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### Abstract

The chemical complexity of decomposing plant litter is a central feature shaping the terrestrial carbon (C) cycle, but explanations of the origin of this complexity remain contentious. Here, we ask: How does litter chemistry change during decomposition, and what roles do decomposers play in these changes? During a long-term (730 days) litter decomposition experiment, we tracked concurrent changes in decomposer community structure and function and litter chemistry using high-resolution molecular techniques. Contrary to the current paradigm, we found that the chemistry of different litter types diverged, rather than converged, during decomposition due to the activities of decomposers. Furthermore, the same litter type exposed to different decomposer communities exhibited striking differences in chemistry, even after > 90% mass loss. Our results show that during decomposition, decomposer community characteristics regulate changes in litter chemistry, which could influence the functionality of litter-derived soil organic matter (SOM) and the turnover and stabilisation of soil C.

### Keywords

Decomposition, enzymes, microarthropods, microbial communities, plant litter, soil carbon sequestration, soil organic matter.

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### INTRODUCTION

While the biotic and abiotic factors controlling litter decomposition rates have been well studied (Adair *et al.* 2008), the drivers of changes in litter chemistry during decomposition – and thus the factors that contribute to the chemical complexity of decomposing litter and litter-derived soil organic matter (SOM) – remain unresolved (Poirier *et al.* 2005; Grandy & Neff 2008; Vancampenhout *et al.* 2009). Nevertheless, determining the origin and consequences of this complexity is critical, as the chemical complexity of decomposing litter and SOM is a central feature shaping the function of soils. For example, litter chemistry influences SOM turnover and carbon (C) stabilisation dynamics (Schmidt *et al.* 2011; Schnitzer & Monreal 2011), as well as microbial biomass and decomposer community structure (Ladd *et al.* 1994; Baumann *et al.* 2009), soil nutrient cycling, and the formation of soil structure (Chivenge *et al.* 2011). Accordingly, changes in the chemistry of litter entering soil have the potential to significantly alter multiple ecosystem characteristics, including organo-mineral interactions, soil C stabilisation dynamics, and soil CO<sub>2</sub> emissions to the atmosphere.

While multiple drivers may be important in regulating organic matter chemistry during decomposition, here we focus on a set of testable hypotheses addressing the major controls over litter chemical transformations (Fig. 1). First, the ‘Chemical Convergence Hypothesis’ suggests that litter chemistry is determined by the stage of decomposition and that, although litter chemistry in the early stages of decomposition may reflect initial litter quality, during decomposition all litter – regardless of differences in initial chemistry – will eventually converge towards a *common* chemistry. This hypothesis has come to represent the current paradigm of litter

decomposition and its rationale is that, regardless of any taxonomic variation that may exist among soil microbial and faunal communities, decomposers share a common and limited set of biochemical pathways and physiological abilities that should constrain the chemical trajectory of decomposing organic matter (Fig. 1; McGill 2007; Fierer *et al.* 2009). Because of these constrained biological controls, differences in chemistry during litter decomposition result from the preferential loss of easily degradable compounds (e.g. starch and proteins) and the persistence and relative accumulation of compounds that are more resistant to decay (e.g. lignin, and lignin-encrusted carbohydrates) (Berg & McClaugherty 2008). Thus, as chemically distinct litter types pass through the decomposer ‘funnel’ (Jenkinson & Ladd 1981; Grandy & Neff 2008; Fierer *et al.* 2009), physiological constraints will lead to converging chemical structures (Fig. 1). Consistent with this hypothesis, many litter bag studies have noted that initially diverse litter types possess remarkably similar chemistries following *c.* 75–80% of mass loss (Melillo *et al.* 1989; Preston *et al.* 2009; Moore *et al.* 2011).

The second hypothesis – the ‘Initial Litter Quality Hypothesis’ – posits that initial litter quality has persistent effects on litter chemical composition during decomposition, and thus differences in initial litter chemistry persist throughout the decay sequence regardless of the structure of the decomposer community or the extent of litter processing (Fig. 1). Consistent with this hypothesis, Berg & McClaugherty (2008) showed that initial differences in litter chemistry, particularly nitrogen (N) and lignin concentrations, persist from early to late stages of decomposition. Other work has also shown that initial differences in litter chemistry may even persist once litter is incorporated into SOM (Angers & Mehuys 1990), and recent studies have observed that chemical differences in litter inputs are reflected in

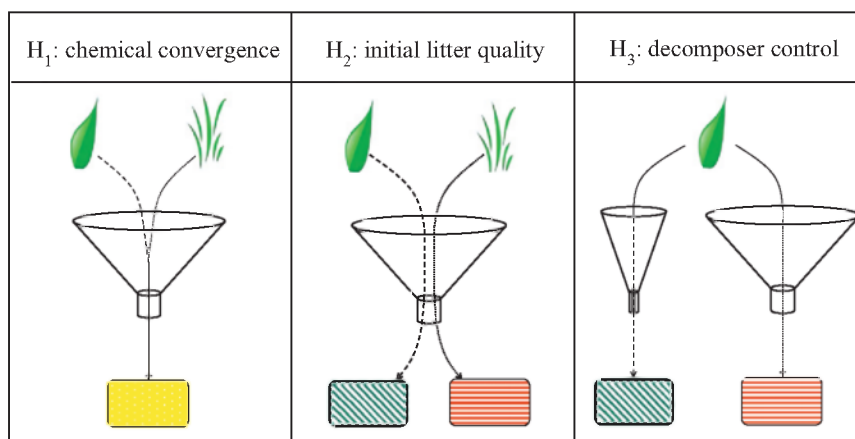
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**Figure 1** Hypothesised controls on litter chemical composition during decomposition. Left panel – chemical convergence hypothesis: due to common physiological constraints among decomposers, initial differences in plant litter chemistry are expected to converge over the course of decomposition; center panel – initial litter quality hypothesis: the chemistry of litter throughout decomposition is a function of initial litter quality; right panel – decomposer control hypothesis: during decomposition plant litter chemistry is shaped by the composition and the metabolic capabilities of the decomposer community.

the chemistry of both aggregated and non-aggregated SOM (Filly *et al.* 2008a; Stewart *et al.* 2011). Taken together, this work demonstrates that initial differences in litter chemistry may persist into late stages of decomposition when plant litter becomes SOM.

The third hypothesis – the ‘Decomposer Control Hypothesis’ – suggests that changes in litter chemistry during decomposition do not simply reflect the effects of time (i.e. the stage of decomposition) and/or initial litter quality, but are also strongly influenced by the functional attributes of distinct decomposer communities (Fig. 1). In other words, this hypothesis predicts that distinct decomposer communities create functionally distinct decomposer ‘funnels’ (and hence diverse litter chemistries) that persist throughout decomposition. If so, changes in environmental conditions that modify decomposer communities (e.g. those associated with human activities) could theoretically result in functionally significant changes in the chemistry of decomposing litter. This may be due to: (1) differences in enzyme activities that could result in selective decomposition of some compounds (Carreiro *et al.* 2000; Gallo *et al.* 2005; Grandy *et al.* 2008); (2) variation in the metabolic capabilities of decomposer organisms (Cavigelli *et al.* 1995; Deleporte & Charrier 1996; Balsler & Firestone 2005); and/or (3) differences in the chemical structure of microbial and macrobial necromass (Kogel-Knabner 2002). There are clear relationships between litter chemistry, microbial communities and enzyme activities (Conn & Dighton 2000; Berg & McLaugherty 2008; Keeler *et al.* 2009) and other work suggests that, in the short-term, decomposer communities can influence changes in C cycling (Strickland *et al.* 2009) and litter chemistry (Wickings *et al.* 2011). However, few decomposition studies have assessed C chemistry dynamics over long-term litter decay in relation to decomposer community composition and activity, although such an effort is critical for assessing the validity of these three competing hypotheses (but see Sinsabaugh *et al.* 2002; Šnajdr *et al.* 2011).

With these hypotheses providing the context, our overall objective was to examine the origin of litter chemical structure during decomposition. Specifically, we decomposed plant litter with varying initial quality in ecosystems with management-induced differences in decomposer communities to better understand shifts in plant-derived organic matter chemistry in the context of decay stage,

initial litter quality and decomposer communities. Our results indicate that the chemistry of plant litter during all stages of decomposition – even over the long-term (i.e. 730 days) – reflects both its initial chemical traits and the unique metabolic traits of its decomposer community, and that distinct decomposer communities may enhance chemical complexity and diversity in decomposing litter rather than reducing it as the current paradigm suggests. By regulating the chemical structure of plant litter inputs to soil, decomposer communities have the potential to influence fundamental soil functions including the formation and stabilisation of SOM.

## MATERIALS AND METHODS

### Study site

The experiment was conducted at the W.K. Kellogg Biological Station (KBS) Long-Term Ecological Research Site (LTER) in Hickory Corners, MI, USA over three growing seasons (730 days) from 2008 through 2010. The experiment was conducted in conventionally tilled (CT), no-till (NT) and early successional old field (OF) treatments (Wickings *et al.* 2011). Treatments consisted of four replicated 1 ha plots arranged in a randomised complete block design (total of 12 plots). Mean annual precipitation at the LTER site is 890 mm per year and soils are classified as Kalamazoo (fine-loamy) and Oshemo (coarse-loamy) mixed, mesic, Typic Hapludalfs (Alfisols) developed on glacial outwash (Crum & Collins 1995).

### Litter bag experiment

Litter bags were constructed from nylon mesh measuring 18 × 18 cm (1.5 mm mesh size) and were filled with 7 g of either corn or grass litter. Grass litter (primarily *Bromus inermis*, Leyss) was collected as live standing shoots (*c.* 50 cm tall at harvest) from old field ecosystems at KBS during May 2008. Corn litter was collected as standing dead biomass during Fall 2007 from large-scale experimental fields at KBS. All litter was air dried and cut into pieces 2–4 cm in length. These litter types were chosen because they are the dominant plant biomass inputs in these systems and also have different chemical compositions. Litter bags were placed in direct

contact with soil in all treatments and secured at their corners with 7 cm stainless steel nails on 5 June 2008. Forty-eight litter bags (24 corn, 24 grass) were placed into each of 12 plots for a total of 576 litter bags according to Wickings *et al.* (2011). Litter bags were collected after 6, 17, 26, 39, 73, 108, 368, 411, 452, 482, 712 and 730 days of decomposition. Upon collection, all litter bags were stored at 4 °C until analysis and all analyses were conducted within 24 h of collection. Litter bags overwintered in all plots but were briefly removed from all sites during spring field activities, placed in individual, open plastic bags, and stored in plastic pails in the field. Once field activities were completed, litter bags were returned to their original locations following the same procedures outlined previously.

### Extracellular enzymes

We analysed the activities of three hydrolytic enzymes [ $\beta$ -*N*-acetylglucosaminidase (NAG),  $\beta$ -glucosidase (BG) and acid phosphatase (PHOS)] and phenol oxidase (POX) at every sample date on subsamples (0.25–0.5 g) of litter using previously published methods (Saiya-Cork *et al.* 2002). Hydrolytic enzymes were assessed using black, 96-well plates and substrates containing the fluorescing molecule 4-methylumbelliferone and phenol oxidase was measured using L-3, 4-dihydroxyphenylalanine in clear, 96-well plates. All enzyme activities were first calculated in units of  $\mu\text{mol h}^{-1} \text{g}^{-1}$  dry litter and were subsequently integrated over all sample dates ( $\text{mol g}^{-1}$  litter) following Sinsabaugh *et al.* (2002) to derive cumulative enzyme activities. Integrated activities are presented as  $\text{mol mg}^{-1}$  litter for simplicity in data presentation.

### DNA extraction and quantitative PCR analysis

Microbial DNA was extracted from litter subsamples (0.05–0.15 g) decomposed for 0, 108, 487, and 730 days using the MoBio PowerSoil DNA kit following the manufacturer's instructions (MoBio Laboratories, Carlsbad, CA, USA; Hofmockel *et al.* 2007; Manerkar *et al.* 2008). The qPCR assays were conducted in 96-well plates on three analytical replicates following the procedures outlined in Fierer *et al.* (2005). Standard curves were created for bacteria and fungi using *E. coli* and *Saccharomyces cerevisiae*, respectively. For all samples, each 25  $\mu\text{L}$  qPCR reaction contained 12.5  $\mu\text{L}$  of PowerSYBR Green PCR Master mix (Applied Biosystems, Foster City, CA, USA); 1.25  $\mu\text{L}$  each of 10  $\mu\text{M}$  forward and reverse primers (Eurofins MWG Operon, Huntsville, AL, USA); 5  $\mu\text{L}$  of sterile, nuclease free water (Promega, Madison, WI, USA); and 5  $\mu\text{L}$  of standard or environmental DNA sample. For bacteria, Eub338 and Eub518 were used as forward and reverse primers, respectively, and for fungi, 5.8s and ITS1f were used as forward and reverse primers, respectively (Fierer *et al.* 2005). Before analysis, sample DNA was diluted to roughly equal concentrations and all reactions were carried out on a StepOne Plus qPCR system (Applied Biosystems). Melting curve analyses and agarose gel electrophoresis were used to confirm that the fluorescence signal resulted from specific PCR products and not amplification artifacts.

### Microarthropod communities and mass loss

Microarthropods were extracted from litter bags at every sample date using Berlese funnels (BioQuip, Inc., Rancho Dominguez, CA, USA) within 3–4 h of litter collection (Wickings *et al.* 2011). Detri-

tivorous microarthropods, including oribatid mites, collembolans, enchytraeid worms, millipedes, and dipteran larvae were tallied and are reported as cumulative number of individuals  $\text{g}^{-1}$  dry litter. Once Berlese extractions were complete, dry litter mass was recorded and subsamples (*c.* 0.5 g) were incinerated at 500 °C in a muffle furnace for the determination of litter ash content. Litter mass loss is reported here as the percentage of ash-free dry mass remaining at a given collection time. An additional subsample (0.25–0.5 g) of air-dried litter was set aside for chemical analysis.

### Litter chemistry

Litter chemistry was assessed using pyrolysis-gas chromatography and mass spectrometry (py-GCMS) on corn and grass litter collected at 0, 108, 487 and 730 days. In addition, we also analysed grass litter chemical composition at 17 days when its mass loss was *c.* 30–35%, which was similar to corn after 108 days (to have an extra data point for testing Hypothesis 1). Samples were pyrolysed at 600 °C for 20 s on a CDS Pyroprobe 5150 pyrolyzer (CDS Analytical, Inc., Oxford, PA, USA), and then transferred to a Thermo Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Austin, TX, USA) and Polaris Q mass spectrometer (Thermo Fisher Scientific). Peaks were analysed using Automated Mass Spectral Deconvolution and Identification System, (AMDIS, V 2.65) and the National Institute of Standards and Technology (NIST) compound library. Compound abundances were determined relative to the total ion signal from all detected and identified peaks and are reported as percentages (Grandy *et al.* 2009; Wickings *et al.* 2011).

### Statistical analysis

We analysed data by both time and decay stage. First, data were analysed using three-way analysis of variance in SAS (Cary, NC, USA) using time, litter and management type as main factors. Treatment means for main effects, and two- and three-way interactions were compared using least square means comparison. Because detritivore densities were not normally distributed they were log-transformed prior to analysis, but back-transformed before presentation here. These analyses provided insight into differences between litter types and management practices over time but litter chemical convergence and its underlying drivers need to be examined at consistent decay stages. Thus, Pearson's correlation in SAS was used to assess relationships between litter chemistry, percent mass remaining, and all biological variables at time points that corresponded with *c.* 30, 80, 95 and 97% mass loss in grass and *c.* 30, 70, and 80% mass loss in corn litter. In addition, apparent enzyme efficiencies were calculated as the slope of the regression between % mass loss and cumulative enzyme activities and reflect the percent change in litter mass per accumulated enzyme activity ( $\text{mol mg}^{-1}$  litter). We also used non-metric multidimensional scaling (NMS) to look for patterns in litter chemistry by litter type, management, and stage of decay and their relationship to litter decomposers.

## RESULTS

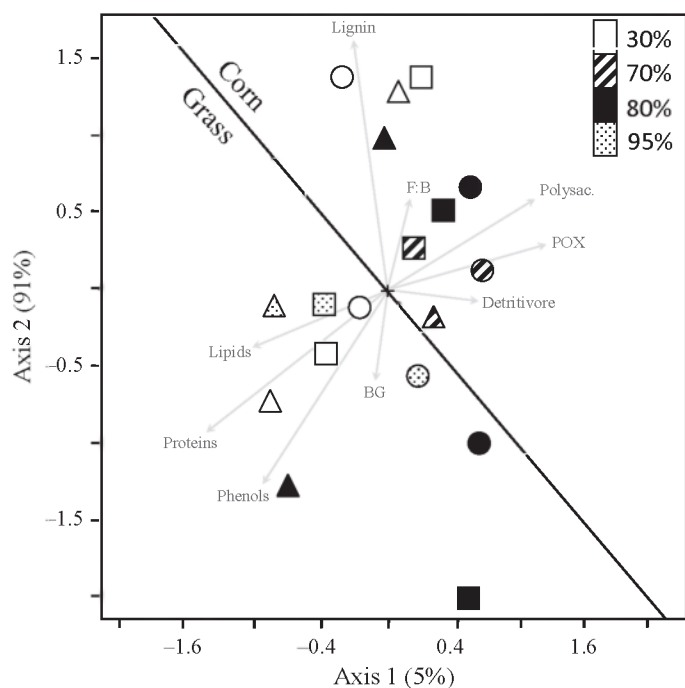
### Decomposition rate and litter chemistry

Initial C:N ratios were greater in corn (61) than in grass litter (18) ( $P < 0.0001$ ; Table 1). Corn litter also had significantly lower relative



**Table 1** Initial and final litter chemistry (carbon-C, nitrogen-N, lignin-L), percentage of litter mass remaining-MREM%, and monthly decay rate- $k \text{ mo}^{-1}$  (means  $\pm$  SE) after 730 days of corn and grass litter decomposition in conventionally tilled (conventional), no-till, and old field treatments

	Corn						Grass					
	C ( $\text{mg g}^{-1}$ )	N ( $\text{mg g}^{-1}$ )	C:N	L:N	% MREM	$k \text{ mo}^{-1}$	C ( $\text{mg g}^{-1}$ )	N ( $\text{mg g}^{-1}$ )	C:N	L:N	% MREM	$k \text{ mo}^{-1}$
Initial	441.1	7.3	60.5	4.2			433.2	23.7	18.3	2.5		
Final												
Conventional	155.5 (23.7)	6.9 (0.8)	22.4 (1.1)	12.2 (4.9)	14.4 (1.8)	0.22 (0.01)	95.7 (12.6)	7.0 (0.4)	13.5 (1.1)	3.0 (1.4)	2.5 (1.4)	0.46 (0.06)
No-till	262.0 (8.5)	9.6 (1.0)	28.0 (2.1)	9.7 (1.8)	19.3 (3.9)	0.19 (0.02)	314.1 (33.8)	22.9 (3.2)	13.8 (0.4)	3.7 (0.6)	0.7 (0.2)	0.56 (0.03)
Old field	311.8 (49.7)	11.0 (1.3)	27.8 (1.5)	17.1 (5.8)	26.6 (2.4)	0.15 (0.01)	350.6 (15.0)	26.2 (1.6)	13.5 (0.5)	3.0 (0.3)	6.5 (2.6)	0.34 (0.05)

**Figure 2** Non-metric multidimensional scaling ordination of litter chemistry by decay stage, litter type and management (also see Fig. S1). Open symbols indicate 30%, line fill indicates 70% (corn only), black indicates 80% and stippled fill indicates 95% (grass only) mass loss. Symbols denote management treatments. Circles – conventionally tilled, squares – no-till, triangles – old field. Primary (chemical) and secondary (biological) variables correlated with ordination axes are plotted over ordinated samples (gray arrows). Distances between sample points reflect variation in litter chemistry (see Fig. S1 for significant management and litter type effects on chemistry).

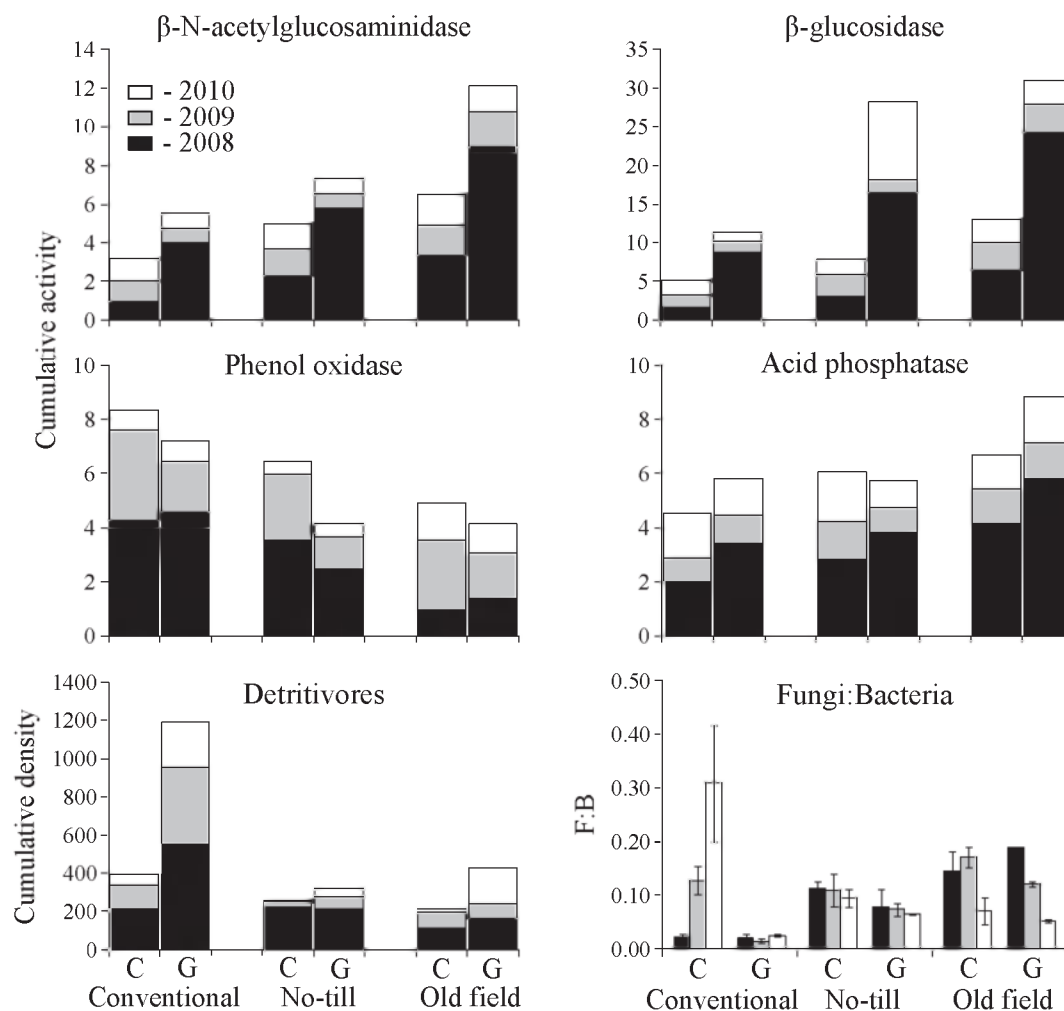
amounts of proteins (0.5 vs. 11%) and a higher abundance of N-bearing compounds of non-protein origin and polysaccharides than grass (10 vs. 4% and 26 vs. 12%; Fig. S1 Supporting Information). Grass litter exhibited greater total mass loss than corn over the three growing seasons (*c.* 3 vs. 20% mass remaining, respectively;  $P < 0.0001$ ; Table 1). Management also exerted modest but significant effects on decomposition of both litter types as total mass loss was significantly greater in CT systems than in OF in corn and greater in both agricultural systems than in OF in grass, ( $P < 0.05$ ; Table 1).

During decomposition, grass litter maintained greater abundances of lipids, phenols, N-bearing compounds, and proteins and lower abundances of lignin and polysaccharides than corn litter (Fig. 2, see Fig. S1). Initial differences in C:N content between litter types also persisted over the course of the experiment ( $P < 0.0001$ ; Table 1).

Litter chemical composition changed substantially over the course of three seasons and differed among the three management systems. Relative to the initial litter, the abundances of lipids and N-bearing compounds declined in corn litter during years 1 and 2. Grass litter showed a general increase in polysaccharides over time and a marginally significant increase from time 0 to year 2 ( $P = 0.058$ ). Total litter C and N content were both significantly greater in OF systems than in agricultural systems ( $P < 0.0001$ ); however, C:N ratios were not affected by management during decomposition (Table 1). At 17 days, the relative abundance of phenols and compounds of unknown origin in grass litter differed significantly among management systems (OF > NT, OF > CT = NT). Additionally, at the end of year one lipids and N-bearing compounds were relatively more abundant in grass litter decomposed in OF than in agricultural systems (Fig. 2, Fig. S1). The relative abundance of N-bearing compounds remained higher in OF than CT systems in year 2, but this effect did not persist into year 3. Polysaccharide relative abundance was higher in CT systems than in OF during year 2 and higher in year 3 than both NT and OF systems. Lipid relative abundance in corn litter also differed significantly during year 3 and was higher in NT than in CT systems (Fig. S1).

### Enzymes and decomposer communities

The activities of all three hydrolytic enzymes were highest in year 1 (Fig. 3). Activities were similar between years 2 and 3, although they were greater in year 3 than year 2 for acid phosphatase, and greater in year 2 than 3 for phenol oxidase (Fig. 3, Table S1 Supporting Information). Activities were also greater in grass than in corn when averaged across all dates (e.g.  $\beta$ -*N*-acetylglucosaminidase: 2.77 vs. 1.62;  $\beta$ -glucosidase: 7.83 vs. 2.89; and acid phosphatase: 2.27 vs. 1.92 mol  $\text{mg}^{-1}$  dry litter, respectively; Fig. 3, Table S1). In contrast, phenol oxidase activity was significantly greater in corn than in grass (2.19 vs. 1.73 mol  $\text{mg}^{-1}$  dry litter, respectively). Litter type  $\times$  year interactions were present for all enzymes;  $\beta$ -*N*-acetylglucosaminidase,  $\beta$ -glucosidase and acid phosphatase activities differed between corn and grass in year 1 and phenol oxidase differed between corn and grass in year 2 (Table S1). Management intensity also had significant effects on enzyme activities regardless of litter type (Fig. 3, Table S1). The activity of  $\beta$ -*N*-acetylglucosaminidase was greater in OF than in NT and greater in NT than in CT.  $\beta$ -glucosidase was greater in OF and NT than in CT, and acid phosphatase activity was greater in OF than both NT and CT systems. In contrast to the response of hydrolases, phenol oxidase activity was greater in CT systems than in NT and OF. Management effects on enzymes also varied by year; while  $\beta$ -glucosidase and acid phosphatase were affected by management in year 1 only, management effects were



**Figure 3** Extracellular enzyme activities ( $\text{mol mg}^{-1}$  litter), detritivore densities ( $\text{individuals g}^{-1}$  litter) and fungal-to-bacterial ratios (F:B) during three years of corn (C) and grass (G) litter decomposition in conventionally tilled (conventional), no-till and old field treatments. For enzymes and detritivores, bars represent cumulative within-year activities (enzymes) or densities (detritivores) (black – 2008, gray – 2009, white – 2010). Fungal-to-bacterial ratios are color coded by year in the same manner.

present in  $\beta$ -N-acetylglucosaminidase and phenol oxidase for years 1 and 2 (Fig. 3, Table S1).

Cumulative detritivore density was greatest in year 1. There was a significant management  $\times$  litter type interaction and densities were higher in CT systems than in NT or OF systems in grass litter (Fig. 3, Table S1). Fungal-bacterial (F:B) ratios were also significantly altered by litter and management type. F:B ratios were consistently higher in corn than in grass; however, the effect of management varied by year; during year 1 F:B ratios were greater in OF than in agricultural systems but in year 3 the pattern was reversed (Fig. 3, Table S1).

#### Interactions between decay rate, chemistry and decomposer communities

The pattern and frequency of relationships between mass loss, litter chemistry, and the composition and activity of the decomposer community differed by litter type (Table 2, Table S2 Supporting Information). Corn and grass litter mass remaining during the experiment were both significantly correlated with integrated

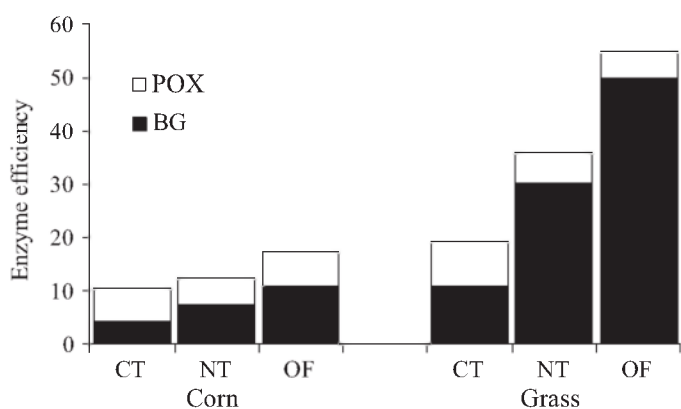
enzyme activities (Table S2). Enzyme efficiencies ( $k_a$ ) calculated from the slope of mass loss against integrated enzyme activities (Sinsabaugh *et al.* 2002), representing the percentage of mass loss per unit enzyme activity, were greater in grass litter than corn litter (Fig. 4, Table S2). Hydrolase efficiencies were also highest in OF treatments followed by NT and CT treatments, while phenol oxidase exhibited the opposite trend (Fig. 4, Table S2). Relationships between chemistry and biology in corn litter within individual stages were rare and only a single significant correlation occurred between 70 and 80% mass loss (Table 2). When all stages were grouped, there were relationships between phenol oxidase activity and the relative abundance of polysaccharides (+) and lignin (–), as well as with the ratio of lignin plus phenols to polysaccharides plus N-bearing compounds (–) (Table 2). In contrast, many correlations between litter chemistry and decomposers occurred within every stage of grass litter decomposition. For example, the activities of  $\beta$ -N-acetylglucosaminidase and  $\beta$ -glucosidase were positively correlated with the relative abundance of N-bearing compounds both within and across decay stages. The ratio of phenol oxidase to total hydrolase activity (the sum of all three hydrolytic enzymes) was also

**Table 2** Pearson coefficients ( $r$ ) for correlations between chemistry (individual classes and ratios), and biology (individual enzyme activities and ratios, detritivore density and F:B) within and across decomposition stages (Full) in corn and grass litter

Correlates	Corn				Grass					
	30%	70%	80%	Full	30%	80%	95%	97%	Full	
Polysaccharides										
POX	0.13	0.33	0.57	<b>0.41</b>	0.33	0.56	<b>0.95</b>	0.61	0.08	
Detritivores	0.35	0.43	0.16	0.34	0.47	<b>0.88</b>	<b>0.70</b>	<b>0.69</b>	0.26	
N-bearing										
NAG	-0.12	0.33	-0.07	-0.04	0.29	<b>0.91</b>	<b>0.77</b>	0.01	<b>0.55</b>	
BG	-0.18	0.36	-0.24	-0.09	<b>0.74</b>	<b>0.89</b>	<b>0.76</b>	-0.32	<b>0.43</b>	
Detritivores	0.53	-0.18	-0.19	0.09	-0.04	<b>-0.71</b>	<b>-0.81</b>	0.44	0.15	
Proteins										
NAG	-0.38	0.12	-0.54	-0.11	<b>0.68</b>	<b>0.85</b>	<b>0.77</b>	-0.14	<b>0.43</b>	
BG	-0.37	0.21	-0.43	-0.07	<b>0.67</b>	<b>0.84</b>	<b>0.86</b>	-0.39	<b>0.37</b>	
POX	0.37	-0.04	0.39	0.28	-0.25	<b>-0.69</b>	-0.56	0.12	0.08	
Lignin										
BG	0.07	-0.14	0.39	-0.03	<b>-0.71</b>	0.24	0.26	0.50	<b>0.37</b>	
POX	-0.23	-0.10	-0.62	<b>-0.42</b>	0.42	-0.12	-0.65	-0.11	0.19	
Lipids										
PHOS	-0.34	0.24	0.57	<b>0.51</b>	0.45	<b>0.75</b>	0.03	-0.26	<b>-0.37</b>	
Phenols										
PHOS	-0.44	0.63	0.47	-0.02	<b>0.77</b>	<b>0.73</b>	0.29	-0.21	<b>-0.66</b>	
lig:N										
POX:HYDRO	-0.05	0.46	-0.36	0.09	<b>0.90</b>	<b>0.83</b>	0.08	-0.01	<b>0.40</b>	
(lig+phen:ps+N)										
POX	-0.26	-0.15	<b>-0.68</b>	<b>-0.39</b>	0.31	0.65	<b>-0.71</b>	-0.20	-0.11	

lig-lignin, N-nitrogen-bearing, POX-phenol oxidase, NAG- $\beta$ -N-acetylglucosaminidase, BG- $\beta$ -glucosidase, PHOS-acid phosphatase, HYDRO-sum of NAG, BG, PHOS.

\* $n = 9$  within stages, 27 and 36 for corn and grass, respectively, across stages (Full). Values in bold indicate significant correlations ( $P < 0.05$ ).



**Figure 4** Phenol oxidase (POX) and  $\beta$ -glucosidase (BG) enzyme efficiencies ( $k_e$ ) calculated over 730 days of corn and grass litter decomposition in conventionally tilled (CT), no-till (NT), and old field (OF) treatments. Enzyme efficiencies are calculated as the slope of the regression between % mass loss and integrated enzyme activity ( $\text{mol mg}^{-1}$  litter). An increase in efficiency indicates greater litter mass loss per unit enzyme activity. The graph shows: (1) greater overall enzyme efficiency in grass than corn litter; (2) variation between management treatments, particularly in grass; and (3) the ratio of phenol oxidase to hydrolase (represented by BG) efficiency is higher in corn litter reflecting its higher lignin content and recalcitrance and higher in CT than NT or OF.

positively correlated with lignin to N ratio during the first year of decomposition, as well as when all stages were combined. Some relationships changed over stages; for example, detritivore densities were correlated with the relative abundance of polysaccharides (+) and N-bearing compounds (-) during two to three different decay stages but not across all the stages. In total, there was one

significant stage-specific correlation between litter biology and chemistry in corn, while there were 25 in grass.

## DISCUSSION

If the 'Chemical Convergence Hypothesis' is true, we would expect that the chemical composition of corn and grass litter would converge during decomposition regardless of differences in initial litter quality or decomposer community characteristics, and that the chemistry of the different litter types would be indistinguishable in late stages of decomposition (Fig. 1). Past studies have argued that convergence in litter chemical composition should occur at roughly 75–80% mass loss (Melillo *et al.* 1989; Preston *et al.* 2009; Moore *et al.* 2011), yet at this stage we found that significant chemical differences persisted (e.g. polysaccharides: 27 vs. 17%; proteins: 1 vs. 5%; and N-bearing compounds: 3 vs. 6% in corn and grass, respectively;  $P < 0.05$  for each) (Fig. 2, Fig. S1). Moreover, novel differences in litter chemistry also emerged during decomposition; lignin and phenols were initially similar in corn and grass litter (Fig. S1) but differed substantially at 75–80% mass remaining (lignin: 47 vs. 28%; and phenols: 2 vs. 8% in corn and grass, respectively;  $P < 0.05$ ). Together, these results indicate that litter chemistry before decomposition influences its chemistry throughout the decay process, supporting the 'Initial Litter Quality Hypothesis' (Fig. 1).

Along with evidence supporting the 'Initial Litter Quality Hypothesis', we also found that the chemistry of a single litter type diverged when it was decomposed in management systems with distinct decomposer communities (Fig. 2). Additionally, once chemistries diverged, they remained distinct over long-term decomposition,

providing strong evidence for the 'Decomposer Control Hypothesis' (Fig. 1). For example, the relative amounts of phenols, lipids, polysaccharides and N-bearing compounds in decomposing grass litter varied between agricultural- and OF-decomposed litter, and the differences in chemistry were associated with lower hydrolytic and higher oxidative enzyme efficiencies, plus greater detritivore densities in agricultural systems (Figs 3, 4 and Table S1). Only a handful of studies have demonstrated that the chemistry of a single litter species can diverge when decomposed in different ecosystems or by different decomposers (Steffen *et al.* 2007; Filley *et al.* 2008b; Wallenstein *et al.* 2010), and ours may be the first to show that management-induced differences in decomposer communities can alter litter chemistry over long-term decomposition.

Our data point to the key role of decomposer communities in regulating changes in litter chemistry during decomposition; however, the specific effects seem to depend strongly on initial litter quality. Thus, the data underscore that the three hypotheses presented here (Fig. 1) are not mutually exclusive, as the controls on litter chemistry during decomposition clearly interact and vary depending on litter quality. Whereas corn litter chemistry suppressed decomposer activity and density, grass litter promoted active biological communities that varied between management practices and exhibited strong relationships with litter chemistry. For example, grass litter polysaccharide abundance differed between management treatments and was related to detritivore density throughout the study. Furthermore, N-bearing compounds differed between treatments in years one and two, corresponding with positive relationships between  $\beta$ -*N*-acetylglucosaminidase and  $\beta$ -glucosidase activities and N abundance. However, the specific relationships between biology and chemistry were dynamic and their strength and direction fluctuated by decomposition stage. For example, while phenol abundance was affected by management and strongly correlated with acid phosphatase at 30% mass loss (17 days in the field; Fig. S1), the relationship appeared to change later in decomposition and the full model correlation between phenol abundance and acid phosphatase was negative. Such fluctuating relationships between biology and chemistry may indicate that decomposition is regulated by both top-down (i.e. emergent properties of decomposer communities) and bottom-up (i.e. litter quality) controls. For instance, while acid phosphatase activity may result in a relative accumulation of phenolic compounds early in decay, litter phenolic concentration may, over the full course of decomposition, suppress phosphatase activities.

In contrast to grass litter, differences in corn litter chemistry among management practices were not apparent until after 730 days of decomposition (corresponding with *c.* 80% mass loss), which was the same time that interactions between corn litter chemistry and decomposers emerged (Table 2). Relationships between corn litter chemistry and biology were stronger across the entire study than within specific stages, and primarily involved either phenol oxidase or lignin. Furthermore, enzyme efficiencies were markedly lower in corn than in grass litter indicating the requirement for greater enzyme activity to decompose the same amount of C (Fig. 4, Table S2). The greater time required for management effects on corn chemistry to emerge, the importance of lignin and lignin-degrading enzymes in corn, and the lower enzyme efficiencies all suggest that corn litter chemistry suppressed decomposer community activity, decomposition rates, and slowed the emergence of decomposer-driven differences in chemistry among management treatments. These findings support the hypothesis that decomposer communities may drive changes in

litter chemistry during decomposition but that their effects are strongly influenced by initial litter quality.

We acknowledge that factors such as climate and soil type might also explain management-induced changes in litter chemistry during decomposition, but to minimise these effects we used a common experimental site with consistent soil types and climate. Nonetheless, nutrient content differed between our agricultural and OF systems due to fertilisation. During corn-growing years, for example, soil N availability was *c.* 4  $\times$  greater in CT and NT treatments than in OF (21 vs. 5  $\mu\text{g g}^{-1}$ , respectively) (Robertson 2009). Furthermore, litter inputs were more diverse in the OF but more abundant in the agricultural sites. Soil nutrient availability can influence microbial N demand and resource partitioning among soil biota with diverse stoichiometric requirements (Paul & Clark 1996; Parton *et al.* 2007), and N availability is known to influence hydrolase and oxidase enzyme activities (Carreiro *et al.* 2000; Sinsabaugh *et al.* 2002; Keeler *et al.* 2009). Consistent with this, we found that OF systems had higher hydrolytic and lower oxidative enzyme activities and efficiencies (Figs 3, 4 and Table S2) relative to agricultural systems. While differences in resource dynamics are unlikely to directly explain variations in litter chemistry during decomposition, they may have played a role in shaping decomposer communities during decomposition.

How do we reconcile our results with other studies that have reported that litter chemistry converges during decomposition irrespective of management type or initial litter quality? First, we suggest that the difference in initial litter quality between this study and others offers one possible explanation. The initial difference in N concentration between corn and grass litter (0.7 vs. 2.4%, respectively) was more than twice as large as that from Melillo *et al.* (1989), and the litter types used in our experiment had lower initial lignin to N ratios than those used in the litter bag studies by others (e.g. Melillo *et al.* 1989; Moore *et al.* 2011). Additionally, we used very different management systems with known differences in decomposer communities whereas past studies have used a narrower range of systems (typically forests).

It is also possible that the high-resolution molecular technique we used to measure litter chemistry explains why we observed differences in chemistry after extensive decay where others have not. Many of the studies reporting convergence in the chemistry of different litter types measured broad elemental classes (e.g. total litter C and N) or used chemical digestion (e.g. proximate analysis) to analyse changes in chemistry over time (e.g. Melillo *et al.* 1989; Moore *et al.* 2011). While changes in total soil C and N concentrations are important and informative, they tell us little about qualitative shifts within these broad elemental groups. For example, we observed substantial differences in the chemical structure of C-containing compounds (e.g. lignin derivatives, lipids and phenols) and N-containing compounds (e.g. proteins and other N-bearing compounds) between and within litter types that could not be detected using elemental and proximate analyses (Fig. S1). Advanced chemical methods such as py-GCMS may, therefore, allow a more mechanistic understanding of the decomposition process. We suggest that more widespread application of these methods in litter decomposition studies could considerably enhance our understanding of litter decomposition dynamics and their relationship to decomposer communities.

There are some caveats associated with the litter bag method (Kampichler & Bruchner 2009; Cotrufo *et al.* 2010) that may have also influenced our results and their interpretation. For example, lit-



ter bag measurements do not encompass organo-mineral complexes, including the incorporation of partially decomposed litter (e.g. particulate SOM) into aggregates (Six *et al.* 2002). Aggregate incorporation of partially decomposed litter could serve to enhance, maintain, or even prevent further differences in litter chemistry (Grandy & Neff 2008). Thus, we cannot confirm whether our observed differences in litter chemistry would persist in old SOM – a contentious aspect of current soil organic matter models (e.g. Six *et al.* 2006; Grandy & Neff 2008; Schmidt *et al.* 2011; Schnitzer & Monreal 2011). However, other py-GCMS studies have observed that chemical variation in SOM pools remains high (e.g. Grandy *et al.* 2007), providing evidence that diverse chemical inputs may have distinct, long-term effects on SOM chemistry. By limiting contact with the surrounding litter, litter bags may also inhibit possible cometabolism of labile and recalcitrant compounds, which may be a common mechanism for the decomposition of recalcitrant compounds (Klotzböcher *et al.* 2011).

This study argues against the current paradigm suggesting that chemical convergence occurs over the course of litter decomposition, and demonstrates that interactions among initial litter quality and decomposer communities increase the chemical complexity of decomposed litter (Figs 1 and 2). Understanding such changes to chemical complexity has significant implications, as the accumulation of chemically distinct organic matter in soil would have numerous important effects on community- and ecosystem-level processes. Perhaps most importantly, this chemical variation could lead to variation in resource utilisation patterns in soil food webs, alter aggregate formation and associated soil characteristics including erosivity and bulk density, and ultimately contribute to variation in SOM stability, soil-atmosphere CO<sub>2</sub> exchange, and global soil C storage.

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## AUTHORSHIP

KW and ASG designed the experiment. KW conducted the experiment and analysed the data. SR and CC conducted quantitative PCR analyses. All authors contributed to interpreting the data and writing the manuscript.

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