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Eleven years of crop diversification alters decomposition dynamics of litter mixtures incubated with soil

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Abstract. Agricultural crop rotations have been shown to increase soil carbon (C), nitrogen (N), and microbial biomass. The mechanisms behind these increases remain unclear, but may be linked to the diversity of crop residue inputs to soil organic matter (SOM). We used a residue mixture incubation to examine how variation in long-term diversity of plant communities in agroecosystems influences decomposition of residue mixtures, thus providing a comparison of the effects of plant diversification on decomposition in the long term (via crop rotation) and short term (via residue mixtures). Three crop residue mixtures, ranging in diversity from two to four species, were incubated for 360 d with soils from five crop rotations, ranging from monoculture corn (mC) to a complex five-crop rotation. In response, we measured fundamental soil pools and processes underlying C and N cycling. These included soil respiration, inorganic N, microbial biomass, and extracellular enzymes. We hypothesized that soils with more diverse cropping histories would show greater synergistic mixture effects than mC. For most variables (except extracellular enzymes), crop rotation history, or the long-term history of plant diversity in the field, had a stronger effect on soil processes than mixture composition. In contrast to our hypothesis, the mC soil had nearly three and seven times greater synergistic mixture effects for respiration and microbial biomass N, respectively, compared with soils from crop rotations. This was due to the low response of the mC soils to poor quality residues (corn and wheat), likely resulting from a lack of available C and nutrients to cometabolize these residues. These results indicate that diversifying crop rotations in agricultural systems alter the decomposition dynamics of new residue inputs, which may be linked to the benefits of increasing crop rotation diversity on soil nutrient cycling, SOM dynamics, and yields.

Key words: biodiversity; crop rotation; decomposition; extracellular enzyme activity; litter mixture; microbial biomass; mixture effect; nitrogen mineralization; plant biodiversity; respiration; soil fertility.

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Introduction

It is critical that we understand the linkages between plant biodiversity and soil processes, especially in ecosystems where aboveground biodiversity is already low, such as in agroecosystems. Even though agroecosystems are notoriously low in plant diversity, farmers have been increasing aboveground biodiversity through crop rotations for millennia. It is unclear, however, whether this form of increasing plant diversity through time alters plant residue decomposition. Increased plant diversity could promote positive soil feedbacks on residue decomposition and soil organic matter (SOM) stabilization, as recently shown in grasslands (Lange et al. 2015), and may contribute to C and N accumulation in soils with rotated crops (McDaniel et al. 2014*b*).

Litter mixtures are a common method employed by ecologists to study aboveground–belowground relationships, but their use in agroecosystems is limited. Given that plant residues differ in their composition, litter mixtures are more diverse in chemical complexity than individual residues, and these differences may strongly affect how they are decomposed and become stabilized SOM (Kögel-Knabner 2002, Wickings et al. 2011, 2012). The variety in litter mixture chemical and physical traits can alter decomposition of the mixtures relative to individual litters in either additive or nonadditive relationships (Wardle et al. 1997, Gartner and Cardon 2004, Meier and Bowman 2008). An additive effect occurs when a litter mixture response is similar to the mean response for all individual litters in the mixture. A nonadditive effect occurs when an observed response to mixtures is different from that predicted by averaging the response of the individual litters. The latter is further classified as either nonadditive synergistic (NAS, positive) or nonadditive antagonistic (NAA, negative), but most observed mixture effects have been NAS (Gartner and Cardon 2004).

The mechanisms behind the NAS mixture effects remain unresolved, but may include: stimulation of decomposition by individual chemical compounds within the litter (Meier and Bowman 2008), fungal networks transferring nutrients between litter species (Frey et al. 2000, Schimel and Hättenschwiler 2007, Lummer et al. 2012), and positive effects on soil fauna due to habitat and resource diversification leading to niche differentiation (Gartner and Cardon 2004, Hättenschwiler et al. 2005). The extent to which any of these mechanisms is controlling the NAS depends on characteristics of the individual litters in the mixture, such as chemical composition (Liu et al. 2007, Ball et al. 2009, Meier and Bowman 2010), the morphology and type of litter (i.e., leaf vs. needle; Kaneko and Salamanca 1999), and diversity of the litter mix (Wardle et al. 1997, Bardgett and Shine 1999). The overall litter mixture effects, however, also depend upon factors external to the litter mixtures, related to the environment where the mixtures are placed. For example, any factor that directly influences soil biota and the availability of soil resources (e.g., land-use change or agricultural management) will likely alter the interactions between decomposing plant litters.

Factors such as climate, parent material, and plant communities have been found to alter the litter mixture effect (Madritch and Cardinale 2007, Jonsson and Wardle 2008, Schuster and Dukes 2014). Using a natural gradient in soil fertility, for instance, Jonsson and Wardle (2008) demonstrated that the direction and magnitude of the litter mixture effect was strongly regulated by differences in soil microbial biomass, microbial activity, and soil N, but this relationship also depended upon the plant community where the litter mixture was placed. Therefore, there are many complex and poorly understood interactions among external factors that regulate the litter mixture effect. Furthermore, soil microbial communities, and the C and nutrients available to them, will likely alter their response to newly added litter mixtures, and these factors can be radically altered by anthropogenic factors such as land-use change and management (Bossio et al. 2005, Jangid et al. 2008, Ramirez et al. 2012).

To date, the majority of litter mixture experiments have been conducted in natural ecosystems, especially in temperate forests (Gartner and Cardon 2004, Hättenschwiler et al. 2005, Handa 2014). One factor likely contributing to the scarcity of litter mixture studies is the low plant diversity in agroecosystems (Cardinale 2012, Tilman et al. 1997), and relative to natural ecosystems, there is less mixing of agroecosystem litter (also known as crop residues). Perhaps the most common way in which residues can mix in agroecosystems is through crop rotations, where plant biodiversity occurs with time (McDaniel et al. 2014*a*, *b*). In a typical crop rotation, fresh residue seldom mixes at the same time, but current crop residues can mix with more decomposed residue fragments from the previous year's crop, creating a soil environment where microbes have access to a diversity of C resources. Fresh crop residues, however, can also mix in a variety of ways on the farm including (but not limited to): intercropping systems, mixing of crop and tree litter from shelterbelts or hedgerows, and wind transport of crop residues even between monoculture fields. These rarely explored forms diversifying crop inputs to SOM may be crucial for maintaining agroecosystem services.

By decomposing crop residue mixtures in soils from historically different crop rotations, we can gain insight into the long-term effects of diversifying rotations, the short-term effects of diversifying crop residue inputs, and how these two may interact. This insight might explain why increasing agroecosystem crop diversity on the farm increases soil C, N, and microbial biomass (McDaniel et al. 2014*b*). Our previous research shows that increases in crop biodiversity at our field site enhanced decomposition and microbial dynamics of individual residues (McDaniel et al. 2014*a*), but the effect of 11 yr of crop rotation on how these soils process crop residue mixtures remains unknown. We added three representative residue mixtures to soils that have been under crop rotations ranging from monoculture corn to a more complex, three-cash-crop-plus-two-cover-crop rotation. We hypothesized that soils with a history of crop rotations would have higher NAS mixture effects, like on CO_2 released during decomposition for example (Fig. 1), due to enhanced chemical diversity of SOM from more diverse litter inputs. In rotations, greater variation in residue quality and chemical diversity (i.e., variation in resources) creates more habitable niches and facilitates a more diverse soil microbial community (Armbrecht et al. 2004, Tiemann et al. 2015) adapted to decomposing mixtures of resources. Monoculture soils, on the other hand, receive little diversity year to year in crop residue inputs and were expected to exhibit little-to-no NAS mixture effects (Fig. 1).

Materials and Methods

Soils for this incubation experiment were collected from the Cropping Biodiversity Gradient Experiment located at the W.K. Kellogg Biological Station (KBS) Long-term Ecological Research site (http://lter.kbs.msu.edu/research/long-term-experiments/biodiversity-gradient/). Mean annual temperature and precipitation at the site are 9.7°C and 890 mm, respectively. 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 2013). Average pH of the soils at 0–10 cm depth is 6.13 ± 0.44 (1:1 w:w). The KBS crop rotation experiment was initiated in 2000, with biodiversity increased through the systematic addition of crops in

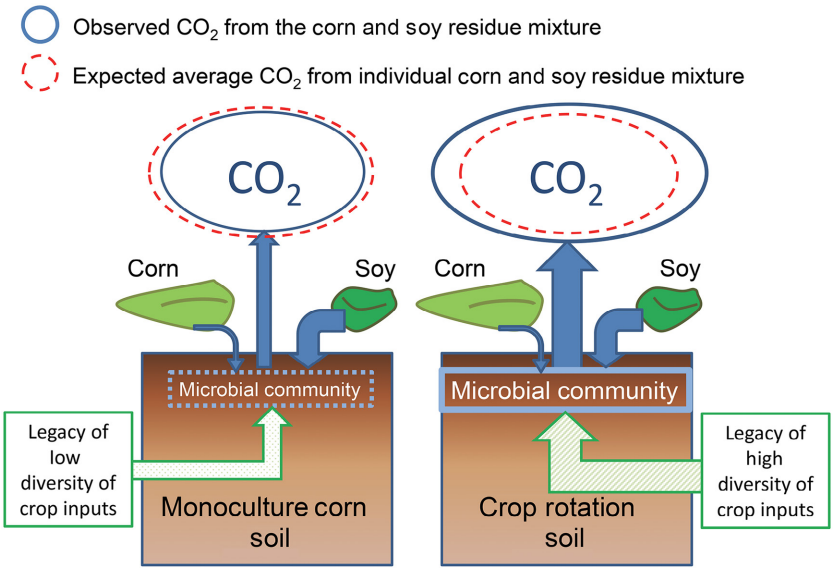

Fig. 1. The hypothesized effect of crop rotations on processing residue mixtures. Our hypothesis is that soils receiving more diverse crop inputs to soil organic matter will have larger, and more diverse, microbial community and that this will increase the observed CO_2 response, relative to the expected average from the individual residues (a nonadditive synergistic mixture effect). On the other hand, monoculture soils will have little to no difference in the observed and expected responses to residue mixtures (additive or nonadditive antagonistic).

rotations ranging from single crop monocultures to complex rotations with three different grain crops and two cover crops for a total of five species in a 3-yr rotation sequence. 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 plots received no external chemical amendments (i.e., fertilizer or pesticides) and the same annual chisel plow tillage to a depth of 15 cm. This approach minimizes the confounding factors of tillage and external fertilization common in other experiments, so that only crop biodiversity differed among treatments, which is a unique feature of this experiment. For further details on the experimental design and agronomic management practices, see Smith et al. (2008).

Experimental design

Soils were collected on 15 November 2011, after corn harvest from the following rotations: monoculture corn (mC), corn–soy (CS), corn–soy–wheat (CSW), corn–soy–wheat with red clover cover crop (CSW1), and corn–soy–wheat with red clover and rye cover crops (CSW2). All soils were collected from plots coming out of the corn phase in order to avoid confounding effects of current crop differences. Three 2.5 cm (diameter) soil cores were collected within each plot (0–10 cm), homogenized in the field, and stored on ice in a cooler until arrival at the laboratory, 2 d later. Fresh soils were sieved to 2 mm, brought to 50% water holding capacity, and then preincubated at 25°C for 5 d to decompose labile substrates released during soil processing.

We chose three residue mixtures, corn and soy (C+S); corn, soy, and wheat (C+S+W); and corn, soy, wheat, and red clover (C+S+W+Rc), which represent common crop rotations used by farmers and that are available to sample at the KBS crop rotation experiment. The crop residues were collected the same year the soils were collected, dried at 60°C for 3 d, and ground to 1 mm using a Wiley mill. After the preincubation period, we added a total of 1.2 g of each dried crop residue or residue mixture per 100 g dry soil and then mixed thoroughly to homogenize. Residue mixtures contained equal amounts of each residue, on a per mass basis. For example, 1.2 g of the C+S+W mixture contained 0.4 g of each of the constituent residues. The residue mixtures were analyzed for relative chemical abundance using pyrolysis gas chromatography/mass spectrometry (Grandy et al. 2007). The soil–residue mixtures were split into three replicates to be randomly sampled throughout the incubation at 30, 90, and 360 d.

Soil chemical and biological responses

We measured several response variables over the 360-d incubation. Some variables were measured multiple times during the incubation, while others were measured once or are a cumulative value of a variable. Soil respiration was measured 30 times, but for simplicity and alignment with other variables, we used three measurements at 30, 90, and 360 d. A CO_2 sample was collected immediately after sealing the jar and 24 h later. Both samples were analyzed on a LI-820 CO_2 analyzer (Li-Cor, Lincoln, Nebraska, USA). The CO_2 generated in the jar headspace over the 24 h was used to calculate the respiration rate.

Inorganic N and dissolved organic C were extracted from soils by shaking on an orbital shaker for 1 h with 40 mL of 0.5 M K_2SO_4 per 5 g fresh soil. Inorganic N extracts were analyzed for ammonium (NH_4^+) using the salicylate and ammonia cyanurate reagent packets (Hach Company, Loveland, Colorado, USA), and for nitrate (NO_3^-) using the single-reagent method (vanadium III, sulfanilamide and *N*-(1-naphthyl)-ethylenediamine dihydrochloride; Doane and Horwáth 2003). Microbial biomass C (MBC) and N (MBN) were measured using the chloroform fumigation-extraction method (Vance 1987) as modified by Scott-Denton et al. (2006). Ethanol-free chloroform (1 mL) was added to 5 g of soil (including a soil-free blank tube) and incubated at room temperature for 24 h in a sealed 50-mL test tube. Following incubation, the tubes were vented in a fume hood for 1 h and extracted as described above. Soil extracts were analyzed for total dissolved N (TDN) and DOC using a Shimadzu TOC-V_{CPN} (Shimadzu Scientific Instruments Inc., Columbia, Maryland, USA). MBC and MBN were calculated as the differences between DOC and TDN, respectively, extracted from fumigated and nonfumigated samples. Extraction efficiency constants of k_{EC} = 0.45 (Joergensen 1996) and k_{EN} = 0.54 (Brookes et al. 1985) were applied.

Soil extracellular enzyme activities (EEA) were measured at pH 5.6 in 96-well microplates using methylumbelliferyl-linked, fluorimetric

substrates for hydrolytic enzymes and two colorimetric substrates for the oxidative enzymes (Saiya-Cork et al. 2002, McDaniel et al. 2014*a*). The hydrolytic enzymes included β-D-1,4-cellobiohydrolase (CBH, cleaves cellobiose from cellulose), β-1,4,-*N*-acetylglucosaminidase (NAG, cleaves *N*-acetyl glucosamine from chitin and peptidoglycan oligomers), acid phosphatase (PHOS, cleaves phosphate groups from organic phosphorus monoesters), and tyrosine aminopeptidase (TAP, cleaves tyrosine from peptides). The oxidative enzymes included phenol oxidase (PO, a lignin-oxidizing enzyme) and peroxidase (PER, a lignin-oxidizing enzyme that uses hydrogen peroxide). More detailed methods for measuring these response variables can be found in McDaniel et al. (2014*a*) and Appendix S1.

Data analysis

We used a common index to measure the residue mixture effect termed the mixture effect index (MEI). This index was applied to each response variable and calculated similar to other studies (Wardle et al. 1997, Meier and Bowman 2010):

$$\text{MEI} = \frac{V_{\text{obs}} - V_{\text{exp}}}{V_{\text{exp}}}$$

where V_{obs} is the observed response of the mixed residues for each individual variable and V_{exp} is the expected response of the variable to mixing (i.e., the average response of each individual residue within the mixture). Thus, a large positive MEI value represents a nonadditive, synergistic mixture effect, a large negative a nonadditive antagonistic mixture effect, and near zero an additive effect. The V_{obs} plotted against the V_{exp} is a good way to illustrate the mixture effect; for readers interested in these values, please see Appendix S3: Figs. S1–S4.

All data were checked for normality and heterogeneity of variances before statistical analyses. Any variables that were non-normal or showed low heterogeneity were appropriately transformed to meet variance analysis assumptions according to Zuur et al. (2010). Response variables were analyzed using a two-way ANOVA, with residue and rotation as main effects, and their interaction. Variables measured at more than one time point during the incubation were analyzed with repeated-measures ANOVA. The ANOVAs were conducted in SAS 9.3 (SAS Institute, Cary, North Carolina, USA) using the *proc mixed* function, and within main effects, we used post hoc *F* tests to determine significant differences between means. Block was assigned as a random effect variable within the model. Correlations between variables were analyzed using *proc corr*, and Pearson's correlation coefficients are reported. Model effects were deemed significant at $\alpha \leq 0.05$, but Type I error associated with multiple ANOVAs was accounted for using the Sidak correction (Šidák 1967).

Results

The residue mixtures showed little difference in total C and N (Table 1); however, the C+S+W residue had a wider C:N than the other two mixtures (31 vs. 26 for C+S and C+S+W+Rc). The C+S+W residue mixture also had more lignin. The C+S residue had the highest relative abundance of N-bearing compounds in general, but the C+S+W+Rc mixture had the highest protein and lipid contents. Before residue mixtures were added, total soil C, N, dissolved forms, and extractable inorganic N showed relatively little

Table 1. Initial residue mixture chemical characteristics.

		%			Relative abundance					
Residue	Abbreviation	C	N	C:N	Lignin	Lipid	Polysaccharide	N-bearing	Protein	Unknown origin
Corn + soy	C+S	47.7	2.4	25.9	0.365	0.011	0.214	0.068	0.037	0.305
Corn + soy + wheat	C+S+W	46.9	1.9	31.1	0.417	0.011	0.183	0.047	0.026	0.316
Corn + soy + wheat + red clover	C+S+W+Rc	46.8	2.4	26.4	0.357	0.025	0.168	0.047	0.051	0.352

difference among rotations (McDaniel et al. 2014*a*, Table 2). However, there were significant differences among crop rotation treatments in the soil C:N, basal respiration, and microbial biomass N, with increasing crop rotation diversity generally increasing the latter two (Table 2; also see McDaniel et al. 2014*a*).

Respiration dynamics

Mixing residues had highly variable effects (both additive and nonadditive) on respiration over the 360-d incubation (Fig. 2). The instantaneous CO_2 MEI tended to increase from 30 to 90 d for most soil and residue combinations. Then from 90 to 360 d, the MEI in all treatment combinations decreased to near additive (not significantly different from zero) or NAS. There were no residue or crop rotation treatment effects on instantaneous CO_2 MEI (Table 3). The MEI for cumulative CO_2, however, varied by crop rotation only ($P = 0.006$) and had no significant effects of residue or the residue by rotation interaction. The total cumulative CO_2 from each soil without residue was, on average, 4–8% of the CO_2 emitted with residues (McDaniel et al. 2014*a*). Monoculture corn had the largest average cumulative CO_2 MEI across all three residue mixtures (0.08, Fig. 2; Appendix S3: Fig. S1), indicating a synergistic effect on decomposition when mixing residues with this soil. Increasing crop rotational biodiversity from mC decreased the MEI for cumulative CO_2 respired (0.04 to −0.01; Fig. 3).

Inorganic nitrogen

Mixture effects on inorganic N also varied over the 360-d incubation (Fig. 4). At 30 d, the NH_4^+ MEI was generally high, and decreased by 90 d across all mixtures and crop rotations (except for CSW rotation, Fig. 4). The NH_4^+ MEI did not show any treatment effects. Contrastingly, the NO_3^- MEI was antagonistic (−0.21) for all mixtures and crop rotations at 30 d, but increased to synergistic (0.61) by 90 d and remained NAS by 360 d (0.54). The NO_3^- MEI showed significant interactive treatment effects with residue mixture and crop rotation history ($P = 0.024$, Fig. 4), with the soil from the CS crop rotation having greatest NO_3^- MEI only with the C+S+W residue and primarily at 90 d.

For final inorganic N (360 d), there were no significant interactive effects of rotation and

Table 2. Initial soil biogeochemical parameters for the five-crop rotation treatments before residue mixtures were added—from McDaniel et al. (2014*a*).

	g/kg				mg/kg			
Crop rotation	Carbon	Nitrogen	C:N	pH	NH_4^+-N	NO_3^--N	DOC	DON
Monoculture corn (mC)	7.9 (0.8)	0.9 (0.1)	**9.0 (0.4)^{a,b}**	6.44 (0.38)	0.18 (0.04)	1.46 (0.63)	19.4 (0.6)	4.2 (1.9)
Corn–soy (CS)	7.6 (0.7)	0.8 (0.1)	**9.6 (0.6)^{a}**	6.28 (0.41)	0.29 (0.13)	0.97 (0.36)	12.8 (1.3)	2.2 (0.4)
Corn–soy–wheat (CSW)	7.8 (0.3)	0.9 (0.1)	**8.7 (0.7)^{b}**	6.15 (0.38)	0.35 (0.07)	0.61 (0.13)	16.4 (1.1)	4.7 (1.2)
Corn–soy–wheat + red clover (CSW1)	9.6 (0.8)	1.0 (0.1)	**9.5 (0.3)^{a}**	6.02 (0.39	0.48 (0.16)	1.3 (0.32)	17.9 (2.9)	4.9 (0.9)
Corn–soy–wheat + red clover and cereal rye cover crops (CSW2)	8.9 (0.9)	1.0 (0.1)	**9.0 (0.4)^{a,b}**	5.79 (0.40)	0.36 (0.15)	0.91 (0.24)	15.4 (2.9)	4.2 (0.9)

	μg·CO_2-C·g·soil^{-1}·d^{-1}	mg/kg		
Crop rotation	Basal respiration	Microbial biomass C	Microbial biomass N	Microbial biomass C:N
Monoculture corn (mC)	**22.02 (4.37)^{b,c}**	260 (56)	**17 (1)^{b}**	15 (3)
Corn–soy (CS)	**21.82 (2.33)^{c}**	234 (29)	**22 (3)^{a}**	11 (2)
Corn–soy–wheat (CSW)	**24.05 (1.9)^{b}**	231 (18)	**21 (3)^{b}**	11 (1)
Corn–soy–wheat + red clover (CSW1)	**33.12 (2.67)^{a}**	306 (32)	**32 (4)^{a}**	10 (1)
Corn–soy–wheat + red clover and cereal rye cover crops (CSW2)	**34.15 (2.33)^{a}**	440 (93)	**29 (3)^{a}**	15 (2)

Notes: DOC is dissolved organic C (and N). Means and SEs ($n = 4$); significant treatment differences are shown in bold ($P < 0.05$), and lower-case letters indicate post-hoc test results.

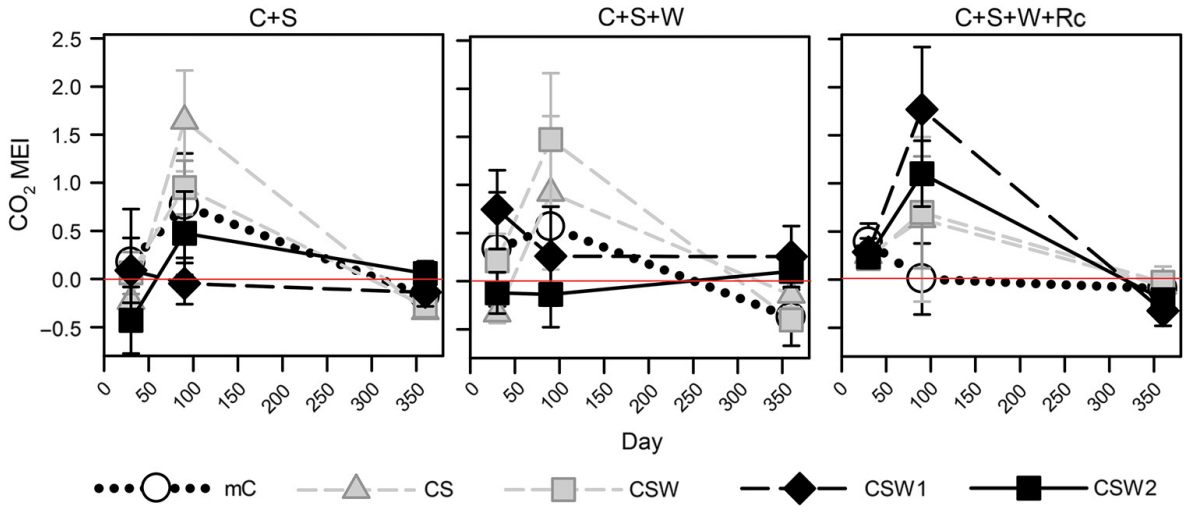

Fig. 2. The mixture effect index (MEI) for instantaneous CO_2 fluxes by residue mixture and crop rotations. Residue mixture abbreviations: corn plus soy (C+S), corn plus soy plus wheat (C+S+W), and corn plus soy plus wheat plus red clover (C+S+W+Rc). Rotation treatment abbreviations are as follows: mC = monoculture corn, CS = corn–soy, CSW = corn–soy–wheat, CSW1 = corn–soy–wheat + rye cover crop, CSW2 = corn–soy–wheat + rye and red clover cover crops. Positive values above the red 0 line are nonadditive synergistic, while negative values below are antagonistic. Expected and observed values used for MEI calculation are shown in Appendix S3: Fig. S1.

residue on NH_4^+ or NO_3^- MEIs (Fig. 5, Table 3). Only crop rotation had a significant effect on final NH_4^+ MEI ($P < 0.001$), which was greatest in the CS soil (Fig. 5; Appendix S3: Fig. S2). Both the main effects of crop rotation and mixture composition influenced NO_3^- MEI (Table 3). Again, within the rotation effect, the highest synergistic MEI was found in CS soil (0.92, $P = 0.005$) vs. antagonistic MEIs for the other crop rotation treatments (Fig. 6; Appendix S3: Fig. S2). The residue mixture also influenced the final NO_3^- MEI, with the average MEI for C+S+W residue (0.08) being significantly greater than the other two mixtures (−0.15 and −0.23 for C+S and C+S+W+Rc; $P = 0.032$).

Microbial parameters and extracellular enzyme activities

The MEIs related to microbial biomass at 30 d showed only significant effects of the crop rotation history, and not the residue mixture. The CSW1 rotation significantly lowered the MEI compared with the other rotations (Fig. 6; Appendix S3: Fig. S3, $P = 0.013$), and this treatment showed the only NAA mixture effect on MBC. The mC rotation had a MBN MEI nearly seven times (681%) of that compared with the other rotations (Fig. 6; Appendix S3: Fig. S3; $P \leq 0.001$). Only the metabolic quotient (or qCO_2), the CO_2 produced per unit of MBC, showed a significant interaction between crop rotation by residue mixture effect ($P \leq 0.001$). Within the C+S+W residue, a strong antagonistic MEI of −4.63 in the CSW2 rotation was much less than the synergistic CS (1.08) and CSW1 (4.03) MEIs (Appendix S3: Figs. S4 and S5).

Extracellular enzyme activities, an indicator for microbial activity and the supply and demand of C and nutrients, showed varied responses among the seven extracellular enzymes we measured (Appendix S3: Fig. S6). Like CO_2 and inorganic N, the EEA MEIs changed over time. The direction and magnitude of change over time differed among the extracellular enzymes, even within the categories of hydrolytic and oxidative enzymes. The range in MEI among all enzymes, within each treatment combination, and across all times was a high NAS with NAG (1.12) to a low NAA with TAP (−0.64). There were significant effects of residue mixture on all EEA MEIs (Table 3), except for BG. The C+S+W residue had higher EEA MEI (0.33) than the other two residue mixtures (0.04 and 0.02 for C+S and C+S+W+Rc, respectively) consistently for nearly all enzymes, except for

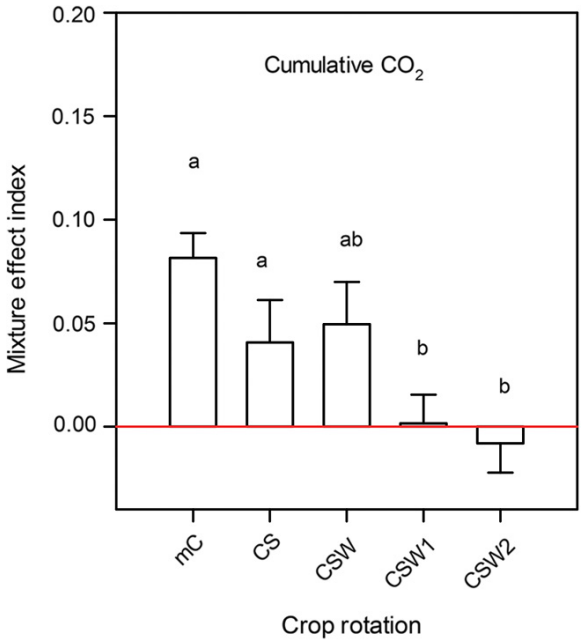

Fig. 3. The mixture effect index (MEI) for cumulative CO_2. See Fig. 1 for crop rotation abbreviations. Positive values above the red 0 line are nonadditive synergistic, while negative values below are antagonistic. Means and standard errors are shown (n=4). Results from post-hoc tests are shown with lower-case letters, indicating significant differences among means. Expected and observed values used for MEI calculation are shown in Appendix S3: Fig. S1.

PO (Appendix S3: Fig. S6). For PO, the highest MEIs were in the C+S+W+Rc ($P < 0.027$). The crop rotation (or soil), in contrast to residue mixture, had little effect on the EEA MEIs (Table 3) except for NAG ($P = 0.003$). Across all residues and time, the NAG MEI was significantly lower in the CS rotation (0.14) compared with all other rotations (MEIs 0.24–0.46; $P < 0.047$).

We grouped enzymes into two categories based on their function in biochemical reactions (Saiya-Cork et al. 2002, Zeglin et al. 2007, Sinsabaugh 2010): (1) hydrolytic enzymes that cleave monomers or dimers from polymers, generally thought to be used in acquisition of labile forms of C and nutrients, and (2) oxidative enzymes that use free-radical reactions to oxidize aromatic C compounds in the degradation of less-labile C forms. There was a strong effect of the residue mixture on the hydrolytic and oxidative enzyme MEIs (Fig. 7, $P < 0.001$), especially the difference between the enzyme MEIs in the C+S+W residue mixture and the other two. All residue mixtures began with relatively similar hydrolytic enzyme MEIs at 30 d, but then the C+S+W residue diverged by 90 d to have higher NAS MEI and remained higher than the other two mixtures. The oxidative enzymes also began with relatively similar MEIs at 30 d, and then the C+S+W remained similar at 90 d while the other two residue mixtures declined (Fig. 7). Then by 360 d, the oxidative enzyme MEI in C+S+W dramatically increased to a NAS effect (0.4), whereas the other two remained similar to 90 d at NAA. Overall, the mixture effects in the C+S+W varied significantly over time and became more synergistic than the other two residue mixtures, especially later in the incubation.

Soil and residue relationship to the mixture effect

Several soil and residue mixture characteristics correlated with the CO_2 MEIs during the year-long incubation (Appendix S2: Table S1). Basal respiration and microbial biomass C and N had significant inverse correlations with the cumulative CO_2-C MEI ($rs > -0.29$). There were few correlations, soil or residue, with inorganic N MEIs. The exceptions being soil microbial biomass C was negatively correlated with final NH_4^+ MEI, and residue lipid and protein contents were negatively correlated with NO_3^- MEI (Appendix S2: Table S1). The residue mixture effect on MBN and qCO_2 both showed several negative correlations with soil characteristics. Overall, the initial soil biogeochemistry correlated better, and more often, with the cumulative CO_2, microbial biomass, and qCO_2 MEIs than the characteristics of the residues (Appendix S2: Table S1).

Unlike the aforementioned variables, the mixture effects on EEA were not related to the soil characteristics (Appendix S2: Table S2), but instead showed strong relationships with the residue mixture characteristics. Both CBH and PHOS mixture effects were negatively related to indicators of high-quality residue (e.g., N, lipid, and proteins), positively related to low quality (e.g., C:N, lignin). The BG MEIs were not related to any variables. TAP MEIs were positively related to many of the residue chemical parameters, but only negatively related to lipids and proteins (Appendix S2: Table S2). Interestingly, even though PO and PER are functionally similar, they showed contrasting relationships to the residue

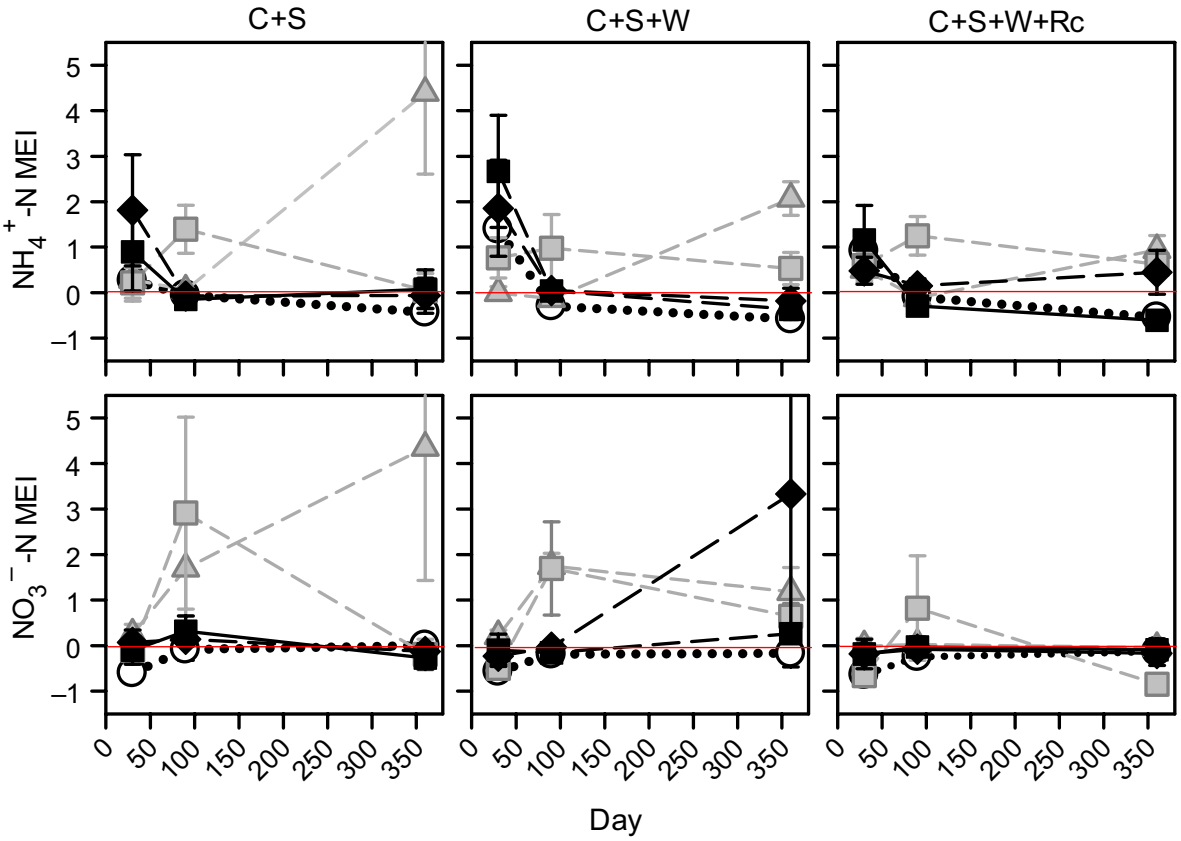

Fig. 4. The mixture effect index (MEI) for NH_4^+ and NO_3^- by residue mixture and crop rotations. See Fig. 1 for crop residue and crop rotation abbreviations. Positive values above the red 0 line are nonadditive synergistic, while negative values below are antagonistic.

mixture chemical characteristics. For instance, PO MEI had the strongest positive relationship with lipids and proteins, but PER MEIs showed strongest positive relationship with C:N and lignin.

Discussion

Effects of crop biodiversity on residue mixture effect

Diversifying crop rotations and residue inputs may enhance soil ecosystem services such as nutrient provisioning and the conversion of residue to SOM (McDaniel et al. 2014*b*, Tiemann et al. 2015). Our overarching goal was to separate the long-term effects of diversifying crop rotations from the shorter-term effects of diversifying residue inputs on residue decomposition processes. We found that crop rotation legacy exerted more control over the mixture effect for CO_2 production, inorganic N, and microbial biomass than the chemical characteristics or diversity of residue mixtures (Table 3), but that residue mixture influenced EEA. Furthermore, mixture effects were not always the same across crop rotations. Among crop rotations, mC showed the strongest synergistic mixture effects on cumulative respiration and microbial biomass (Figs. 3 and 6, Table 3). These results contrast with our primary hypothesis that more biodiverse cropping systems would have greater synergistic mixture effects, due to 11 yr of receiving a greater variety of crop residue inputs. We expected crop diversity would increase the heterogeneity and chemical complexity of microbial substrates and thus expand microbial niche breadth and depth, giving rise to a more functionally diverse decomposer community (Wardle et al. 2004, Hättenschwiler et al. 2005, Hooper 2005) that would increase the synergistic mixture effects in more diverse crop

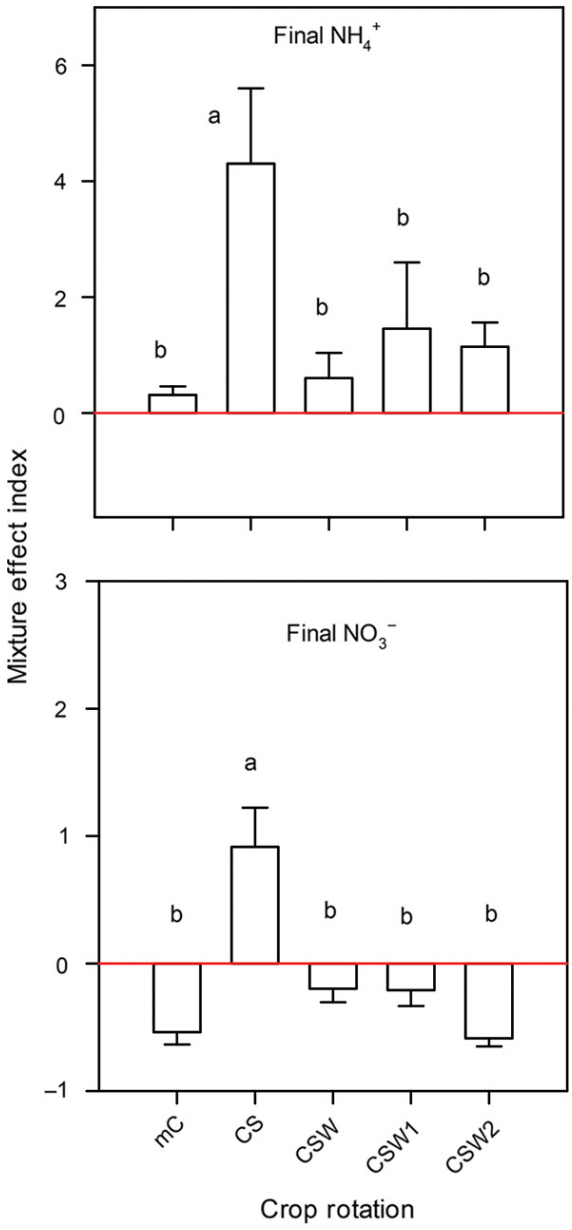

Fig. 5. The mixture effect index (MEI) for final NH_4^+ and NO_3^-. See Fig. 1 for crop rotation abbreviations. Positive values above the red 0 line are nonadditive synergistic, while negative values below are antagonistic. Results from post-hoc tests are shown with lower-case letters, indicating significant differences among means. Expected and observed values used for MEI calculation are shown in Appendix S3: Fig. S2.

rotations. Instead, we saw the opposite trend—increasing crop diversity decreased the synergistic mixture effect for several variables.

The chemical characteristics of our residue mixtures were not a large factor in determining the mixture effect on most variables, but they were a significant factor affecting nitrate, and especially extracellular enzymes (Fig. 7, Table 3). The strong residue mixture effect for extracellular enzymes, and not other variables, emphasizes the direct link between extracellular enzymes and supply and demand of substrates used by microorganisms. This is supported by the observation that many of the chemical parameters of the mixtures correlated well with the enzyme mixture effects (Appendix S2: Table S2). The observation that most of our residue mixture effects, excluding enzymes, were unrelated to mixture composition contrasts with several other studies that found litter or residue characteristics were important in controlling the mixture effect (Wardle et al. 1997, Gartner and Cardon 2004, Liu et al. 2007, Redin et al. 2014). These other studies, however, typically included litter mixtures with a wider range of chemical compositions (Wardle et al. 1997, Liu et al. 2007).

While the chemical composition of residue mixtures had little effect on respiration and microbial biomass, the soil biological and chemical differences created by 11 yr of crop rotation did (Appendix S2: Table S1). The stark difference between soils from monoculture and more diverse cropping systems likely reflects differences in both the soil biota and the quantity, and quality, of substrates available to microorganisms. For instance, in a previous paper from the same crop rotation experiment, we reported that increasing cropping system biodiversity increased soil microbial biomass, increased potentially mineralizable C and N, and increased the activities of extracellular enzymes that are responsible for labile vs. recalcitrant C decomposition (McDaniel et al. 2014*a*). In a more recent paper, we also found that bacteria and fungi (excluding arbuscular mycorrhizae) increased with crop rotation diversity (Tiemann et al. 2015). Similarly, a study in a low-land tropical forest found that after 9 yr, tree diversity largely controlled how fast litter mixtures decomposed (Carney and Matson 2005). The change in litter

Table 3. ANOVA results using residue and crop rotation as main effects (and interaction).

Response variable	Residue mixture			Crop rotation			Mixture × rotation		
	df	*F* value	*P* value	df	*F* value	*P* value	df	*F* value	*P* value
Instantaneous†									
CO_2	2	0.34	0.716	4	0.61	0.658	8	0.52	0.843
NH_4^+	2	0.13	0.877	4	1.05	0.385	8	0.06	0.999
NO_3^+	2	**9.06**	**< 0.001**	4	**16.20**	**< 0.001**	8	**2.29**	**0.024**
Beta-glucosidase (BG)	2	1.78	0.172	4	1.34	0.258	8	0.62	0.76
Cellobiohydrolase (CBH)	2	**9.58**	**< 0.001**	4	0.58	0.697	8	0.31	0.962
N-acetyl-glucosaminidase (NAG)	2	**10.44**	**< 0.001**	4	**4.18**	**0.003**	8	0.23	0.985
Tyrosine aminopeptidase (TAP)	2	**13.85**	**< 0.001**	4	1.77	0.139	8	0.60	0.776
Acid phosphatase (PHOS)	2	**14.57**	**< 0.001**	4	0.82	0.513	8	0.41	0.912
Polyphenol oxidase (PO)	2	**7.92**	**< 0.001**	4	0.66	0.622	8	0.65	0.734
Peroxidase (PER)	2	**16.89**	**< 0.001**	4	0.63	0.64	8	1.73	0.096
Hydroxylases	2	**15.67**	**< 0.001**	4	0.46	0.767	8	0.7	0.674
Oxidoreductases	2	**17.61**	**< 0.001**	4	0.59	0.691	8	0.63	0.751
At 30 d									
Microbial biomass C	2	1.01	0.371	4	**3.58**	**0.013**	8	1.91	0.0831
Microbial biomass N	2	0.70	0.502	4	**13.87**	**< 0.001**	8	1.40	0.227
Microbial biomass C:N	2	1.59	0.215	4	1.89	0.130	8	0.94	0.492
Metabolic quotient (qCO_2)	2	2.10	0.136	4	**6.23**	**< 0.001**	8	**3.80**	**0.002**
Cumulative or final (at 365 d)									
CO_2	2	0.73	0.489	4	**4.21**	**0.006**	8	1.09	0.386
NH_4^+	2	1.39	0.259	4	**14.15**	**< 0.001**	8	1.51	0.183
NO_3^-	2	**3.75**	**0.032**	4	**4.31**	**0.005**	8	1.90	0.086

Notes: *P* values in bold are significant at $P < 0.05$; with Sidak correction for multiple significance, the significance is at $P < 0.0051$.

† Two-way repeated-measures ANOVA.

mixture decomposition, the authors suggested, was likely due to tree diversity increasing the soil microbial community size and altering its composition and the pool of potentially mineralizable C and N. Taken together, these studies show that aboveground biodiversity, whether tree or crop, enhances soil microbial activity and resource availability; this, in turn, alters how litter is decomposed, whether in mixtures or not.

Because all soils showed large responses to the high-quality residue, regardless of crop rotation, it was a soil's response to low-quality residue that typically determined the MEI. For instance, take the cumulative CO_2 produced in the incubation with a corn and soy residue mixture. Soils from the monoculture and diverse rotations had equally large amounts of CO_2 respired from high-quality residues (e.g., soy, Fig. 8a). However, it was with lower quality residues (e.g., corn, Fig. 8b) where there was a large difference between the CO_2 produced by monoculture and diverse soils, with the diverse soils respiring up to as much as 20% more CO_2 from corn residues (McDaniel et al. 2014*a*). This smaller response to corn residue in monoculture soils then lowers the expected average (V_{exp}) down for the residue mixtures (Fig. 8c). This has two effects on the MEI equation which cause a large nonadditive, synergistic mixture effect for mC soils: (1) it increases the difference between the observed and expected values ($V_{obs} - V_{exp}$, the numerator in the MEI equation), and (2) it also decreases the denominator (V_{exp}), resulting in a higher MEI value for the monoculture soils. We attribute the lower cumulative CO_2 with monoculture soils and low-quality residues (and high resulting MEI) to the lower concentrations of potentially mineralizable SOM (McDaniel et al. 2014*a*) and smaller microbial biomass in these soils. Thus, it appears that the decomposition of low-quality residue, like corn, is enhanced with greater microbial biomass and labile organic matter resources in the soil under more diverse crop rotations.

Overall patterns in residue mixture effects from agroecosystems

Despite being highly managed in a low-plant-diversity ecosystem, crop residues do often

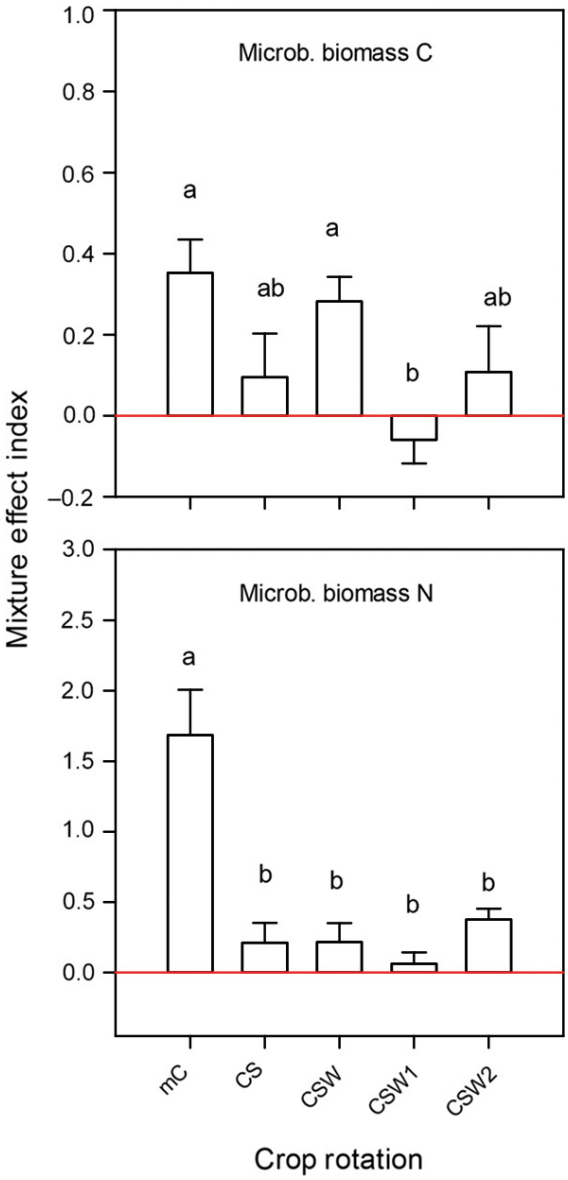

Fig. 6. The mixture effect index (MEI) for microbial biomass C and N. See Fig. 1 for crop rotation abbreviations. Positive values above the red 0 line are nonadditive synergistic, while negative values below are antagonistic. Results from post-hoc tests are shown with lower-case letters, indicating significant differences among means. Expected and observed values used for MEI calculation are shown in Appendix S3: Fig. S3.

come into contact with other crop residues, as well as other plant litter. While there have been relatively few residue mixture studies performed in agroecosystems, they represent the diverse modes of residue mixing in farm fields. For example, some studies have mixed crop residues with tree litter from a natural buffer zone that may blow onto the field (Zeng et al. 2010, Mao and Zeng 2012). Another study mixed different crop tissues (stem or leaves) from the same species to look at the mixture effect between biochemically different tissues (Collins et al. 1992). Like their counterparts in natural systems, the relatively few residue mixture studies in agroecosystems have also shown a predominance of NAS mixture effects (Sakala et al. 2000, Thippayarugs et al. 2008, Zeng et al. 2010, Guo et al. 2011, Mao and Zeng 2012). In order to put our results in a broader context, we compiled a summary of the mean residue mixture effects found in the few available studies in agroecosystems (Table 4).

Across all of the residue mixture studies summarized in Table 4, cumulative mass loss showed synergistic effects with a global mean of +0.13, C mineralization showed a mean nil or slight additive effect, and N mineralization showed a strong mean antagonistic effect (−1.97). This compares to values in a meta-analysis on natural ecosystems of a mean for cumulative mass loss of +0.03, C mineralization of +0.04, and N mineralization of −0.02 (Gartner and Cardon 2004). The direction (or type of mixture effect) is similar among natural and agricultural ecosystems, but the magnitudes in means are quite different. For instance, the synergistic MEI is nearly four times greater for mass loss and the antagonistic MEI is nearly 99 times greater in agroecosystems compared with all natural ecosystems.

What could account for the discrepancy between the natural ecosystems and agroecosystems summarized here? First, these are mixture effect means for all observations from natural ecosystems, but there was an enormous amount of variability among the studies in this meta-analysis (Gartner and Cardon 2004). Second, the majority of the litter mixture experiments in natural ecosystems were conducted using litter bags, while all of the residue mixture experiments in agroecosystems were carried out in the laboratory (Table 4). The stronger mixture effects for mass loss and N mineralization could be a reflection of these laboratory conditions. By controlling soil moisture and incubating at the same temperature in the laboratory,

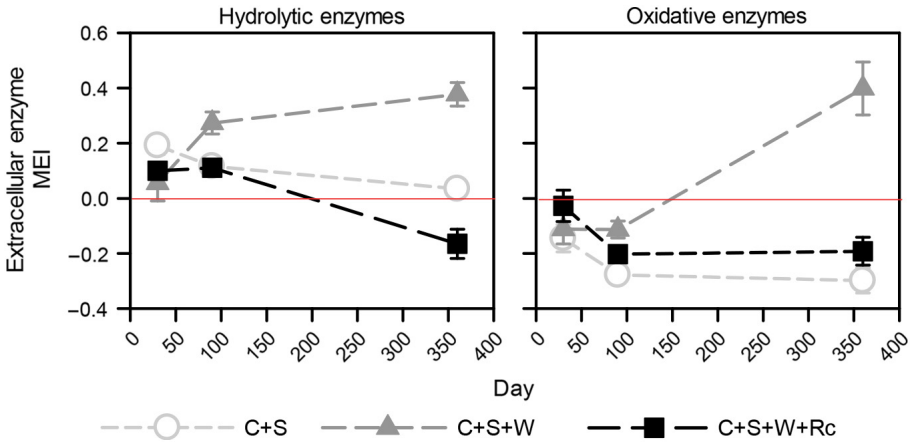

Fig. 7. The mixture effect index (MEI) for hydrolytic and oxidative extracellular enzyme activities by residue mixture (Table 3). See Fig. 1 for crop residue and crop rotation abbreviations. Positive values above the red 0 line are nonadditive synergistic, while negative values below are antagonistic. For MEI on individual enzyme activities, see Appendix S3: Fig. S6.

one eliminates the abiotic environmental factors that would influence the mixture effect in the field. Further, in our incubation, we ground residue through a fine (1 mm) mesh and thoroughly mixed it with the soil which also controlled for any apparent habitat complexity differences (Hoorens et al. 2003, Liu et al. 2007, Rinkes et al. 2011) and made the residue substrates more accessible. Third, the larger mean mixture effect values in agroecosystems could be due to the consequences of land-use change and management practices. Land-use change from natural to agricultural use involves intense soil disturbance and external fertilizer and pesticide inputs, which often lead to reductions in soil biological diversity and biomass. All these factors have been shown to affect soil biota and available resources (Yao et al. 2000, Bossio et al. 2005, Paula et al. 2014), and could enhance residue mixture effects within agroecosystems relative to less disturbed forests and grasslands.

Another factor shown to influence the mixture effect is the time at which the litter or residue response is measured, given that dynamics change rapidly during the course of decomposition (Wickings et al. 2012, Rinkes et al. 2013). The agricultural studies we summarized measured residue mixture responses between 18- and 500-d incubations, which may account for the highly variable mixture effect indices. There is some mounting evidence showing that the mixture effect changes over the duration of an experiment. For example, one study measured the mixture effect within the first 6 months and found mostly nonadditive, synergistic effects (Butenschoen et al. 2014), but at 12 and 24 months the mixture effect was mostly additive. Another study on invasive plant species litter also showed that the mixture effect changes over time, but the direction varies (Schuster and Dukes 2014). Although not completely understood, the temporal variability in the mixture effect is likely indicative of both changes in litter chemistry and decomposer communities as litter decomposes (Wickings et al. 2011, 2012).

In our study, we measured responses both early in the incubation (30 d) and later in the incubation (360 d). We also observed wide variation in the MEIs with time (Figs. 2, 4, and 7). For instance, inorganic N showed large variation in the MEIs among residue, crop rotation history, and time measured (Fig. 4), but this variation appears to be largely idiosyncratic with no apparent pattern. Because C resources are plentiful and decomposition is fast (i.e., higher respiration rates, higher enzyme activities, greater MBC) early in decomposition (Rinkes et al. 2011), there are more resources available for transfer between resource-rich and resource-poor microsites in order to facilitate a NAS or NAA effect. In later stages of decomposition, C resources are less available and nutrients (e.g., N) are relatively more available (Aerts 1997) and thus might not be shared

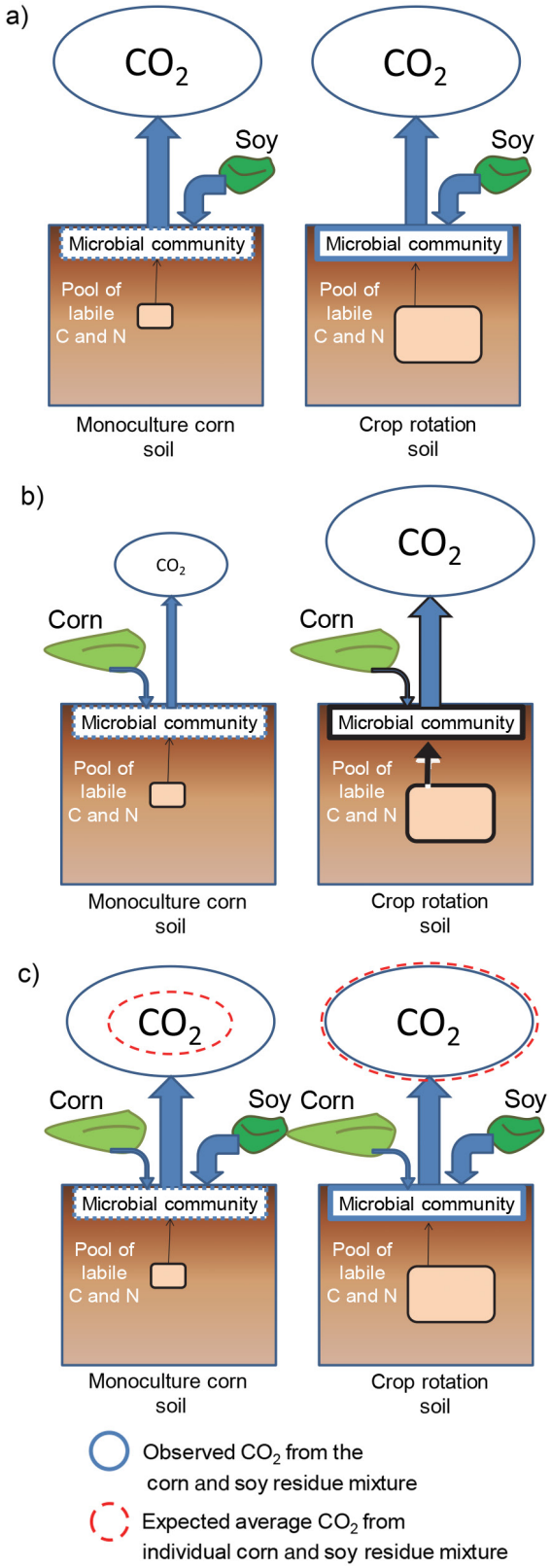

Fig. 8. Conceptual diagram showing the mechanism for a reduced mixture effect in soils from more diverse crop rotations relative to monoculture soils. Showing the cumulative CO_2-C response in a) soy, b) corn, and c) C+S residues. In a), microbes from both soils easily decompose the high quality soy residue. In b), the microbial community from the crop rotation has more available soil resources (labile C and N) to enhance decomposition of poor-quality, corn residue. In c), both soils have high cumulative CO_2 when a mixture of corn and soy is added, but because the decomposition of corn is hampered in monoculture soils it ends up with a high synergistic mixture effect (or high observed CO_2 relative to the expected average of the residues).

between litter types, resulting in more of an additive effect. Some of our response variables do show this tapering of the MEI over time to a more additive effect (CO_2, inorganic N for some soils; Figs. 2 and 4), while others do not (extracellular enzymes; Fig. 7). The extracellular enzyme MEIs also indicate changes over time are largely regulated by the residue mixture chemical characteristics. For example, the lowest quality mixture (C+S+W) showed a different trajectory compared with the other two mixtures (C+S and C+S+W+Rc; Fig. 7). The stoichiometric and microbial drivers of these changes in the mixture effect over time remain unclear and warrant further study.

Conclusions

By controlling for abiotic variables and physical differences in crop residue structure, we explored soil biogeochemical mechanisms that might explain the tendency for soils under more complex crop rotations to accrue more C, N, and microbial biomass (McDaniel et al. 2014*a*). We show that crop rotations have the ability to alter how newly added crop residues and residue mixtures are processed, likely due to the availability of labile soil C and N, as well as microbial abundance and community composition. In the long run, these differences in residue decomposition dynamics will affect crucial agroecosystem services such as the stabilization of residue C and N, reduction in soil–atmosphere greenhouse gas fluxes, and even the maintenance of soil fertility given that crop residues are an important source of C and nutrients to agricultural soils.

Table 4. Summary of residue mixture studies in agroecosystems.

Study	Litter/residues	Method	Secondary treatments	Responses measured and mean mixture effect indices		
				Mass loss	C mineralized	Net N mineralized
Mao and Zeng (2012)	Poplar, soybean leaves, soybean stems	84-d incubation	Two species	+0.07		+0.06
			Three species	+0.04		+0.12
Quemada and Cabrera (1995)	Rye, wheat, oat leaves, and stems	160-d incubation	Clover	+0.05		−0.01
			Rye	+0.06		−0.58
			Wheat	+0.05		−0.06
			Oat	+0.15		−0.03
Redin et al. (2014)	25 crop species' leaves and stems	120-d incubation	Minimum of 25		−0.1	−0.2
			Maximum of 25		+0.2	+0.2
Sakala et al. (2000)	Pigeonpea leaves, corn leaves	237- to 500-d incubation	Soil 1 (26% clay)			−9.2
			Soil 2 (28% clay)			+0.36
			Soil 3 (4% clay)			−7.11
Shi and Marschner (2014)	*Stipa* sp. grass and barley roots and shoots	18-d incubation	Stipa shoots and roots		+ 0.22	−0.26
			Barley shoots and roots		−0.21	+0.17
			Stipa shoots and barley shoots		+0.02	−0.37
Thippayarugs et al. (2008)	Peanut, pigeonpea, hairy indigo stem, leaves, litter, and roots	133-d incubation	Peanut	+0.37		−0.24
			Pigeonpea	+0.23		−8.63
			Hairy indigo	+0.17		−7.75
Zeng et al. (2010)	Poplar leaves, peanut roots and stem, corn roots and stem	50-d incubation	Peanut roots		−0.03	
			Peanut straw		−0.02	
			Corn roots		−0.02	
			Corn straw		−0.04	
Global mean				+0.13	+0.01 to 0.00	−1.97
This study	Corn, soy, wheat, red clover leaves and stems combined	360-d incubation	Soil from monoculture corn		+0.08	−0.54
			Soil from diverse crop rotation		−0.01	−0.59

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1426/supinfo