyses can be found in McDaniel, Grandy et al. (2014) and McDaniel and Grandy (2016).

Statistics and data analyses

To determine the source of CO_2 carbon we used the Keeling plot method (Keeling, 1958). The $\delta\ ^{13}C$ signature of CO_2 was calculated using a linear regression of the $\delta^{13}C$ and inverse of CO_2 concentration with a minimum of three time points for each chamber. This was calculated for both the wheat-added and control chambers. The $\delta^{13}C_{treatment}$ is the source value of CO_2 from the $^{13}C_{wheat}$-added soil using a Keeling plot. $\delta^{13}C_{control}$ is the source value of CO_2-^{13}C from endogenous SOC (derived from Keeling plot of the control).

To calculate ^{13}C or ^{15}N in all measured soil pools—SOC, total N, microbial biomass, and extractable C from soil with salt (K_2SO_4)—we used $\delta^{13}C$ or $\delta^{15}N$ values from labeled and control soils. For example, the $\delta^{13}C_{MBC}$ values for both treatment and control soils were calculated based on mass balance as

$$\delta^{13}C_{MBC} = \frac{\delta^{13}C_F \times [C]_F - \delta^{13}C_{NF} \times [C]_{NF}}{[MBC]}, \quad (1)$$

where $\delta^{13}C_F$ and $\delta^{13}C_{NF}$ and $[C]_F$ and $[C]_{NF}$ are the delta values and concentrations for fumigated and nonfumigated samples, respectively, and [MBC] is the calculated concentration of microbial biomass.

All wheat C and N pools and fluxes (from Equation 1) were then used in a two-source mixing model, where treatment (wheat added) and control (no wheat added) were used to calculate f_{wheat}, where f_{wheat} is the fraction of ^{13}C or ^{15}N derived from wheat residue. Here we show just ^{13}C for an example, but this equation also applies to ^{15}N:

$$f_{wheat} = \frac{\delta^{13}C_{treatment} - \delta^{13}C_{control}}{\delta^{13}C_{wheat} - \delta^{13}C_{control}}, \quad (2)$$

where $\delta^{13}C_{treatment}$ is the delta value from the wheat-added sample of interest, $\delta^{13}C_{control}$ is the respective

sample from the mesocosm control, and $\delta^{13}C_{wheat}$ is the delta value of the wheat residue ($\delta^{13}C = 5126$ or $\delta^{15}N = 29{,}709$). Accordingly, f_{wheat} can be applied to a pool or flux to derive the proportion of C or N coming from the added wheat residue (e.g., C_{wheat} leached as DOC). Primed SOC was calculated as control subtracted from treatment CO_2 flux multiplied by $1 - f_{wheat}$.

We also calculated three measures of efficiency of the soil decomposer community to convert the wheat residue into SOM. First, a soil stratification index (SSI) (sensu Franzluebbers, 2002 and Jarecki et al., 2005) was calculated as the percentage of wheat C and N in the top 0–5 cm depth divided by that in the 5–20 cm depth. The SSI in this case is a measure of the efficiency of the top 5 cm of soil to retain wheat C and N and not lose it through leaching to the 5–20 cm depths. Second, we used the change in total mesocosm wheat residue-derived C and N (0–20 cm) between Years 1 and 2 as a measure of whether the soil was accumulating or losing residue-derived C and N within the 1- to 2-year timeframe. Third, as a measure of carbon-retention efficiency (CRE) at the soil profile level, we simply divided the remainder of the residue-derived C stored in each soil mesocosm at 2 years by the total amount lost as CO_2-C plus DOC and that of C remaining in soil. The CRE is similar to the C-use-efficiency metric that is typically used in more controlled incubation studies and reflects efficiency specific to microbial biomass (Geyer et al., 2019; Manzoni et al., 2012; Spohn et al., 2016). Here, however, the CRE reflects soil-profile-level processes and gives the proportion of C that persisted for 2 years (at 0–20 cm depth).

Because of infrequent measurements of CO_2 (due to logistics and the high cost of $\delta^{13}C$-CO_2 analyses), we also used a two-pronged modeling approach to derive the cumulative losses of CO_2-C_{wheat}. First, to interpolate daily CO_2 fluxes, we used known CO_2 flux measurements with soil temperature and moisture data from nearby sensors (<0.5 km) in a stepwise multiple linear regression (MLR) model. The MLR variables included 239 log-normal CO_2 flux ($lnCO_2$) with empirically linked measurements of the year of experiment (Y, values of 0 or 1), soil temperature (T, 5.3 to 31.2°C), and gravimetric soil moisture (θ, 0.024 to 0.42 g g^{-1}). Terms not significant at $\alpha < 0.05$ were dropped from the model. CO_2 fluxes can be modeled quite accurately from soil temperature and moisture alone (McDaniel, Kaye, et al., 2014; Sullivan et al., 2008; Tang et al., 2005). Second, these predicted CO_2 fluxes from the MLR were used with an interpolated f_{wheat} to gap fill and calculate cumulative losses of CO_2-C_{wheat}. We can also fit f_{wheat} to three-parameter exponential decay models ($f_{wheat} = y_0 + ae^{-kx}$) to measure wheat decomposition kinetics, including decay rate (k) and mean residence time (Adair et al., 2008). These modeled cumulative CO_2-C_{wheat} were also used to calculate CRE.

Data were checked for normality and heterogeneity of variances in R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria) using Q-Q plots (qqnorm), a Shapiro test (shapiro.test), and a Bartlett test (bartlett.test), and outliers were removed under a greater than 1.5× interquartile range and transformed if tests showed $p < 0.05$. Based on these tests, there were no outliers (some missing values), and all the data were normally distributed and did not need transformation. ANOVAs among plant diversity treatments were carried out with the R package aov, and means were compared using TukeyHSD. Due to the high variability of field studies with stable isotopes in general and our specific highly variable soils, we used an $\alpha = 0.05$ for significance and $\alpha = 0.1$ for marginal significance (Enjalbert et al., 2013; Freedman & Zak, 2015). Where means are reported, so are SE (after ±) for reference. Repeated-measures ANOVA was used for CO_2 flux data in SAS version 9.4 using the MIXED procedure, and lsmeans was used for the separation of means and treatment effects by dates. To determine the main drivers of wheat residue dynamics, Pearson correlation coefficients were calculated between decomposition/retention dynamics and 36 soil properties measured and published previously in McDaniel and Grandy (2016). We used SigmaPlot version 13 (Systat Software, Inc., San Jose, CA) for linear and nonlinear correlations among variables and visualization of data.

RESULTS

Weather patterns at the KBS-LTER from autumn 2011 to 2013 deviated substantially from 30-year historical records (Appendix S1: Figure S2). More specifically, 2012 was one of the driest years on record for the entire US Midwest (Hamilton et al., 2015), and precipitation at the KBS-LTER in 2012 was only 742 mm (mean annual precipitation [MAP] for 1988 to 2013 was 902 mm). By comparison, 2011 and 2013 were both rather wet and exceeded the long-term mean (MAP of 1125 and 1177 mm, respectively). These patterns were also reflected in gravimetric soil moisture measurements collected during our experiment in 2012 (McDaniel & Grandy, 2016). This contrasts with our mesocosm moisture measurements, where our first mesocosm collection (4 November 2012), gravimetric water contents were 0.152 to 0.173 g g^{-1}, whereas at the second collection (14 October 2013, during a wetter year) the range was 0.115 to 0.141 g g^{-1}.

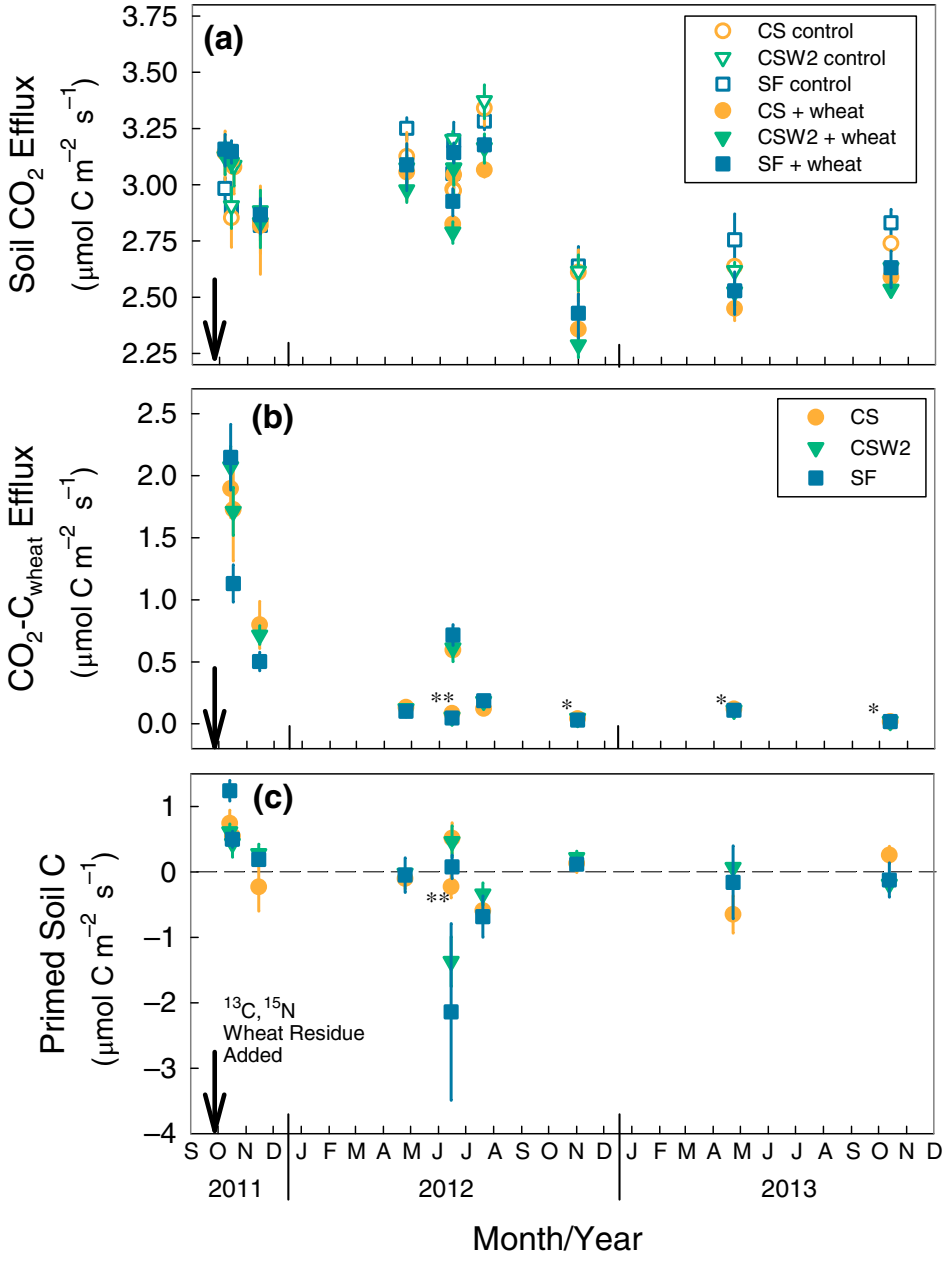

FIGURE 1 Soil-to-atmosphere carbon dioxide fluxes after dual (^{13}C, ^{15}N)-labeled wheat was added. (a) Total CO_2 flux measurements from both the control (no residue) and wheat residue added. (b) Residue-derived CO_2-C (C_{wheat}) flux emitted from mesocosms. (c) Native soil organic carbon lost, via priming, from residue addition. Means with SE shown ($n = 4$). Treatment abbreviations: CS = maize–soybean, CSW2 = maize-soybean-wheat + red clover and Rye cover crops, SF = spring fallow or tilled in spring and naturally regenerated seed bank (7–10 species). Asterisks indicate significant difference between treatments at <0.05 (*), <0.01 (**), and <0.001 (***).

C_{wheat} losses from soil

In the 2 months after wheat residue was added to the mesocosms, surface CO_2 fluxes were greater than the no-residue controls (Figure 1a) by 1.10 ± 0.21 μmol C m^{-2} s^{-1}. This trend was reversed by the 2012 growing season, where the controls tended to have greater CO_2 fluxes (~0.6 ± 0.08 μmol C m^{-2} s^{-1}). The wheat C lost as CO_2 (CO_2-C_{wheat}) decreased exponentially from October 2011 to October 2013. There was a marginally significant interactive effect of time and crop diversity on CO_2-C_{wheat} ($p = 0.085$), due mostly to the overwhelming effect of time. Analyzed by date, the SF lowered CO_2-C_{wheat} compared to CS in three out of 10 measurements (253, 329, 565 days).

Our MLR models, based on soil temperature and moisture (and their interactions), predicted CO_2 fluxes moderately well but underestimated observed fluxes by

TABLE 2 Wheat carbon budget and decomposition kinetics from each treatment (means ± SE*, $n = 4$).

Parameter	Percentage of total wheat C added		
	Maize–soy (CS)	Maize–soy–wheat + red clover and cereal rye cover crops (CSW2)	Spring fallow (SF)
Fate of wheat C after 2 years			
Cumulative CO_2-C_{wheat} loss	75.0 ± 14.8	51.8 ± 8.8	65.4 ± 15.5
Cumulative DOC_{wheat} loss	2.0 ± 0.5	2.1 ± 0.3	1.3 ± 0.1
Soil C_{wheat} retained	15.9 ± 1.4	20.5 ± 1.7	19 ± 2.2
Microbial biomass C_{wheat}	0.19 ± 0.018	0.228 ± 0.012	0.16 ± 0.018
Salt-extractable organic C_{wheat}	0.070 ± 0.002b	0.084 ± 0.006a	0.080 ± 0.006a
Total C_{wheat} accounted for	92.9 ± 16.1	74.4 ± 9.2	85.7 ± 17.1
Wheat C decomposition kinetics (three-parameter exponential decay, $f_{wheat} = y_0 + ae^{-kx}$)			
y_0 (f_{wheat}, %)	3.49 ± 0.79	5.15 ± 1.74	3.61 ± 0.26
a (f_{wheat}, %)	58.73 ± 2.19a	61.27 ± 2.55a	47.99 ± 2.22b
k (day^{-1})	0.57 ± 0.04	1.03 ± 0.39	0.66 ± 0.04
Mean residence time (days)	179 ± 15	132 ± 31	153 ± 9

Abbreviation: DOC, dissolved organic C.

*Significant difference among treatments indicated by lowercase letters ($p < 0.05$).

18% ($p < 0.0001$, $R^2 = 0.52$, Appendix S1: Table S1 and Figure S3a). After applying the model to CO_2-C_{wheat} data to gap-fill, the cumulative percentage of CO_2-C_{wheat} loss at the end of 2 years was 75% ± 15%, 52% ± 9%, and 65% ± 16% for CS, CSW2, and SF treatments, respectively (Table 2, $p = 0.334$; Appendix S1: Figure S3b–d). The f_{wheat} from each plot showed a significant fit with a three-parameter exponential decay model—R^2 from 0.90 to 0.97, and p values from 0.067 to 0.009 (Table 2)—not surprising since f_{wheat} is similar to the fraction of mass loss in traditional litter-bag studies. The CSW2 treatment had twice the decomposition rate compared to CS, but only the initial f_{wheat} (α) was significantly lower in SF compared to the two cropped treatments ($p = 0.006$). This was also illustrated by the lower measured CO_2-C_{wheat} values ~1 year after wheat was added (Figure 1b).

The amount of endogenous SOC lost due to the addition of wheat residue (e.g., priming effect) was strongly positive during the first 2–3 months of the study based on $^{13}CO_2$ fluxes (Figure 1c). Within the first 2 weeks, primed SOC loss averaged 0.68 ± 0.06 μmol C m^{-2} s^{-1} (ranging from 0.2 to 1.5 μmol C m^{-2} s^{-1}). However, by the second year, nearly all soils were either near zero or negative native soil C loss, suggesting that the wheat residue addition primed native SOC in the first few weeks but then subsided. We found significant effects of crop diversity/perenniality on primed SOC at only two dates: 15 June 2012 (Appendix S1: Figure S4c) and 13 October 2013, where added residue depressed endogenous CO_2 losses in the CSW2 relative to the CS treatment ($p = 0.033$), −1.37 ± 0.22 versus −0.23 ± 0.16 μmol C m^{-2} s^{-1}, respectively. The SF endogenous CO_2 flux was −2.14 ± 1.35 μmol C m^{-2} s^{-1} but not different due to high variability.

Very little C_{wheat} leached below a depth of 20 cm as dissolved organic carbon (DOC_{wheat}) (<2%, Appendix S1: Figure S5, Table 2). Most of the cumulative leached DOC_{wheat} was lost in the first couple of months after the addition of wheat residue at the end of 2011; from 37% to 76% of total DOC_{wheat} loss occurred during these first few months. The SF had 39% less DOC_{wheat} leach from the soil profile than CS, although the difference was not significant.

C_{wheat} and N_{wheat} retained in soil

After 2 years and across all treatments, most of the wheat C and N retained in the soil was in the top 0–5 cm (Figure 2a,b). There were no significant differences in the retention of either element among treatments at depths. Summing the 0–20 cm depth, all soils retained more N_{wheat} (29.6%–50.5%) than C_{wheat} (12.4%–25.0%), reflecting the greater mobility and demand for N in these soils. The total percentage of C_{wheat} retained in each treatment was 15.9% ± 1.4%, 20.5% ± 1.7%, and 19.2% ± 2.2% for CS, CSW2, and SF, respectively ($p = 0.233$). The total percentage of N_{wheat} remaining after 2 years was 38.7% ± 3.4%, 44.0% ± 2.0%, and 41.7% ± 4.0% for the CS, CSW2, and SF treatments, respectively

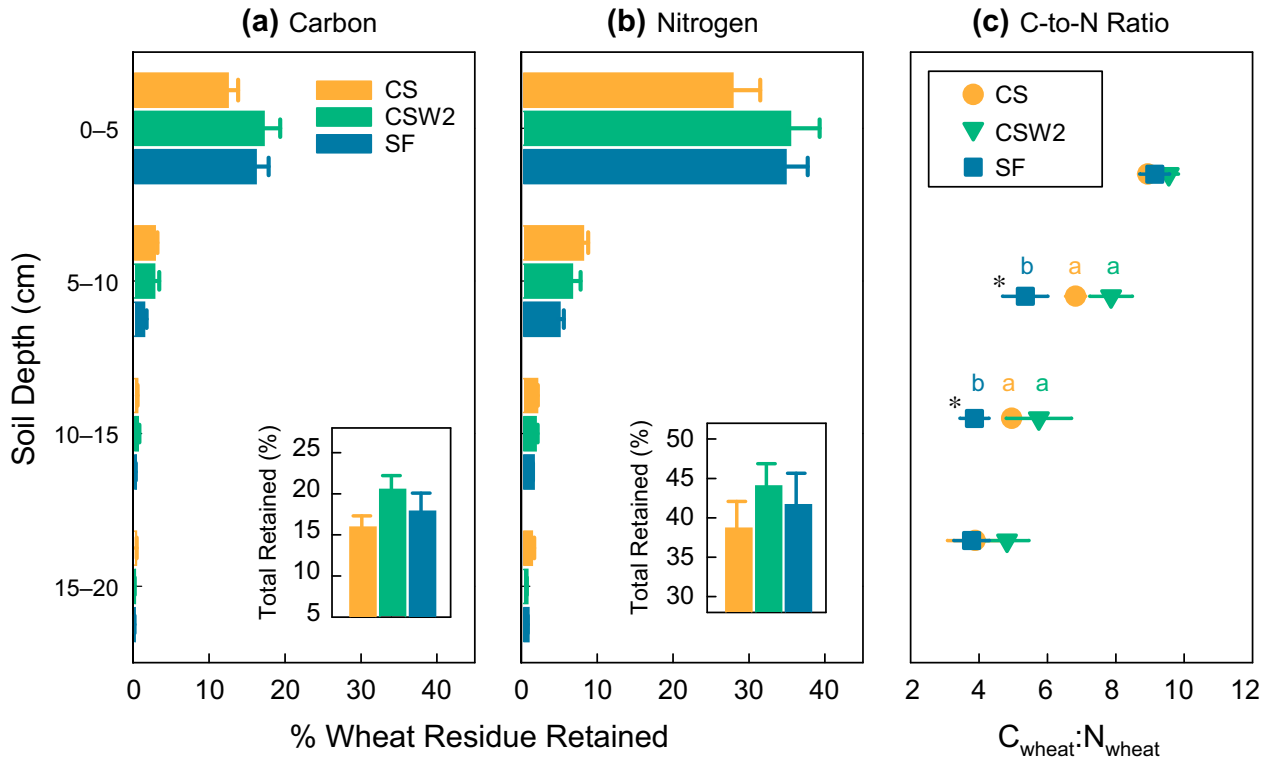

FIGURE 2 Percentage of wheat-derived (a) carbon and (b) nitrogen, and (c) carbon-to-nitrogen ratio of retained residue after 2 years of decomposition. Means with SE shown ($n = 4$). Insets show total residue C and N over entire mesocosm. See Figure 1 caption for treatment abbreviations and asterisk significance.

($p = 0.561$). There were no significant differences in soil wheat retention among cropping systems, largely due to high variability across depths and among blocks (the coefficient of variation ranged from 12.3% to 86.3%).

The C-to-N ratio (C:N) of wheat residue retained ($C:N_{wheat}$) at various depths provides a measure of microbial supply and demand of both elements. At 0–5 cm the $C:N_{wheat}$ across all treatments was 8.3–10.2, but at 15–20 cm the range of C:N was 2.6–6.7 (Figure 2c). SF had a significantly lower $C:N_{wheat}$ at 5–10 and 10–15 cm depths than the other two treatments ($p < 0.03$, Figure 2c), indicating greater demand for N_{wheat} compared to C_{wheat}.

Soil MBC and SEOC varied from Year 1 to 2, as did the percentage of wheat C retained therein (Figure 3). Microbial biomass C decreased with depth in both years with ranges from 91 to 387 and 39–150 mg C kg^{-1} across all depths. SEOC also varied from 158 to 589 and 107 to 302 mg C kg^{-1} across all depths but increased with depth (Figure 3a,c). There were no significant differences in MBC among treatments at all depths but a few significant differences in percentage C_{wheat} in microbial biomass (Figure 3b,d). There were significant SEOC differences among treatments—with SF having greater SEOC at lower depths than the other two treatments (and % C_{wheat} therein). Only a few depths showed significant treatment effects, but with no consistent trend with treatments. Summed across the 0- to 20-cm soil profile, and though a small portion of total C_{wheat} was added (<0.1%), the CSW2 and SF treatments had 25% more $SEOC_{wheat}$ than CS (Figure 3d, Table 2).

Dynamics and efficiency of accumulating new C_{wheat} and N_{wheat}

We used three methods to quantify the dynamics of C_{wheat} and N_{wheat} accumulation in soil over the 2-year study (Figure 4). First, we used a SSI to reflect the stratification or distribution of C_{wheat} and N_{wheat} with soil depth. The greater amount of residue retained in 0–5 cm (relative to 5–20 cm) reflects the internal efficiency of soil to retain C_{wheat} and N_{wheat} and reduce losses to lower soil depths (Figure 4a,b). SF increased the SSI of C_{wheat} by 155% ($p = 0.035$) and N_{wheat} by 87% compared to CS ($p = 0.195$), and though the CSW2 was greater on average than CS for both C and N, they were nonsignificant ($p < 0.404$). Second, we evaluated the percentage change in wheat residue C and N between Years 1 and 2 and found no significant differences due to the large variation (Figure 4c,d). Finally, we used C_{wheat} loss (as CO_2 and

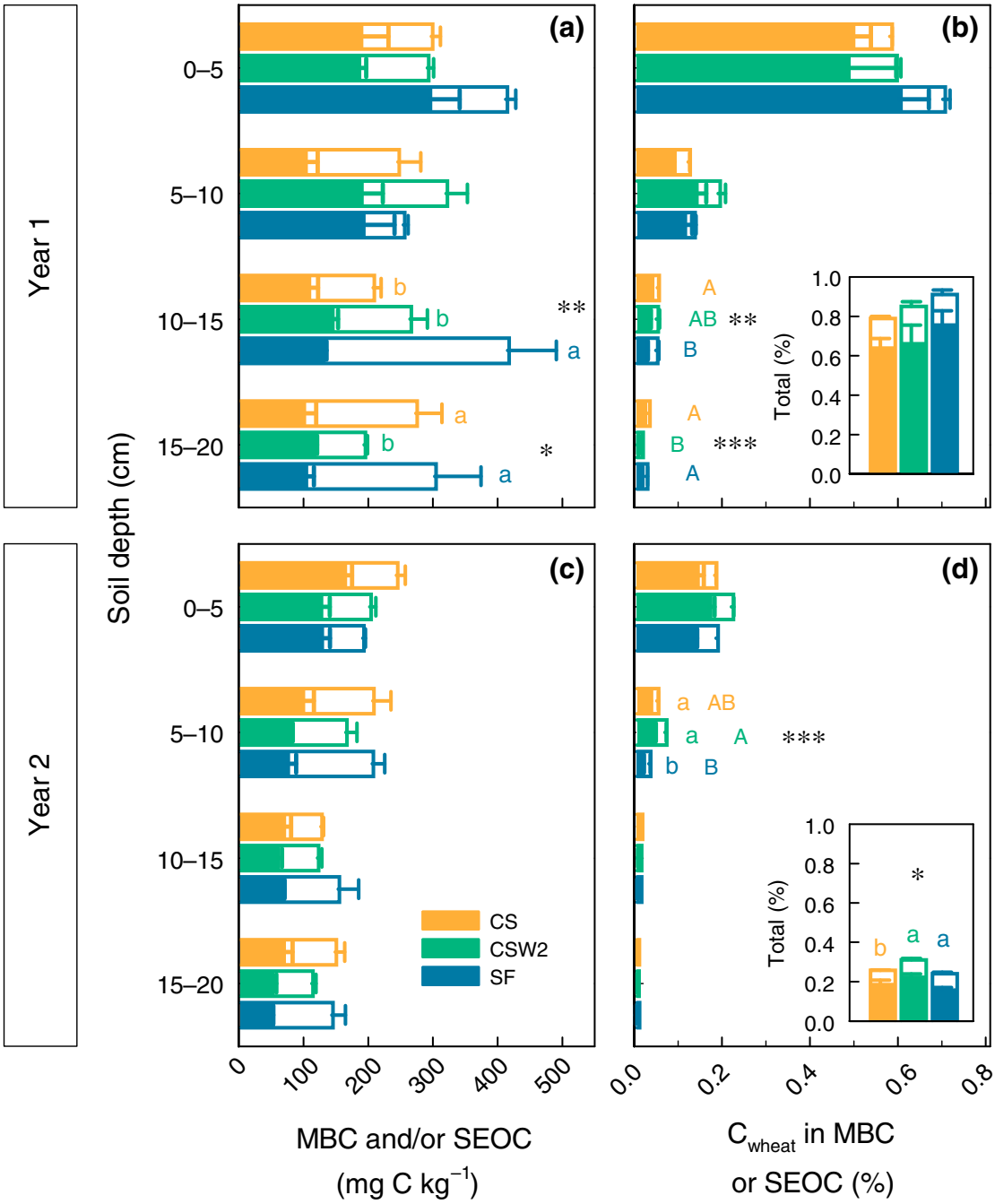

FIGURE 3 Microbial biomass carbon (MBC, filled bars) and salt-extractable organic C (SEOC, open bars) after (a) Year 1 and (c) Year 2. The percentage of wheat residue carbon in MBC and SEOC after (b) Year 1 and (d) Year 2. Insets show total residue retained in MBC and SEOC over the entire depth of the mesocosms, in Years 1 and 2, respectively. Means with SE shown ($n = 4$). Uppercase letters indicate significance with MBC, lowercase letters indicate significant difference with SEOC. See Figure 1 caption for treatment abbreviations and asterisk significance.

DOC) compared to that remaining in the soil profile to calculate a CRE at 0–20 cm (Figure 4e). The mean CRE ranged from 0.18 to 0.27 and was 49.1% greater in the CSW2 soil than in the CS rotation ($p = 0.001$), but not significantly different from the SF treatment.

We used univariate, linear correlations to examine the relationships between wheat residue decomposition/retention to various soil properties (Figure 5, methods and findings previously outlined in McDaniel & Grandy, 2016). Decomposition kinetics were not related to physical properties but were negatively correlated with soil pH and microbial biomass. Stratification of C_{wheat} and N_{wheat} was more related to physical characteristics, especially soil texture (Figure 5), but N stratification

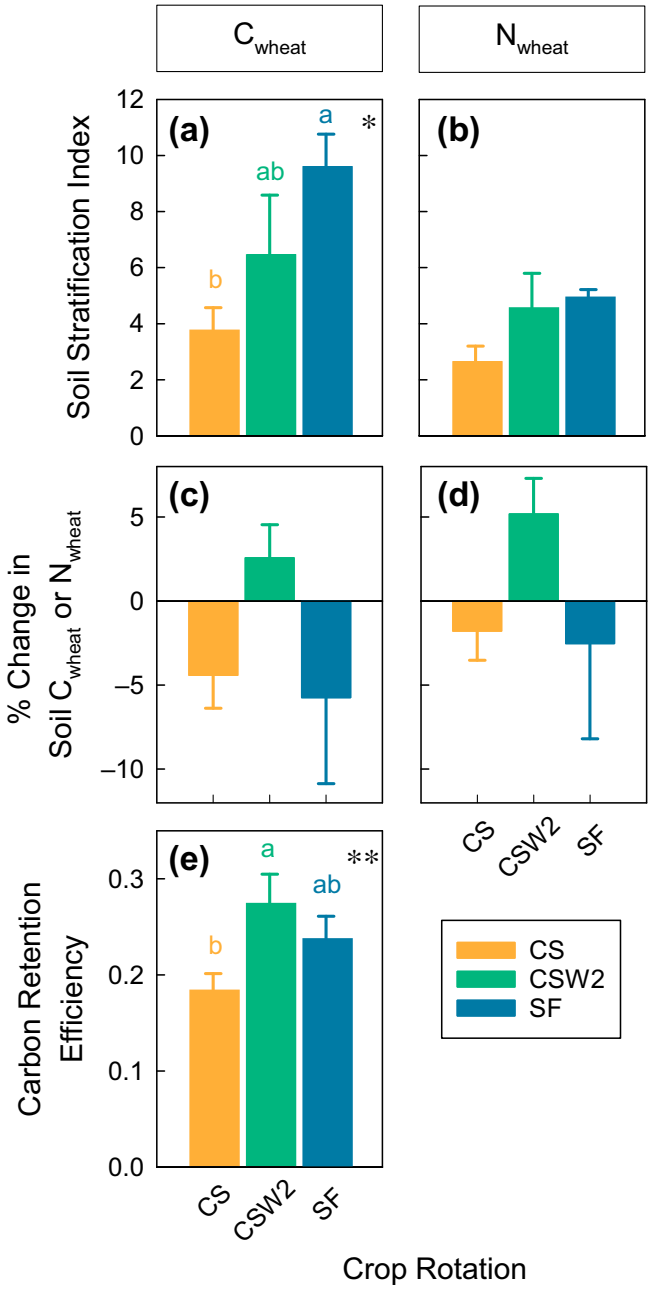

FIGURE 4 (a, b) Soil stratification index (SSI) of wheat residue C and N. The SSI is calculated as the percentage of residue retained in the top 5 cm of the mesocosms divided by that in the bottom 5–20 cm. See Franzluebbers (2002) and Jarecki et al. (2005) for more information on this index. (c, d) Rate of residue carbon and nitrogen accumulation or change between Years 1 and 2. This is calculated by subtracting the total residue C and N retained in each mesocosm in Year 1 from Year 2. (e) Carbon retention efficiency calculated at Year 2—derived from C_{wheat} retained in soil divided by how much was lost as DOC_{wheat} and CO_2-C_{wheat}. Means with SE shown ($n = 4$). See Figure 1 caption for treatment abbreviations and asterisk significance.

was negatively related to labile C as measured by permanganate oxidatizable C and basal respiration. Wheat residue CRE, at the mesocosm scale, was strongly negatively correlated with water content, pH, basal respiration, and peroxidase (a lignin-modifying extracellular enzyme); CRE was positively correlated with SEOC and leucine aminopeptidase (a N-acquiring extracellular enzyme). These correlations with $n = 12$ must be taken with caution, but they do provide fodder for mechanisms and hypothesis testing.

DISCUSSION

Diversifying agroecosystems with plants can take place both in space or through time, that is, with early successional fallow or diversified rotations (e.g., CSW2), respectively. Both treatments also extended the length of time living plant roots covered the soil (or increased perenniality). In our study, both modes of enhancing plant diversity and perenniality subtly altered how soil biota decomposed and stabilized new residues, and these management practices altered the decomposition dynamics in different ways. In support of our primary hypothesis, greater plant diversity and perenniality (whether in crop rotations or early successional grassland species) not only subtly increased the retention of C_{wheat} and N_{wheat} but also decreased losses compared to the business-as-usual, CS rotation. We use the word subtly to describe the effects of 12 years of diversification because neither treatment statistically increased the net retention of either C_{wheat} and N_{wheat} in the soil, at a significance level of $p < 0.1$ (Figure 2a,b). However, we did find that two nuanced indices of retention highlighted the observable effects of plant diversification at the profile scale: SF increased the proportion of C_{wheat} stabilized in 0–5 cm soil (+155%, Figure 4a), and CSW2 increased the CRE from 0.18 to 0.27 (+49%, Figure 4e) compared to CS. These important effects of diversification and perennialization were facilitated through a positive interaction between the soil decomposer community (and the resources available to them) and the retention of new residue C and N.

Effects of diversifying and perennializing agroecosystems on C_{wheat} and N_{wheat} losses

In our study, soils from more diverse agroecosystems generally lost less C_{wheat} as CO_2. These declines were −31% and −13% for CSW2 and SW compared to CS rotations, but not statistically different (Table 2, $p > 0.334$). Under SF, the reduced loss of C_{wheat} primarily occurred early in decomposition relative to the CS rotation (Figure 1b and Table 2). The C_{wheat} losses from the diversified/perennialized crop rotation (CSW2), however, were lower

Soil Parameter	Wheat decomposition kinetics (f_{wheat}) y_0	a	k	Wheat C and N retention dynamics SSI_C	SSI_N	CRE
Physical Soil Characteristics						
Bulk density	0.668	0.309	0.618	0.725	0.544	0.168
Gravimetric water content	**0.085**	0.301	0.449	0.327	**0.069**	**0.028**
Clay	0.692	0.603	0.25	0.394	0.136	0.757
Silt	0.78	0.652	0.288	0.312	**0.089**	0.492
Sand	0.857	0.701	0.336	0.268	**0.069**	0.331
53–125 µm	0.554	0.867	0.753	0.101	**0.022**	0.103
125–250 µm	0.83	0.693	0.364	0.445	0.19	0.356
250–500 µm	0.799	0.629	0.641	0.198	**0.040**	**0.094**
500–1000 µm	0.485	0.76	0.15	0.422	0.154	0.968
1000–2000 µm	0.257	0.541	**0.069**	0.54	0.220	0.978
Gravel (> 2000 µm)	0.842	0.266	0.595	**0.021**	**0.072**	0.118
Chemical Soil Characteristics						
Total organic carbon (C)	0.58	0.690	0.132	0.762	0.484	0.771
Total nitrogen (N)	0.685	0.724	0.204	0.569	0.361	0.532
Total phosphorus	0.888	0.544	0.568	0.749	0.759	0.86
C-to-N ratio	0.793	0.283	0.951	0.556	0.61	0.773
Salt-extractable organic C	0.509	0.363	0.335	0.637	0.366	**0.031**
Salt-extractable organic N	0.255	0.196	**0.073**	0.478	0.562	0.599
Permanganate-oxidizable C	0.801	0.607	0.342	0.113	**0.041**	0.198
Ammonium-N	0.831	0.297	0.282	0.146	0.176	0.126
Nitrate-N	0.571	0.879	0.809	0.121	0.162	0.695
pH in H_2O (1:1 w:w)	**0.065**	**0.019**	**0.062**	0.792	0.636	**0.061**
pH in 0.01M $CaCl_2$ (1:1 w:w)	**0.020**	**0.018**	**0.018**	1.000	0.473	**0.019**
Particulate organic matter	**0.077**	**0.034**	0.456	0.405	0.808	0.728
Biological Soil Characteristics						
Basal respiration	0.319	0.96	0.705	0.148	**0.043**	**0.050**
Potential mineralizable C	0.671	0.647	0.777	0.668	0.998	0.320
Potential mineralizable N	0.429	**0.067**	0.333	0.314	0.717	0.320
Microbial biomass C	0.625	**0.007**	0.774	**0.089**	0.344	0.705
Microbial biomass N	0.445	**0.004**	0.658	**0.067**	0.297	0.588
β-Glucosidase	0.992	0.456	0.225	0.781	0.382	0.952
Cellobiohydrolase	0.729	0.408	0.420	0.727	0.71	0.955
Leucine aminopeptidase	0.306	0.455	0.207	0.415	0.411	**0.038**
β-N-acetylglucosaminidase	0.835	0.156	0.549	0.713	0.946	0.961
Acid Phosphatase	0.855	0.324	0.736	0.788	0.472	0.159
Tyrosine aminopeptidase	0.992	0.950	0.310	0.335	0.409	**0.081**
Polyphenol Oxidase	0.921	0.970	0.522	0.979	0.574	0.611
Peroxidase	0.441	0.227	0.861	0.128	0.126	**0.015**

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FIGURE 5 *p*-values based on Pearson correlation coefficient (*r*) between wheat decomposition/retention dynamics and ancillary soil parameters ($n = 12$), measured on 1 November 2012 (1 year after wheat addition and ancillary soil data published in McDaniel & Grandy, 2016). Decomposition dynamics are from three-parameter exponential decay constants from f_{wheat} (Table 2). Wheat residue retention dynamics: soil stratification index for carbon (SSI_C) and nitrogen (SSI_N), and carbon retention efficiency (CRE). Bold values are significant at $p < 0.1$.

than CS and mostly in the second year. These contrasting temporal dynamics of C_{wheat} losses indicate differences in spatial versus temporal diversification and perennialization of agroecosystems and their legacy effects on soil biology and resources. A study by Wickings et al. (2011, 2012) showed decomposing two different residues in litterbags (maize and grass—*Bromus inermis*) in widely varying agroecosystems (conventional, no-till, and restored grassland) altered not only decomposition rates but also changed the chemical composition of residues at

very late stages of decomposition. They showed that history of no-tillage and restored grasslands reduced residue mass loss at 108 days by 31%–49% and 217%–252% in maize and grass residues, respectively. Our findings support these previous results and show that less intensive agriculture practices, whether from reduced tillage or crop rotations, can lead to reduced residue C losses.

Priming, or the enhanced mineralization of endogenous C after exogenous C inputs, is important to the global C cycle, yet the magnitude and mechanisms driving it remain uncertain and remain a challenge to measure, especially in situ and in agroecosystems (Bastida et al., 2019; Guenet et al., 2018; Sun et al., 2019). We found evidence of a moderate positive priming response initially after adding the wheat and then measured a shift to neutral or "negative" priming (Figure 1c). This aligns with most priming studies that show positive priming occurring soon after the addition of new C inputs (~20 days), followed by negative, near-zero, or no priming later in incubations (Luo et al., 2016).

There was little difference in priming among management practices over our 2-year study. The exception occurred in June 2012 when soils were extremely dry and soils in the two most diverse ecosystems had on average 262%–509% greater negative priming, or in other words, the more diverse/perennial cropping system (CSW2) had slower endogenous SOC decomposition compared to the CS rotation (Appendix S1: Figure S4c). This short-lived but significant event may point to how and when diversified and perennialized cropping systems tend to accumulate SOC relative to conventional systems during drought or stress. Losses of C_{wheat} through leached DOC were minimal (Table 2; Appendix S1: Figure S5). A very small fraction of mineralized C_{wheat} was found to be soluble (2%); most was recovered either in soil microbial biomass or in nonliving but mineral-adsorbed C (Gaillard et al., 1999; Figure 3; Appendix S1: Figure S5).

While we did not quantify losses of N_{wheat}, there may have been gaseous losses of N_{wheat} through volatilization, nitrification, and denitrification. Other studies using ^{15}N-labeled residue showed these gaseous losses to be negligible (<1%, Eickenscheidt & Brumme, 2013). Rather, most N_{wheat} losses are likely leached products of mineralization that were not retained in microbial biomass or adsorbed to soil particles (Gaillard et al., 1999), and these likely include small organic ^{15}N-containing molecules, ammonium-^{15}N, or, most likely, nitrate-^{15}N due to its greater mobility in soils. In support of our third hypothesis, all three systems retained more N_{wheat} than C_{wheat} (Figure 2).

Subtle effects of diversifying/perennializing agroecosystems on C_{wheat} and N_{wheat} retention

Generally, diversifying and perennializing cropping systems resulted in greater profile-level retention of new C_{wheat} and N_{wheat}, depending on diversification/perennialization with the rotation of more crops or restored grassland species (Figure 2 insets). High spatial variability, however, obscured statistically significant treatment effects on net C_{wheat} and N_{wheat} retention ($p > 0.561$). A study from a systematically diversified grassland experiment showed that an increase in plant species richness resulted in nearly two- to four-fold increase in SOC between monoculture and >16 species, attributed to increased root carbon inputs and elevated microbial activity (Lange et al., 2015). In a more agriculturally relevant context, Kong and Six (2010) showed that low-input and organic cropping in tomato rotations significantly increased the retention of hairy vetch root C by 85% and 161%, respectively. In their study, similarly to ours, retention of aboveground residue retention showed no difference among management practices.

In our study, management effects on wheat residue dynamics were subtle and only observed via nuanced indices of retention (e.g., $C:N_{wheat}$, stratification of residue, and when measuring C_{wheat} retention efficiency [CRE]). First, SF decreased $C:N_{wheat}$, whereas CSW2 generally increased it, compared to soils under 12 years of CS rotations (Figure 2). This was most likely driven by management effects on labile resources among all three agroecosystems. Indeed, labile sources of C and nutrients differ among these systems (Table 1, McDaniel & Grandy, 2016), and this could likely drive the differences between C_{wheat} versus N_{wheat} retention (Figure 3, discussed more in next section). Second, this divergent effect on $C:N_{wheat}$ and other findings may be due to documented differences in soil microbial communities among treatments (Peralta et al., 2018; Tiemann et al., 2015). Soil microorganisms can differ in their C and N use efficiencies (Saifuddin et al., 2019), and the retention of new C_{wheat} and N_{wheat} may be a reflection of differences in microbial community composition and/or resource availability.

We used a CRE metric similar to carbon-use efficiency (CUE), which in our study was simply the C_{wheat} retained in the mesocosm divided by the total measured. Past studies showed that management practices that minimize disturbance and maximize organic nutrients tend to increase CUE (Kallenbach et al., 2015; Sauvadet et al., 2018; Xiao et al., 2021), but not always (Jenkinson, 1965; Miao et al., 2021). Our study was the first, to our knowledge, to

observe a 49% increase in CRE from diversifying/perennializing a maize–soybean rotation by adding small grain (winter wheat) and a mixed cover crop of red clover and cereal rye. The mechanisms that might drive this observation are discussed more in the next section.

The surface soil (0–5 cm) is the "first line of defense" against C and N losses via leaching with soil water. Labeled litter studies across ecosystem types have found that C and N from surface residues accumulate disproportionately in surface soils (Eickenscheidt & Brumme, 2013; Fröberg et al., 2009; Kammer & Hagedorn, 2011), and this zone of rapid physicochemical retention and biological activity has been called the "detritusphere" (Gaillard et al., 1999). In previous studies in agroecosystems but without labeled residue, the importance of this zone has been quantified using a stratification index (Franzluebbers, 2002; King & Hofmockel, 2017; Lazicki et al., 2016). In our study, the SSI reflects retention dynamics within the top 0–20 cm of soil but also a tendency for losses of C_{wheat} and N_{wheat} as a whole. Diversifying/perennializing agroecosystems increased C_{wheat} and N_{wheat} stratification by 72%–155% and 72%–87%, respectively, but only SF was significantly greater than traditional maize–soybean rotation (Figure 4a). Our finding of an increase in the stratification of new residue C_{wheat} and N_{wheat} aligns with previous studies that showed that practices like reduced tillage (Franzluebbers, 2002) or diversification through crop rotation and manure (King & Hofmockel, 2017; Lazicki et al., 2016) increased the stratification of other soil properties like total organic C, particulate organic matter, and biological activity. Although soil science research has embraced deeper soils and rhizosphere dynamics for many good reasons (Jilling et al., 2018; Tautges et al., 2019; Wallenstein, 2017), studies measuring soil vertical stratification (including ours) highlight the importance of the detritusphere and surface soils in sustainable agroecosystem management.

Underlying mechanisms for improved residue retention with increased agroecosystem diversity and perenniality

We determined the proportion of C_{wheat} that ended up in microbial biomass and nonliving, salt-extractable (i.e., low-molecular-weight) C compounds (<1% after 1 year; Figure 3). Microbial biomass can be a more rapid and efficient pathway for creating persistent SOM through the retention of necromass and microbial byproducts, as well as through abiotic processes (Cotrufo et al., 2013; Grandy & Neff, 2008; Kallenbach et al., 2015, 2016, 2019). This pathway could underly the enhanced C_{wheat} and N_{wheat} retention dynamics we observed under more diversified cropping systems. In a meta-analysis of 18 laboratory studies, the initial microbial biomass was positively related to CUE and one of the most important factors explaining CUE (Geyer et al., 2020). Additionally, previous reports on this experiment showed persistent increases in soil microbial biomass from the more diversified crop rotation, CSW2, compared to CS (McDaniel & Grandy, 2016), although, contrary to our second hypothesis in this study, we observed no consistent trends in agroecosystem diversity effects on MBC_{wheat}. However, we did measure slightly more C_{wheat} in this labile pool under both diversified agroecosystems (Figure 3b,d), and microbial biomass was negatively related to the initial fraction of C being released as CO_2 (Figure 5).

It is possible that there were stronger effects on MBC_{wheat} earlier than when we first sampled (<1 year after adding wheat residue), how here we focused on longer-term residue-to-SOM dynamics. The most parsimonious mechanisms for greater C_{wheat} and N_{wheat} retention in diversified/perennialized agroecosystems is the greater soil microbial activity and/or biomass. However, it is not possible to tease apart the effects of perenniality from crop diversity in this study because they are both increasing under the two management practices (SF and CSW2 compared to CS). Greater quality of inputs (via leguminous crops), extending the duration with living roots, and perhaps even greater diversity of crop inputs (e.g., residues and rhizodeposits) all likely contributed to increased soil microbial activity and biomass in these soils (McDaniel & Grandy, 2016; McDaniel, Tiemann, & Grandy, 2014; Tiemann et al., 2015).

From microbial to ecosystem scales, the efficiency with which new C inputs are transformed into persistent C (rather than lost as CO_2-C) has been shown to be regulated by the availability of other nutrients, especially N (Fernández-Martínez et al., 2014; Manzoni et al., 2012; Sinsabaugh et al., 2013). In our experiment, enhanced biologically available N may be positively linked to CRE. Although soybeans do fix atmospheric N_2, there are additional, and perhaps more efficient, N-fixing, leguminous species (*T. pratense* and *Trifolium incarnatum*) in the more diverse agroecosystem (CSW2). These alternative legumes are also nonharvested crops with their residue returned to the soil, also known as green manure, so their fixed N was added throughout the 12-year experiment. Spohn et al. (2016) showed that 44 years of adding 120 kg N ha^{-1} $year^{-1}$ in fertilizer (regardless of P or K inputs) increased microbial C use efficiency from 0.32 to 0.43 (+37%) in silty loam soils from Austria. Overall, we found strong evidence for management altering the efficiency of residue-to-SOM conversion either directly through plant diversity/perenniality effects on soil

microbial biomass or activity or indirectly through soil resources available for cometabolism of new residues.

CONCLUSION

This study provides the first direct mechanistic evidence of plant diversity and perenniality increasing the retention of residues in soil and may be responsible for the oft-observed trend of increasing SOM under these practices (King & Blesh, 2018; McClelland et al., 2021; McDaniel, Tiemann, & Grandy, 2014; West & Post, 2002). Although we showed that standard measurements of C_{wheat} and N_{wheat} retained in soils were generally greater in diversified agroecosystems, and CO_2-C_{wheat} losses were lower, these patterns were statistically insignificant with standard statistical benchmarks (Figure 2a,b). Only by using more subtle indices of how the C and N were distributed in the profile or normalized for total CO_2-C_{wheat} lost were we able to resolve treatment differences (Figures 2c and 4). Based on these more nuanced metrics, it is clear that when residue quantity and quality are kept constant, the history of crop/plant diversity alters the decomposition and retention dynamics of new residue C and N. This highlights the importance of increasing crop or plant diversity/perenniality to increase the retention of new residue C and N inputs.

Beyond crop/plant diversity and perenniality, other agroecosystem management factors may also increase the efficiency of crop residue retention. We encourage further exploration of the management effects on residue-to-SOM trajectory, and future studies should delve more deeply into mechanistic drivers and interactions with roots, especially the microbial dynamics involved. Furthermore, we showed an interesting interaction between management and drought, whereby more diverse crops/plants lessen the pulse of primed mineral SOC losses during rainfall preceding a long drought (Figure 1c, Appendix S1: Figure S4), suggesting the importance of diversifying/perennializing agroecosystems to enhance soil C and N retention in more extreme climates. It is critical to further understand how residue is converted to persistent SOM in order to optimize agroecosystem management practices so that they are regenerative and sustainable; this is even more important considering the current context of farming under a rapidly changing climate.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (McDaniel et al., 2022) are archived by the Iowa State University at https://doi.org/10.25380/iastate.21259206.v1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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ECOLOGICAL APPLICATIONS

Appendix S1: SUPPLEMENTAL MATERIALS

Diversifying and Perennializing Plants in Agroecosystems Alters Retention of New C and N from Crop Residues

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Table S1. Soil CO_2 efflux stepwise multiple linear regression model output†

Coefficient	Estimate	Std. Error	t value	*p* value‡
Intercept	-3.551	0.564	-6.294	< 0.0001*
Year	-0.176	0.959	-0.183	0.855
Temperature (Temp.)	0.168	0.025	6.731	< 0.0001*
Gravimetric water content (GWC)	11.428	4.539	2.518	0.013*
Year ×Temp.	-0.059	0.043	-1.378	0.169
Year × GWC	0.310	6.843	0.045	0.964
Temp.× GWC	-0.399	0.195	-2.047	0.042*
Year ×Temp. × GWC	0.092	0.311	0.295	0.768

† Overall model: Used $\ln CO_2$, $R^2 = 0.52$, Adjusted $R^2 = 0.69$, $p < 0.0001$

‡ If significant(*), used in final model (Fig. S2)

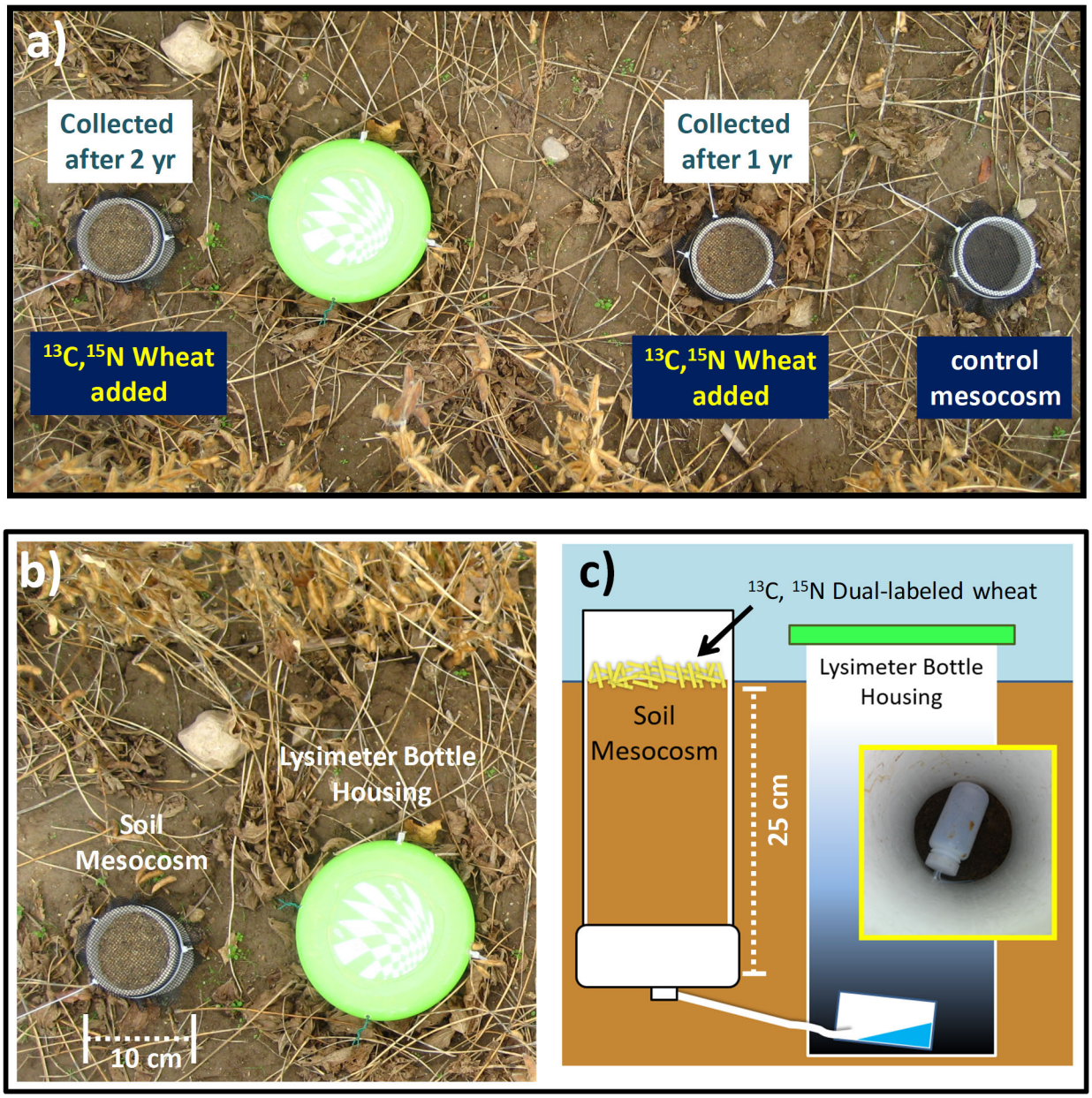

Figure S1. Overhead photos showing mesocosm layout in each plot (*a*, *b*) and vertical cross section of (*b*) showing the experimental design (*c*). Photo Credits: Marshall McDaniel.

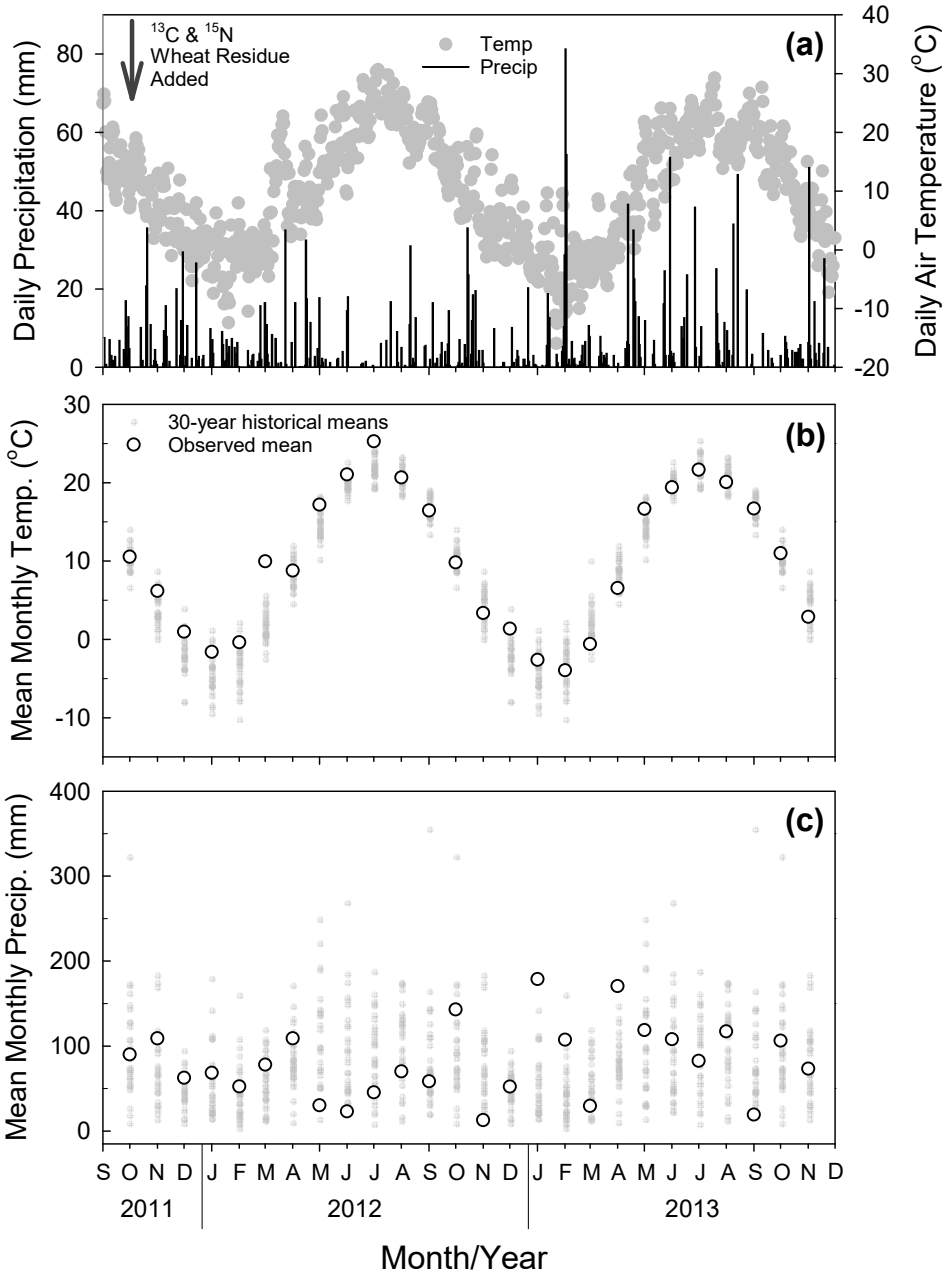

Figure S2. Precipitation and air temperature over the duration of the two-year experiment. (*a*) Daily precipitation and air temperature for over two years. Gray circles are temperature, and black bars precipitation. Downward arrow showing dual-labeled wheat residue addition. (*b*) Observed and historical (1988-2018) mean monthly temperatures. (*c*) Observed and historical (1988-2018) mean monthly precipitation. For (*b*) and (*c*), gray circles are historical annual means and open circles are observed for that month/year. From nearby weather station at Kellogg Biological Station Long-term Ecological Research site.

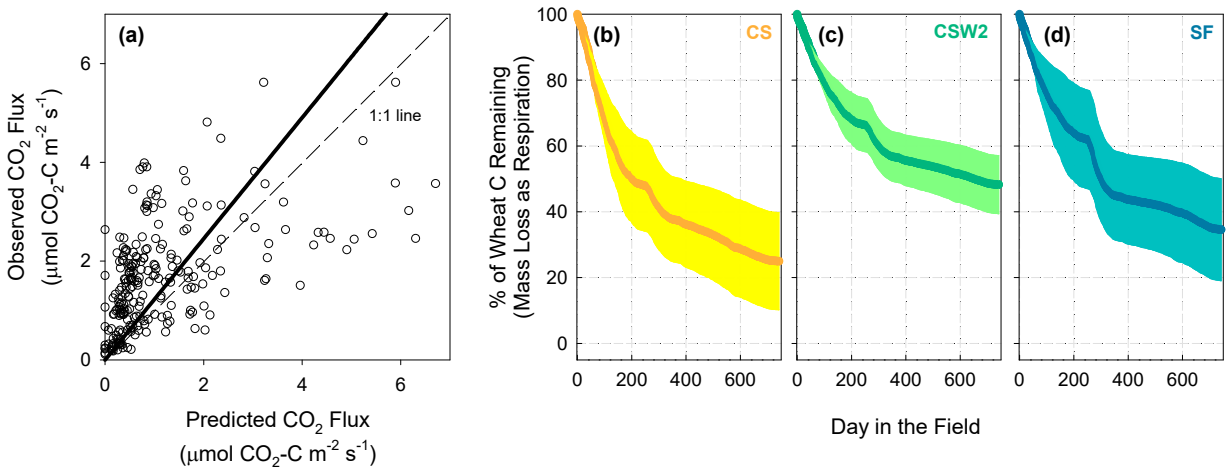

Figure S3. (*a*) Observed versus modeled (or predicted) CO_2 flux from mesocosms using soil temperature and moisture (5 and 10 cm depth). (*b*, *c*, *d*) Modeled residue loss (*solid lines*) with standard errors (*lighter area*). Treatment abbreviations are: CS = Maize-Soybean, CSW2 = Maize-Soybean-Wheat + Red Clover and Rye Cover Crops, SF = Spring fallow or tilled in spring and naturally regenerated seed bank (7-10 species).

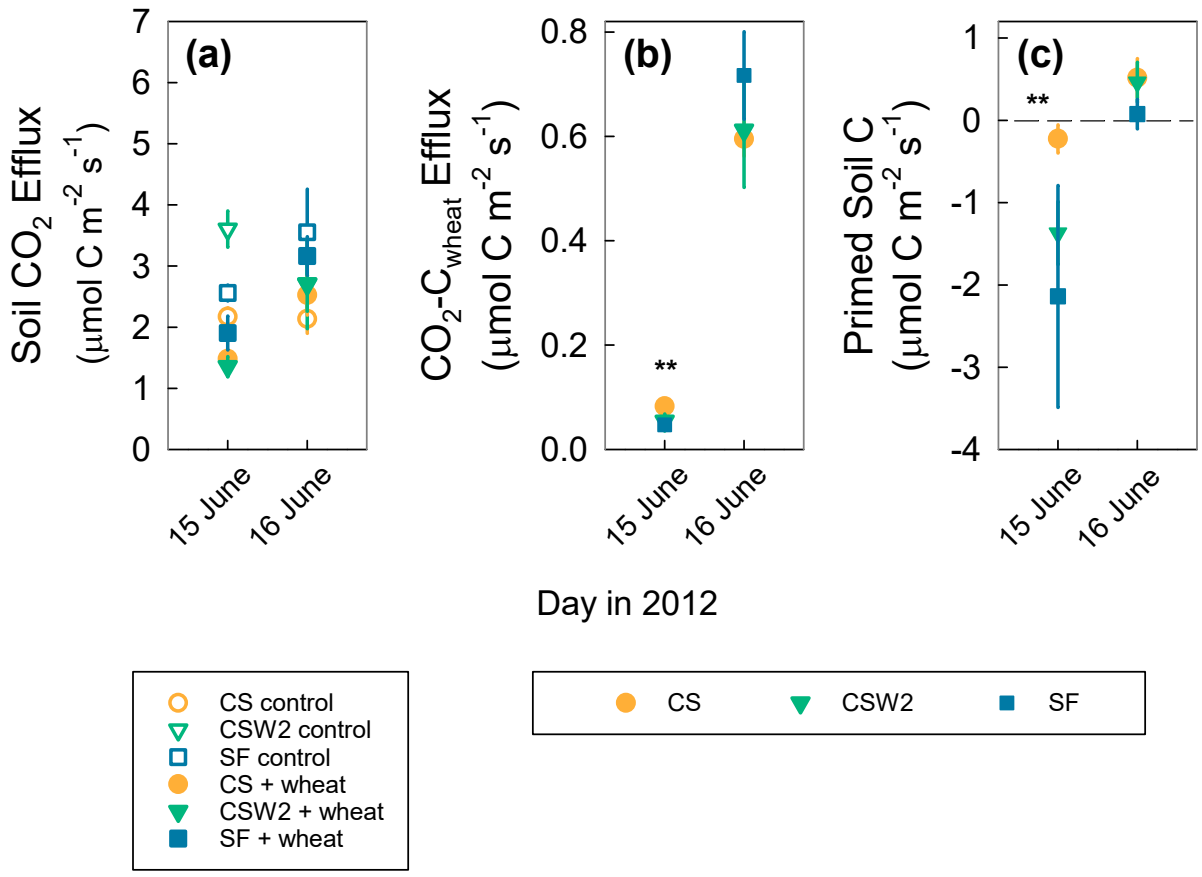

Figure S4. Soil CO_2 dynamics before (15 June) and after (16 June) manipulated wetting event in 2012 (from Figure 1 in main manuscript). Soil respiration was measured at 8:00 to 13:00 on 15 June. Then 2.5 cm of water was added to all soil mesocosms at 13:30. CO_2 was measured again 16 June between 8:00 to 13:00. (*a*) Total CO_2 flux measurements from both the control (no residue) and wheat residue added. (*b*) Residue-derived CO_2-C (C_{wheat}) flux emitted from mesocosms. (*c*) Native soil organic carbon lost, via priming, from residue addition.

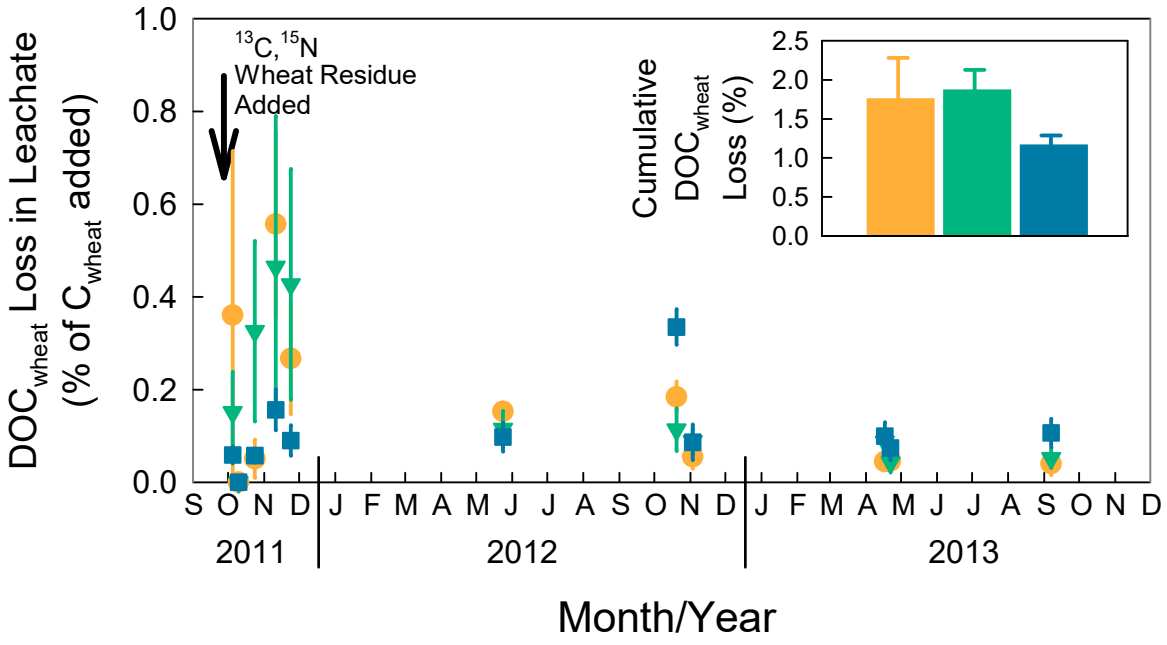

Figure S5. Percent of wheat carbon (C) lost as dissolved organic C (DOC) leached through soil profile and into zero-tension lysimeter (Fig. S1). *Inset* shows cumulative losses as % wheat C.