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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-labelled ( $^{13}\text{C}$  and  $^{15}\text{N}$ ), isotopically enriched wheat (*Triticum aestivum*) residue *in situ* for two years as it decomposed in three agroecosystems: maize-soybean rotation (CS), maize-soybean-wheat plus red clover and cereal rye cover crops (CSW2), and spring fallow management with regeneration of natural grassland species (7-10 species; SF). We measured losses of wheat residue ( $C_{\text{wheat}}$  and  $N_{\text{wheat}}$ ) 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 tease apart subtle differences. For example, SF retained a greater portion of  $C_{\text{wheat}}$  in 0-5 cm surface soils (155%,  $p=0.035$ ) and narrowed the  $C_{\text{wheat}}$  to  $N_{\text{wheat}}$  ratio ( $p<0.030$ ) compared to CS. CSW2 increased an index of carbon-retention-efficiency,  $C_{\text{wheat}}$  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 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-labelled 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<sub>2</sub> concentrations in the atmosphere (Guo and Gifford 2002, De et al. 2020). Furthermore, N losses from these same agroecosystems have contributed to both water quality impairment and climate change (Rabalais et al. 2001, Boesch et al. 2009). Since aboveground crop litter (or residue) is important to soil organic carbon (C) and nitrogen (N) pools, managing agroecosystems to retain these elements is key to mitigate 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. While belowground plant inputs have received much recent attention, in agroecosystems, aboveground crop residues are 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 and 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 under Michigan, USA Alfisol soils, two decades of no-tillage and reestablishment of grassland plant communities altered soil biota and their activities, and this affected chemical composition of residue and its mass loss (Wickings et al. 2011, 2012). Another example, in a nearby Michigan, USA experiment, organic management increased the retention of new plant inputs, simulated with chemical compounds [e.g., glucose, phenol, cellulose; Kallenbach et al. (2015)]. Finally, in another study from California, USA, 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 and Six 2010).

Other studies report no difference in retention of newly added plant residues due to historical, long-term management practices (Jenkinson 1965, Kong and Six 2010). For example, in the 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 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 (Swift et al. 1979; Bradford et al. 2016).

Diversifying and extending crop rotations has been widely shown to enhance soil biological activity and alter microbial community structure (McDaniel et al. 2014c, Tiemann et al. 2015, Venter et al. 2016, Kim et al. 2020), and is a likely management candidate to

drive 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 et al. 2014c, 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 (West and Post 2002, McDaniel et al. 2014c). 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 et al. 2014c).

These benefits from plant diversity and perenniality that increase SOM, soil biological activity, and even plant growth, may be the result of a positive feedback between soils and plants; a feedback which 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 history of plant or crop diversity. For example, McDaniel et al. (2014a) showed in a laboratory setting that after 12 years, crop rotations altered how new residues are decomposed. McDaniel et al. (2014a) found soils with history of diverse and longer crop rotations had a more rapid and complete decomposition, particularly of low-quality residues (i.e., C-to-N ratios > 38). This begs the question: Could a history of greater agricultural plant diversity and perennialization 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 affects soil retention of new residue C and N. We incubated dual-labeled ( $^{13}\text{C}$  and  $^{15}\text{N}$ ), isotopically enriched wheat (*Triticum aestivum* L.)

residue *in situ* for two 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 two 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 and Grandy 2016).

## Materials and Methods

### *Site and Experiment Description*

This experiment was conducted at the W.K. Kellogg Biological Station Long-term Ecological Research site (KBS-LTER) 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 set-up 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<sub>2</sub>O) ranges from 5.2-6.9, soil C from 1.4-4.6 g C kg<sup>-1</sup>, and bulk densities from 0.99-1.65 g cm<sup>-3</sup> (Table 1). For 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 (*Triticum aestivum*) + two cover crops (red clover and cereal rye, *Trifolium pratense* and *Secale cereale*) rotation is the managed cropping system with greatest crop diversity and cover (CSW2). In the third treatment, spring fallow treatment (SF), is a regeneration of natural grassland species after tillage. Total biodiversity in these plots can be up to 10 species of plants with the three most abundant plants being pigweed (*Chenopodium album*), foxtails (*Setaria pumila* and *Setaria faberi* Herrm.), and crabgrass (*Digitaria sanguinalis*) (Smith and Gross 2006). All treatments are chisel-plowed in the spring to a depth of 15 cm. Unlike many commercial farms, however, all treatments receive no external chemical amendments (i.e. fertilizer or pesticides).

#### *Dual-labelled (<sup>13</sup>C and <sup>15</sup>N) wheat residue and mesocosm set-up*

We used dual-labelled (<sup>13</sup>C and <sup>15</sup>N) wheat (*Triticum aestivum*) straw to track the fate of the residue's C and N as it decomposed over two 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 <sup>13</sup>CO<sub>2</sub> (10 atom percent, At%) in plexiglass growth chambers under similar conditions to Bird et al. (2003). The wheat was fertilized weekly with K<sup>15</sup>NO<sub>3</sub> (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% <sup>13</sup>C) and total N content of 1.8% (10.9 At% <sup>15</sup>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: Fig. 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 PVC tube (30 cm long, 10 cm diameter) with ten equally-spaced, 2 mm diameter holes drilled into the side for soil fauna movement. This tube was bevelled 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, 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: Fig. 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 two months to allow for recovery from the installation disturbance. Then on 1 October 2011, we added 7.29 g of the dual-labelled 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 y and a second retrieved 2 y after application of the wheat straw (along with the control mesocosms). At collection, the mesocosms were split in half vertically within 2-3 d 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 y some visually observable wheat residue remained in the surface soils, but by 2 y most of visually observed wheat residue was absent. Soils were placed at 4 °C for one or two days while being sieved to < 2 mm, and rocks removed. 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, a sub-sample of this was dried to 105 °C, and then ground.

#### *Soil, gas and water sample collection and analyses*

Emissions of  $^{13}\text{CO}_2$  were measured 10 times throughout the 2-y experiment.  $^{13}\text{CO}_2$  was measured using a non-steady state, static chamber placed on top of the mesocosm. The chamber height was 15 cm with a volume of 1.3 L. A  $^{13}\text{CO}_2$  measuring event began with placing 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 analysed for  $\text{CO}_2$  concentrations and  $\delta^{13}\text{C-CO}_2$  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  $^{13}\text{CO}_2$  fluxes before and then 1 d 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  $\text{CO}_2$ . We wanted to determine if a strong drying-wetting event changed the source of  $^{13}\text{CO}_2$  equally among treatments.

Lysimeter water samples were checked regularly during the growing season (~1 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, 707 d after adding the wheat residue. Lysimeter samples were transported in coolers and kept frozen until analysis.

At each microcosm harvest (1 and 2 y 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 et al. 2014a). Briefly, two sets of fresh, sieved soil (5 g) were weighed into 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<sub>2</sub>SO<sub>4</sub>. The chloroform fumigated and non-fumigated extracts were analysed on a TOC-TN analyzer (TOC-V-CPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). We used 0.45 (Joergensen 1996) and for the fumigation extraction efficiency. The non-chloroformed sub-sample is also used for salt extractable organic C (SEOC), a pool of labile and moderately labile organic C.

All solid and liquid samples (MBC extracts and lysimeter samples) were analyzed at the University of California at Davis Stable Isotope Facility. Bulk soil isotope signatures (<sup>13</sup>C and <sup>15</sup>N) were measured on a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 IRMS (Sercon Ltd., Cheshire, UK). A subsample of each soil was also analysed for total organic C and N on a Costech ECS 4010 CHNSO analyser at University of New Hampshire for quality assurance. Dissolved organic C (DOC) was analysed for <sup>13</sup>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 analysed surface soils outside the mesocosms to characterize their physical, chemical, and biological properties on 1 November 2012. These soils were composites of three 0-10 cm deep cores collected with a 5 cm diameter PVC tube. Soil was emptied into a plastic bag and placed into 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, salt-extractable organic C (N), 0.5 M K<sub>2</sub>SO<sub>4</sub>-extractable ammonium, 0.5 M K<sub>2</sub>SO<sub>4</sub>-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 remainder of 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 et al. (2014a) and McDaniel & Grandy (2016).

### *Statistics and Data Analyses*

To determine the source of CO<sub>2</sub> carbon we used the Keeling plot method (Keeling 1958). The  $\delta^{13}\text{C}$  signature of CO<sub>2</sub> was calculated using a linear regression of the  $\delta^{13}\text{C}$  and inverse of CO<sub>2</sub> concentration with a minimum of three time points for each chamber. This was calculated for both the wheat-added and control chambers. The  $\delta^{13}\text{C}_{\text{treatment}}$  is the source value of CO<sub>2</sub> from the  $^{13}\text{C}_{\text{wheat-added}}$  soil using a Keeling plot.  $\delta^{13}\text{C}_{\text{control}}$  is the source value of CO<sub>2</sub>-<sup>13</sup>C from endogenous soil organic C (derived from Keeling plot of the control).

To calculate <sup>13</sup>C or <sup>15</sup>N in all measured soil pools – SOC, total N, microbial biomass and extractable C from soil with salt (K<sub>2</sub>SO<sub>4</sub>) – we used  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values from labelled and controlled soils. For example, the  $\delta^{13}\text{C}_{\text{MBC}}$  values for both treatment and control soils were calculated based on mass balance as

$$\delta^{13}\text{C}_{\text{MBC}} = \frac{\delta^{13}\text{C}_{\text{F}^*[\text{C}]_{\text{F}}} - \delta^{13}\text{C}_{\text{NF}^*[\text{C}]_{\text{NF}}}{[\text{MBC}]} \quad [1]$$

where  $\delta^{13}\text{C}_F$  and  $\delta^{13}\text{C}_{NF}$ , and  $[C]_F$  and  $[C]_{NF}$  are the delta values and concentrations for fumigated and non-fumigated 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  $^{13}\text{C}$  or  $^{15}\text{N}$  derived from wheat residue. Here we show just  $^{13}\text{C}$  for an example, but this equation also applies for  $^{15}\text{N}$ .

$$f_{wheat} = \frac{\delta^{13}\text{C}_{treatment} - \delta^{13}\text{C}_{control}}{\delta^{13}\text{C}_{wheat} - \delta^{13}\text{C}_{control}} \quad [2]$$

$\delta^{13}\text{C}_{treatment}$  is the delta value from the wheat-added sample of interest,  $\delta^{13}\text{C}_{control}$  is the respective sample from the mesocosm control, and  $\delta^{13}\text{C}_{wheat}$  is the delta value of the wheat residue ( $\delta^{13}\text{C} = 5,126$  or  $\delta^{15}\text{N} = 29,709$ ). Accordingly,  $f_{wheat}$  can be applied to a pool or flux to derive 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  $\text{CO}_2$  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 % 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 depth. Second, we used the change in total mesocosm wheat residue-derived C and N (0-20 cm) between Year 1 and Year 2 as a measure of whether the soil was accumulating or losing residue-derived C and N within the 1-2 y timeframe. Third, as a measure of carbon-retention-efficiency (CRE) at the soil profile level, we simply divided the remainder of residue-derived C stored in each soil mesocosm at 2 y by the amount emitted as CO<sub>2</sub>-C plus DOC and that of C remaining in soil. The CRE is similar to C-use-efficiency metric that is typically used in more controlled incubation studies and reflects efficiency specific to microbial biomass (Manzoni et al. 2012, Spohn et al. 2016, Geyer et al. 2019). Here, however, the CRE reflects a soil-profile-level processes, and gives proportion of C that persisted for two years (at 0-20cm depth).

Because of infrequent measurements of CO<sub>2</sub> (due to logistics and the high cost of  $\delta^{13}\text{C}$ -CO<sub>2</sub> analyses), we also used a two-prong modelling approach to derive cumulative losses of CO<sub>2</sub>-C<sub>wheat</sub>. First, to interpolate daily CO<sub>2</sub> fluxes, we used known CO<sub>2</sub> flux measurements with soil temperature and moisture data from nearby sensors (< 0.5 km) in a step-wise multiple linear regression (MLR) model. The MLR variables included 239 log normal CO<sub>2</sub> flux (lnCO<sub>2</sub>) with empirically linked measurements of year of experiment (Y, values of 0 or 1), soil temperature (T, 5.3 to 31.2 °C), gravimetric soil moisture ( $\theta$ , 0.024 to 0.42 g g<sup>-1</sup>). Terms not significant at  $\alpha < 0.05$  were dropped from the model. CO<sub>2</sub> fluxes can be modelled quite accurately from soil temperature and moisture alone (Tang et al. 2005, Sullivan et al. 2008, McDaniel et al. 2014b). Second, these predicted CO<sub>2</sub> fluxes from the MLR were used with an interpolated  $f_{wheat}$  to gap-fill and calculate cumulative losses of CO<sub>2</sub>-C<sub>wheat</sub>. We can also fit  $f_{wheat}$  to 3-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 modelled cumulative CO<sub>2</sub>-C<sub>wheat</sub> were also used to calculate CRE.

Data were checked for normality and heterogeneity of variances in R (v3.4.3, the 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 removed if 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 comparison of means with *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 and Zak, 2015). Where means are reported, so are standard errors (after  $\pm$ ) for reference. Repeated measures ANOVAs were used for CO<sub>2</sub> flux data in SAS (v9.4) using *proc mixed*, and *lsmeans* for separation of means and treatment effects by dates. To determine 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 & Grandy (2016). We used SigmaPlot (v.13, Systat Software, Inc., San Jose, CA) for linear and non-linear 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: Fig. S2). More specifically, 2012 was one of the driest years on record for the entire Midwest US (Hamilton et al. 2015) and precipitation at the KBS-LTER in 2012 was only 742 mm (MAP for 1988 to 2013 was 902 mm). By comparison, 2011 and 2013 were both rather wet and exceeded the long-term mean (1125 and 1177 mm respectively). These patterns were also reflected in gravimetric soil moisture measurements collected during our experiment (McDaniel and Grandy, 2016). At our 1<sup>st</sup> mesocosm collection (4 November 2012) gravimetric water contents were 0.152 to 0.173 g g<sup>-1</sup>

<sup>1</sup>, whereas at the 2<sup>nd</sup> collection (14 October 2013, during a wetter year) the range was 0.115 to 0.141 g g<sup>-1</sup>.

#### *C<sub>wheat</sub> losses from the soil*

In the two months after wheat residue was added to mesocosms, surface CO<sub>2</sub> fluxes were greater than the no-residue controls (Fig. 1a) by  $1.10 \pm 0.21 \mu\text{mol C m}^{-2} \text{ s}^{-1}$ . This trend was reversed by the 2012 growing season where the controls tended to have greater CO<sub>2</sub> fluxes ( $\sim 0.6 \pm 0.08 \mu\text{mol C m}^{-2} \text{ s}^{-1}$ ). The wheat C lost as CO<sub>2</sub> (CO<sub>2</sub>-C<sub>wheat</sub>) decreased exponentially from October 2011 to October 2013. There was a marginally significant interactive effect of time and crop diversity on CO<sub>2</sub>-C<sub>wheat</sub> ( $p = 0.085$ ), due mostly to the overwhelming effect of time. Analysed by date, the SF lowered CO<sub>2</sub>-C<sub>wheat</sub> compared to CS in three out of ten measurements (253, 329, 565 d).

Our multiple linear regression models, based on soil temperature and moisture (and their interactions), predicted CO<sub>2</sub> fluxes moderately well but underestimated observed fluxes by 18% ( $p < 0.0001$ ,  $R^2 = 0.52$ , Appendix S1: Table S1, Fig. S3a). After applying the model to CO<sub>2</sub>-C<sub>wheat</sub> data to gap-fill, the cumulative percent CO<sub>2</sub>-C<sub>wheat</sub> loss at the end of two years was  $75 \pm 15$ ,  $52 \pm 9$ , and  $65 \pm 16$  % for CS, CSW2, and SF treatments respectively (Table 2,  $p = 0.334$ ; Appendix S1: Fig. S3b-d). The  $f_{wheat}$  from each plot showed significant fit with a 3-parameter exponential decay model –  $R^2$  from 0.90-0.97, and  $p$  values from 0.067-0.009 (Table 2) – not surprising since  $f_{wheat}$  is similar to fraction of mass loss in traditional litter-bag studies. The CSW2 treatment had twice the decomposition rate compared to CS, but only the initial  $f_{wheat}$  ( $\alpha$ ) was significantly lower in SF compared to the two cropped treatments ( $p = 0.006$ ). This was also illustrated by the lower measured CO<sub>2</sub>-C<sub>wheat</sub> values  $\sim 1$  y after wheat was added (Fig. 1b).

The amount of endogenous soil organic C 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



<sup>13</sup>CO<sub>2</sub> fluxes (Fig. 1c). Within the first two weeks, primed SOC loss averaged  $0.68 \pm 0.06$   $\mu\text{mol C m}^{-2} \text{ s}^{-1}$  (ranging from 0.2 to  $1.5 \mu\text{mol C m}^{-2} \text{ s}^{-1}$ ). 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 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: Fig. S4c) and 13 October 2013, where added residue depressed endogenous CO<sub>2</sub> losses in the CSW2 relative to compared to CS treatment ( $p = 0.033$ ),  $-1.37 \pm 0.22$  versus  $-0.23 \pm 0.16 \mu\text{mol C m}^{-2} \text{ s}^{-1}$  respectively. The SF endogenous CO<sub>2</sub> flux was  $-2.14 \pm 1.35 \mu\text{mol C m}^{-2} \text{ s}^{-1}$  but not different due to high variability.

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

#### *C<sub>wheat</sub> and N<sub>wheat</sub> retained in the soil*

After two years and across all treatments, most of the wheat C and N retained in the soil was in the top 0-5 cm (Fig. 2a,b). There were no significant differences in retention of either element among treatments at depths. Summing the 0-20 cm depth, all soils retained more  $N_{\text{wheat}}$  (29.6-50.5%) than  $C_{\text{wheat}}$  (12.4-25.0%), reflecting the greater mobility and demand for N in these soils. The total percent of  $C_{\text{wheat}}$  retained in each treatment was  $15.9 \pm 1.4$ ,  $20.5 \pm 1.7$ , and  $19.2 \pm 2.2$  % for CS, CSW2, and SF respectively ( $p = 0.233$ ). The total percent  $N_{\text{wheat}}$  remaining after 2 years was  $38.7 \pm 3.4$ ,  $44.0 \pm 2.0$ , and  $41.7 \pm 4.0$  % for the CS, CSW2, and SF treatments respectively ( $p = 0.561$ ). There were no significant differences in soil wheat retention among cropping systems, largely due to high variability across depths and amongst blocks (the coefficient of variation ranged from 12.3 to 86.3%).

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

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

#### *Dynamics and efficiency of accumulating new $C_{\text{wheat}}$ and $N_{\text{wheat}}$*

We used three methods to quantify the dynamics of  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  accumulation in soil over the 2-y study (Fig. 4). First, we used a soil stratification index (SSI) to reflect the stratification or distribution of  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  with soil depth. The greater amount of residue retained in 0-5 cm (relative to 5-20 cm) reflects internal efficiency of soil to retain  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  and reduce losses to lower soil depths (Fig. 4a,b). SF increased the SSI of  $C_{\text{wheat}}$  by 155% ( $p = 0.035$ ) and  $N_{\text{wheat}}$  by 87% compared to CS ( $p = 0.195$ ), and while the CSW2 was greater on average than CS for both C and N they were non-significant ( $p < 0.404$ ). Second, we evaluated the percent change in wheat residue C and N between Years 1 and 2, and found

there were no significant differences due to large variation (Fig. 4c,d). Finally, we used  $C_{\text{wheat}}$  loss (as  $\text{CO}_2$  and DOC) compared to that remaining in the soil profile to calculate a CRE for the 0-20 cm (Fig. 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 relationship between wheat residue decomposition/retention to various soil properties (Fig. 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  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  was more related to physical characteristics especially soil texture (Fig. 5), but N stratification was negatively related to labile C as measured by permanganate-oxidization and basal respiration. Wheat residue CRE, at the mesocosm scale, was strongly negatively related to water content, pH, basal respiration, and peroxidase (a lignin-modifying extracellular enzyme); CRE was positively related to SEO C 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, i.e., with early successional fallow (SF) or diversified rotations (CSW2) respectively. Both treatments also extended length of time living plant roots covered the soil (or increased perennality). In our study, both modes of enhancing plant diversity and perennality subtly altered how soil biota decompose and stabilize new residue, and these management practices altered decomposition dynamics in different ways. In support of our primary hypothesis, greater plant diversity and perennality (whether in crop rotations or early successional grassland species), not only subtly increased the retention of  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  but also

decreased losses compared to the business-as-usual, maize-soybean (CS) rotation. We use the term ‘subtly’ to describe the effects of 12 years of diversification because neither treatment statistically increased net retention of either  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  in the soil, at a significance level of  $p < 0.1$  (Fig. 2a, 2b). 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  $C_{\text{wheat}}$  stabilized in 0-5 cm soil (+155%, Fig. 4a), and CSW2 increased carbon-retention efficiency (CRE) from 0.18 to 0.27 (+49%, Fig. 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 retention of new residue C and N.

#### *The effects of diversifying and perennializing agroecosystems on $C_{\text{wheat}}$ and $N_{\text{wheat}}$ losses*

In our study, soils from more diverse agroecosystems generally lost less  $C_{\text{wheat}}$  as  $\text{CO}_2$ . 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  $C_{\text{wheat}}$  primarily occurred early in decomposition relative to the CS rotation (Fig. 1b and Table 2). The  $C_{\text{wheat}}$  losses from the diversified/perennialized crop rotation (CSW2), however, were lower than CS and mostly in the second year. These contrasting temporal dynamics of  $C_{\text{wheat}}$  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 litter bags (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 d 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 (Guenet et al. 2018, Bastida et al. 2019, 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 (Fig. 1c). This aligns with most priming studies which 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 two-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% to 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: Fig. S4c). This short-lived but significant event may point to how and when diversified and perennialized cropping systems tend to accumulate soil organic C relative to conventional systems – during drought or stress. Losses of  $C_{\text{wheat}}$  through leached DOC were minimal (Table 2; Appendix S1: Fig. S5). A very small fraction of mineralized  $C_{\text{wheat}}$  was found to be soluble (2%); most was recovered either in soil microbial biomass or in non-living but mineral-adsorbed C (Gaillard et al. 1999; Figs. 3 and S5).

While we did not quantify losses of  $N_{\text{wheat}}$ , there may have been gaseous losses of  $N_{\text{wheat}}$  through volatilization, nitrification, and denitrification. Other studies using  $^{15}\text{N}$  labelled residue have shown these gaseous losses to be negligible (< 1%, Eickenscheidt and

Brumme 2013). Rather, most  $N_{\text{wheat}}$  losses are likely leached products of mineralization that were not retained in microbial biomass nor adsorbed to soil particles (Gaillard et al. 1999); and these likely include small organic  $^{15}\text{N}$ -containing molecules, ammonium- $^{15}\text{N}$ , or most likely nitrate- $^{15}\text{N}$  due to its greater mobility in soils. In support of our third hypothesis, all three systems retained more  $N_{\text{wheat}}$  than  $C_{\text{wheat}}$  (Fig. 2).

#### *The subtle effects of diversifying/perennializing agroecosystems on $C_{\text{wheat}}$ and $N_{\text{wheat}}$ retention*

Generally, diversifying and perennializing cropping systems resulted in greater profile-level retention of new  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$ , depending on diversification/perennialization with rotating more crops or restored grassland species (Fig. 2 *insets*). High spatial variability, however, obscured statistically significant treatment effects on net  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  retention ( $p > 0.561$ ). A study from a systematically diversified grassland experiment showed an increase of plant species richness resulted in nearly 2-4 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, similar 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.,  $C:N_{\text{wheat}}$ , stratification of residue, and when measuring  $C_{\text{wheat}}$  retention efficiency (CRE)]. First, SF decreased  $C:N_{\text{wheat}}$ , while CSW2 generally increased it, compared to soils under 12 years of CS rotations (Fig. 2). This was most likely driven by management effects on labile resources amongst all three agroecosystems. Labile sources of C and nutrients differ amongst these systems, (Table 1, McDaniel and Grandy 2016), and likely drive the differences between  $C_{\text{wheat}}$  versus  $N_{\text{wheat}}$

retention (Fig. 3). Second, this divergent effect on  $C:N_{\text{wheat}}$  and other findings may be due to documented differences in soil microbial communities amongst treatments (Tiemann et al. 2015, Peralta et al. 2018). Soil microorganisms can differ in their C and N use efficiencies (Saifuddin et al. 2019), and the retention of new  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  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  $C_{\text{wheat}}$  retained in the mesocosm divided by the total measured. Past studies have shown 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 corn-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. Labelled litter studies across ecosystem types find that C and N from surface residues accumulate disproportionately in surface soils (Fröberg et al. 2009, Kammer and Hagedorn 2011, Eickenscheidt and Brumme 2013); and this zone of rapid physicochemical retention and biological activity has been called the ‘detritosphere’ (Gaillard et al. 1999). In previous studies, the importance of this zone has been quantified using a stratification index (Franzluebbers 2002, Lazicki et al. 2016, King and Hofmockel 2017). In our study, the SSI reflects retention dynamics within the top 0-20 cm soil but also tendency for losses of  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  as a whole. Diversifying/perennializing agroecosystems increased  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  stratification by 72-155% and 72-87%, but only SF was significantly greater than traditional maize-soybean rotation (Fig. 4a). Our increase in stratification of new residue  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  aligns with previous studies which show

practices like reduced tillage (Franzluebbers 2002) or diversification through crop rotation and manure (Lazicki et al. 2016, King and Hofmockel 2017) increase 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 (Wallenstein 2017, Jilling et al. 2018, Tautges et al. 2019), studies measuring soil vertical stratification (including ours) highlight the importance of the detritosphere and surface soils in sustainable agroecosystem management.

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

We determined the proportion of  $C_{\text{wheat}}$  that ended up in microbial biomass and non-living, salt-extractable (i.e., low-molecular weight) C compounds (< 1 % after 1 year; Fig. 3). Microbial biomass can be a more rapid and efficient pathway for creating persistent SOM through retention of necromass and microbial byproducts, as well as through abiotic processes (Grandy and Neff 2008, Cotrufo et al. 2013; Kallenbach et al. 2015, 2016, 2019). This pathway could be underlying the enhanced  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  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). And previous reports from this experiment show 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 did not observe any consistent trends in agroecosystem diversity effects on  $MBC_{\text{wheat}}$ . However, we did measure slightly more  $C_{\text{wheat}}$  in this labile pool under both diversified agroecosystems (Fig. 3b,d), and microbial biomass was negatively related to the initial fraction of C being released as  $CO_2$  (Fig. 5).



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

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 availability of other nutrients, but especially N (Manzoni et al. 2012, Sinsabaugh et al. 2013, Fernández-Martínez et al. 2014). In our experiment, enhanced biologically available N may be positively linked to CRE. While soybeans do fix atmospheric  $N_2$ , there are additional, and perhaps more efficient N-fixing, leguminous species (*Trifolium pratense* and *Trifolium incarnatum*) in the more diverse agroecosystem (CSW2). These alternative legumes are also non-harvested crops with 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  $120 \text{ kg N ha}^{-1} \text{ y}^{-1}$  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 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 co-metabolism of new residues.

## Conclusion

This study provides the first direct mechanistic evidence of plant diversity and perennality increasing retention of residue in soil and may be responsible for the often-observed trend of increasing SOM under these practices (West and Post 2002, McDaniel et al. 2014c, King and Blesh 2018; McClelland et al. 2021). While we showed standard measurements of  $C_{\text{wheat}}$  and  $N_{\text{wheat}}$  retained in soils were generally greater in diversified agroecosystems, and  $\text{CO}_2\text{-}C_{\text{wheat}}$  losses lower, these patterns were statistically insignificant with standard statistical benchmarks (Fig. 2a,b). Only by using more subtle indices of how the C and N were distributed in the profile or normalized for total  $\text{CO}_2\text{-}C_{\text{wheat}}$  lost were we able to resolve treatment differences (Fig. 2c and Fig. 4). By using 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/perennality to increase retention of new residue C and N inputs.

Beyond crop/plant diversity and perennality, other agroecosystem management factors may also increase efficiency of crop residue retention. We encourage further exploration of the management effects on residue-to-SOM trajectory, and future studies should delve more into the mechanistic drivers and interactions with roots, especially including microbial dynamics involved. Furthermore, we showed an interesting interaction between management and drought, whereby more diverse crops/plants lessen pulse of primed mineral soil organic C losses during rainfall preceding a long drought (Fig. 1c, Appendix S1: Fig. S4). Portending 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, especially doing this under a rapidly changing climate.

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Table 1. Treatment and soil characteristics for 0-10 cm depth (means  $\pm$  standard error\*, n = 4)

| Crop Rotation or Plant Diversity Treatment                         | Abbreviation | No. Crop (or Plant) Species | pH <sup>†</sup> | Bulk density (g cm <sup>-3</sup> ) | Carbon (g kg <sup>-1</sup> ) | Nitrogen (g kg <sup>-1</sup> ) | Carbon-to-Nitrogen Ratio | Microbial Biomass Carbon (mg C kg <sup>-1</sup> ) <sup>‡</sup> |
|--------------------------------------------------------------------|--------------|-----------------------------|-----------------|------------------------------------|------------------------------|--------------------------------|--------------------------|----------------------------------------------------------------|
| Maize-Soy                                                          | CS           | 2                           | 6.66 $\pm$ 0.12 | 1.38 $\pm$ 0.13                    | 7.70 $\pm$ 2.11              | 0.73 $\pm$ 0.26                | 10.91 $\pm$ 1.97         | 257 $\pm$ 116b                                                 |
| Maize-Soy-Wheat + Red Clover & Cereal Rye Cover Crops              | CSW2         | 5                           | 6.37 $\pm$ 0.25 | 1.39 $\pm$ 0.13                    | 9.63 $\pm$ 1.29              | 0.91 $\pm$ 0.04                | 10.58 $\pm$ 1.00         | 427 $\pm$ 122ab                                                |
| Spring Fallow (spring tilled and natural regeneration of seedbank) | SF           | 7-10                        | 6.70 $\pm$ 0.28 | 1.28 $\pm$ 0.10                    | 8.74 $\pm$ 2.41              | 0.77 $\pm$ 0.16                | 11.29 $\pm$ 0.80         | 682 $\pm$ 181a                                                 |

\* Significant difference among treatments indicated with lowercase letters ( $p < 0.05$ )

<sup>†</sup> pH in 1:1 DI H<sub>2</sub>O (w:w)

<sup>‡</sup> Chloroform-fumigation extraction collected in autumn 2012

Table 2. Wheat carbon budget and decomposition kinetics from each treatment (means  $\pm$  standard error\*, n = 4)

| Parameter                                           | Maize-Soy (CS)     | Maize-Soy-Wheat + Red Clover & Cereal Rye Cover Crops (CSW2) | Spring Fallow (SF) |
|-----------------------------------------------------|--------------------|--------------------------------------------------------------|--------------------|
| <b>% of total wheat C added</b>                     |                    |                                                              |                    |
| <b>Fate of Wheat C after 2 years</b>                |                    |                                                              |                    |
| Cumulative CO <sub>2</sub> -C <sub>wheat</sub> loss | 75.0 $\pm$ 14.8    | 51.8 $\pm$ 8.8                                               | 65.4 $\pm$ 15.5    |
| Cumulative DOC <sub>wheat</sub> loss                | 2.0 $\pm$ 0.5      | 2.1 $\pm$ 0.3                                                | 1.3 $\pm$ 0.1      |
| Soil C <sub>wheat</sub> retained                    | 15.9 $\pm$ 1.4     | 20.5 $\pm$ 1.7                                               | 19 $\pm$ 2.2       |
| Microbial biomass C <sub>wheat</sub>                | 0.19 $\pm$ 0.018   | 0.228 $\pm$ 0.012                                            | 0.16 $\pm$ 0.018   |
| Salt-extractable organic C <sub>wheat</sub>         | 0.070 $\pm$ 0.002b | 0.084 $\pm$ 0.006a                                           | 0.080 $\pm$ 0.006a |
| Total C <sub>wheat</sub> accounted for              | 92.9 $\pm$ 16.1    | 74.4 $\pm$ 9.2                                               | 85.7 $\pm$ 17.1    |
| <b>Wheat C Decomposition Kinetics</b>               |                    |                                                              |                    |
| <b>(3-parameter exponential decay,</b>              |                    |                                                              |                    |
| <b><math>f_{wheat} = y_0 + ae^{-kx}</math>)</b>     |                    |                                                              |                    |
| $y_0$ ( $f_{wheat}$ , %)                            | 3.49 $\pm$ 0.79    | 5.15 $\pm$ 1.74                                              | 3.61 $\pm$ 0.26    |
| $a$ ( $f_{wheat}$ , %)                              | 58.73 $\pm$ 2.19a  | 61.27 $\pm$ 2.55a                                            | 47.99 $\pm$ 2.22b  |
| $k$ (d <sup>-1</sup> )                              | 0.57 $\pm$ 0.04    | 1.03 $\pm$ 0.39                                              | 0.66 $\pm$ 0.04    |
| Mean residence time (d)                             | 179 $\pm$ 15       | 132 $\pm$ 31                                                 | 153 $\pm$ 9        |

\* Significant difference among treatments indicated with lowercase letters ( $p < 0.05$ )

## List of Figures (Figure Captions)

Figure 1. Soil-to-atmosphere carbon dioxide fluxes after dual ( $^{13}\text{C}$ ,  $^{15}\text{N}$ )-labeled wheat was added. (a)

Total  $\text{CO}_2$  flux measurements from both the control (no residue) and wheat residue added. (b)

Residue-derived  $\text{CO}_2\text{-C}$  ( $\text{C}_{\text{wheat}}$ ) flux emitted from mesocosms. (c) Native soil organic carbon lost,

via priming, from residue addition. Means with standard error shown ( $n = 4$ ). 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). Asterisks indicate significant difference between treatments at  $<0.05$  (\*),  $<0.01$  (\*\*), and

$<0.001$ (\*\*\*).

Figure 2. Percentage of wheat-derived (a) carbon and (b) nitrogen, and (c) carbon-to-nitrogen ratio of

the retained residue after 2-years of decomposition. Means with standard error shown ( $n = 4$ ). *Insets*

show total residue C and N over the entire mesocosm. See Fig. 1 caption for treatment abbreviations

and asterisk significance.

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 percent 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 Year 1 and 2 respectively. Means with standard error shown ( $n = 4$ ). Capital

letters indicate significance with MBC, lower-case indicate significant difference with SEOC. See

Fig. 1 caption for treatment abbreviations and asterisk significance.

Figure 4. (a, b) Soil stratification index (SSI) of wheat residue carbon and nitrogen. 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 et al. (2002) and Jarecki et al. (2005) for more information on this

index. (c, d) Rate of residue carbon and nitrogen accumulation or change between Year 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  $C_{\text{wheat}}$  retained in soil divided by how much was lost as  $\text{DOC}_{\text{wheat}}$  and  $\text{CO}_2\text{-}C_{\text{wheat}}$ . Means with standard error shown ( $n = 4$ ). See Fig. 1 caption for treatment abbreviations and asterisk significance.

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 (one-year after wheat addition and ancillary soil data published in McDaniel & Grandy 2016). Decomposition dynamics are from 3-parameter exponential decay constants from  $f_{\text{wheat}}$  (Table 2). Wheat residue retention dynamics: soil stratification index for carbon ( $\text{SSI}_C$ ) and nitrogen ( $\text{SSI}_N$ ), and carbon retention efficiency (CRE). Bold values are significant at  $p < 0.1$ .

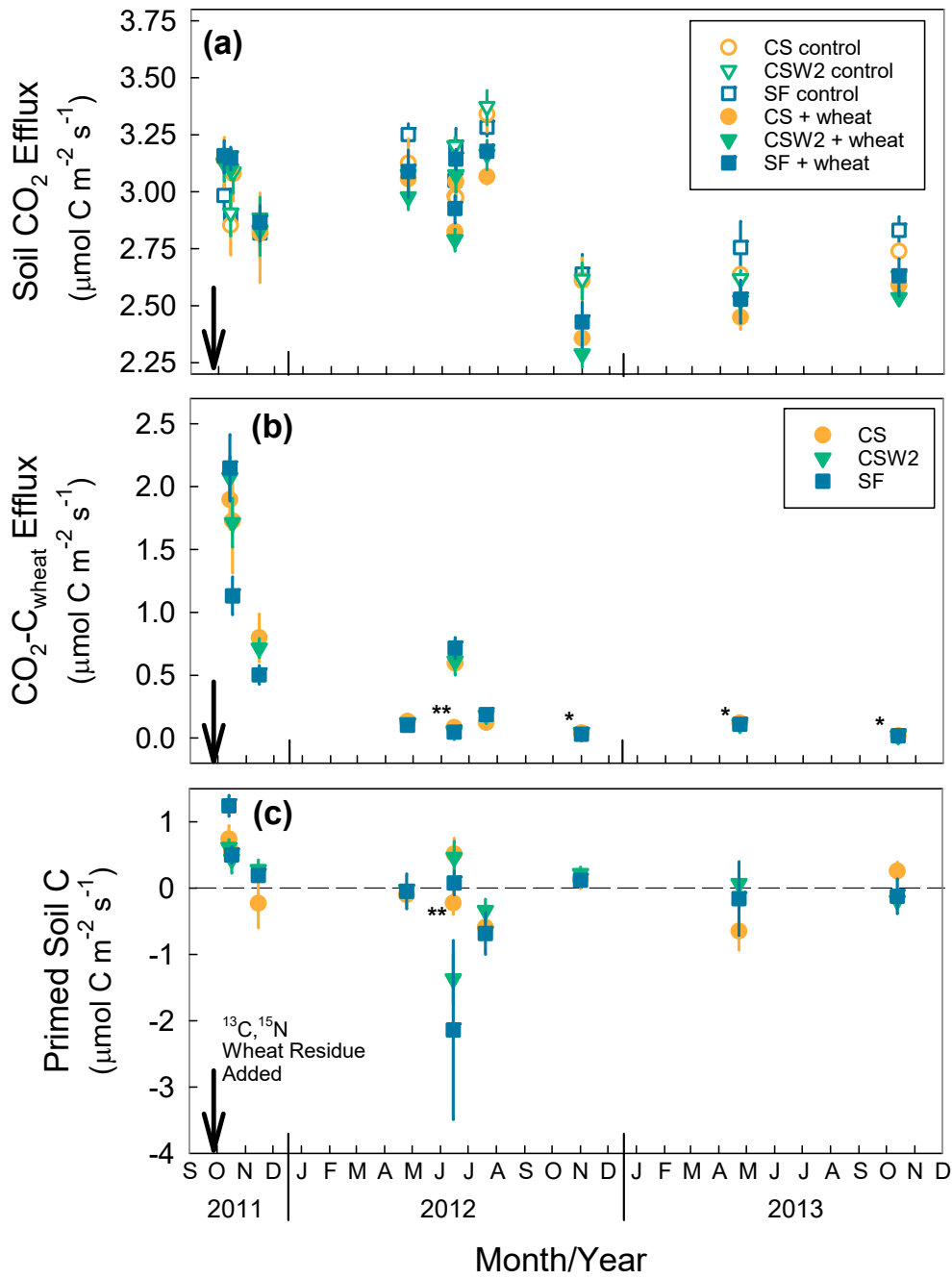


Figure 1.

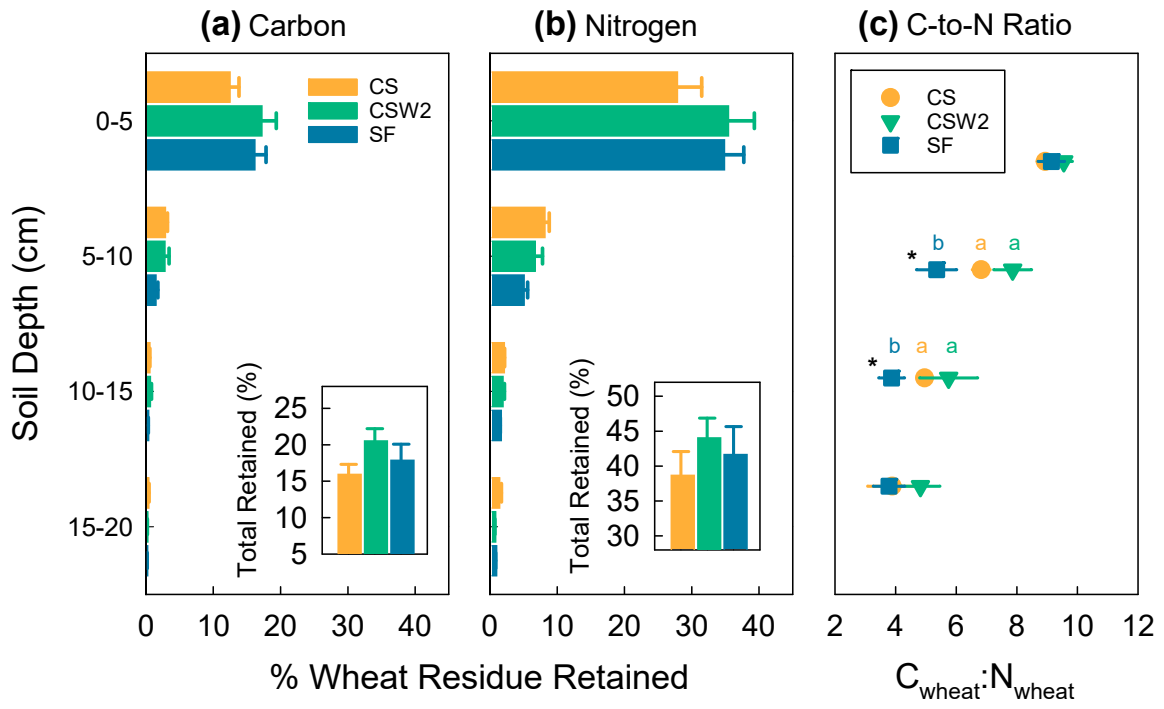
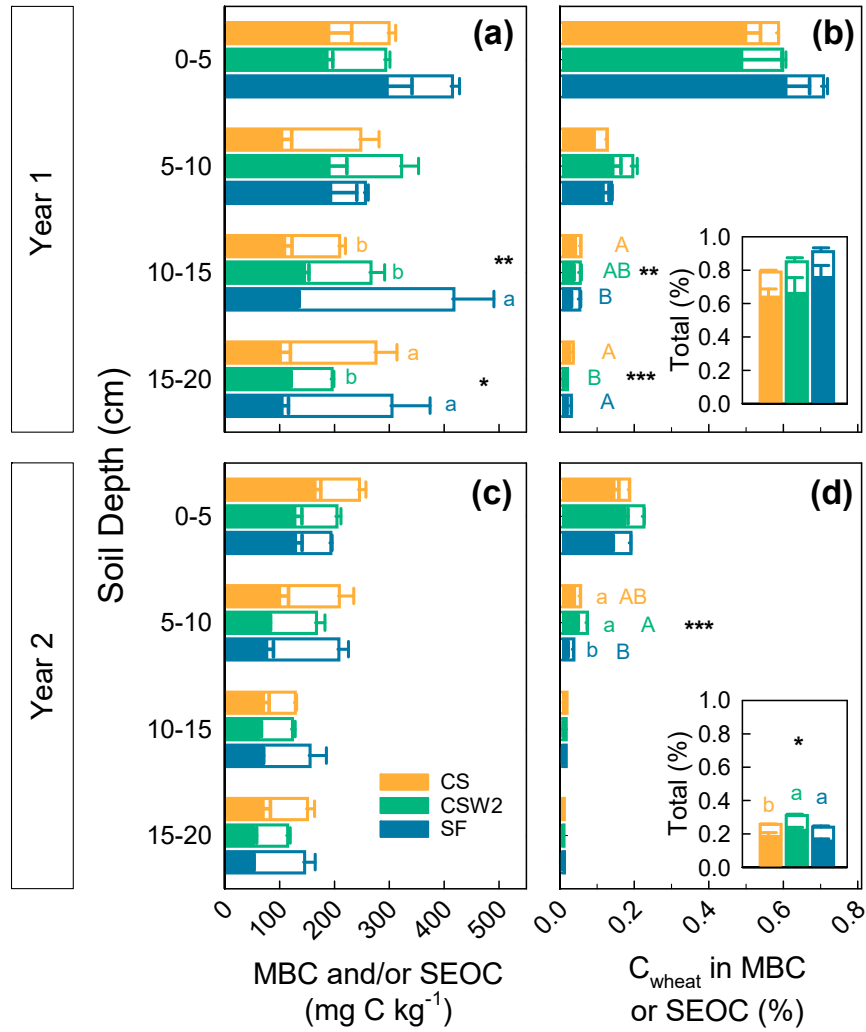


Figure 2.



**Figure 3.**

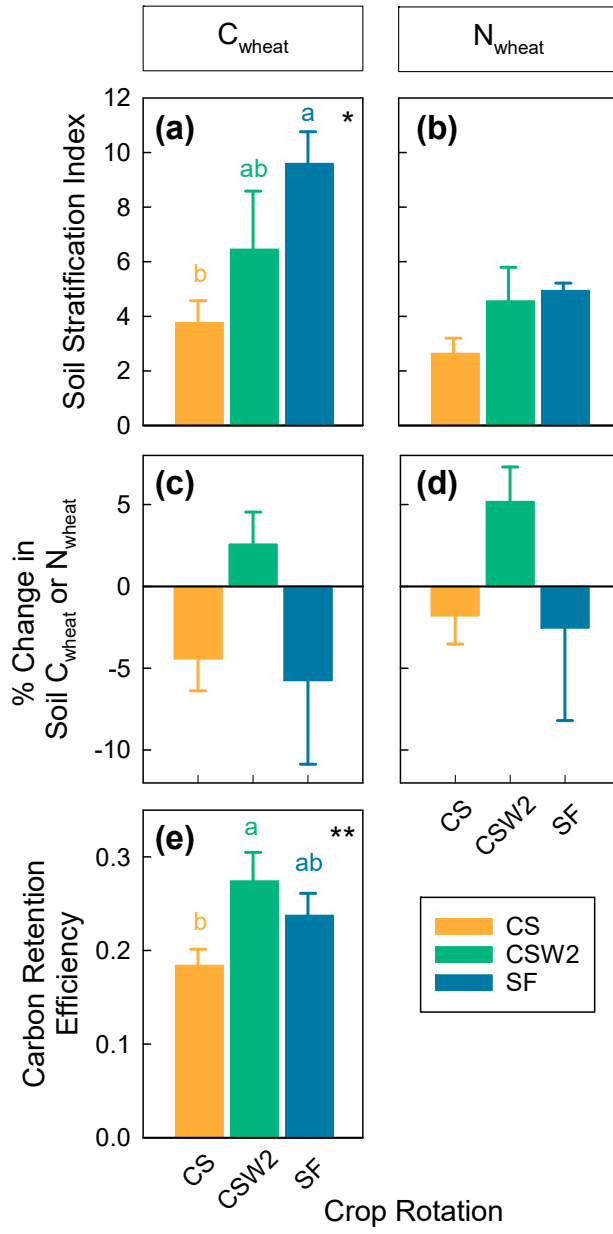


Figure 4.

| Soil Parameter                          | Wheat decomposition kinetics ( $f_{\text{wheat}}$ ) |              |              | Wheat C and N retention dynamics |                  |              | Color Key<br>r |
|-----------------------------------------|-----------------------------------------------------|--------------|--------------|----------------------------------|------------------|--------------|----------------|
|                                         | $y_0$                                               | $a$          | $k$          | SSI <sub>C</sub>                 | SSI <sub>N</sub> | CRE          |                |
| <i>Physical Soil Characteristics</i>    |                                                     |              |              |                                  |                  |              |                |
| Bulk density                            | 0.668                                               | 0.309        | 0.618        | 0.725                            | 0.544            | 0.168        |                |
| Gravimetric water content               | <b>0.085</b>                                        | 0.301        | 0.449        | 0.327                            | <b>0.069</b>     | <b>0.028</b> |                |
| Clay                                    | 0.692                                               | 0.603        | 0.25         | 0.394                            | 0.136            | 0.757        |                |
| Silt                                    | 0.78                                                | 0.652        | 0.288        | 0.312                            | <b>0.089</b>     | 0.492        |                |
| Sand                                    | 0.857                                               | 0.701        | 0.336        | 0.268                            | <b>0.069</b>     | 0.331        |                |
| 53-125 $\mu\text{m}$                    | 0.554                                               | 0.867        | 0.753        | 0.101                            | <b>0.022</b>     | 0.103        |                |
| 125-250 $\mu\text{m}$                   | 0.83                                                | 0.693        | 0.364        | 0.445                            | 0.19             | 0.356        |                |
| 250-500 $\mu\text{m}$                   | 0.799                                               | 0.629        | 0.641        | 0.198                            | <b>0.040</b>     | <b>0.094</b> |                |
| 500-1000 $\mu\text{m}$                  | 0.485                                               | 0.76         | 0.15         | 0.422                            | 0.154            | 0.968        |                |
| 1000-2000 $\mu\text{m}$                 | 0.257                                               | 0.541        | <b>0.069</b> | 0.54                             | 0.220            | 0.978        |                |
| Gravel (> 2000 $\mu\text{m}$ )          | 0.842                                               | 0.266        | 0.595        | <b>0.021</b>                     | <b>0.072</b>     | 0.118        |                |
| <i>Chemical Soil Characteristics</i>    |                                                     |              |              |                                  |                  |              |                |
| 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            | <b>0.031</b> |                |
| Salt-extractable organic N              | 0.255                                               | 0.196        | <b>0.073</b> | 0.478                            | 0.562            | 0.599        |                |
| Permanganate-oxidizable C               | 0.801                                               | 0.607        | 0.342        | 0.113                            | <b>0.041</b>     | 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 <sub>2</sub> O (1:1 w:w)        | <b>0.065</b>                                        | <b>0.019</b> | <b>0.062</b> | 0.792                            | 0.636            | <b>0.061</b> |                |
| pH in 0.01M CaCl <sub>2</sub> (1:1 w:w) | <b>0.020</b>                                        | <b>0.018</b> | <b>0.018</b> | 1.000                            | 0.473            | <b>0.019</b> |                |
| Particulate organic matter              | <b>0.077</b>                                        | <b>0.034</b> | 0.456        | 0.405                            | 0.808            | 0.728        |                |
| <i>Biological Soil Characteristics</i>  |                                                     |              |              |                                  |                  |              |                |
| Basal respiration                       | 0.319                                               | 0.96         | 0.705        | 0.148                            | <b>0.043</b>     | <b>0.050</b> |                |
| Potential mineralizable C               | 0.671                                               | 0.647        | 0.777        | 0.668                            | 0.998            | 0.320        |                |
| Potential mineralizable N               | 0.429                                               | <b>0.067</b> | 0.333        | 0.314                            | 0.717            | 0.320        |                |
| Microbial biomass C                     | 0.625                                               | <b>0.007</b> | 0.774        | <b>0.089</b>                     | 0.344            | 0.705        |                |
| Microbial biomass N                     | 0.445                                               | <b>0.004</b> | 0.658        | <b>0.067</b>                     | 0.297            | 0.588        |                |
| $\beta$ -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            | <b>0.038</b> |                |
| $\beta$ -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            | <b>0.081</b> |                |
| 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            | <b>0.015</b> |                |

Figure 5.