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Short communication

Soil respiration and litter decomposition responses to nitrogen fertilization rate in no-till corn systems

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ABSTRACT

Litter decomposition dynamics are influenced by soil nutrient status, yet the specific effects of soil nitrogen (N) on litter decomposition in agricultural systems are not well understood. We explored litter decomposition and related soil organic matter dynamics in no-till, corn-based Midwestern U.S. cropping systems receiving 0, 134, and 291 kg N ha^{-1} y^{-1}. We found that total soil carbon (C) and N, light fraction organic matter, and permanganate oxidizable C were similar among treatments, but N fertilization at rates of 134 and 291 kg N ha^{-1} y^{-1} reduced potentially mineralizable C by as much as 37% and 58%, respectively, compared to the unfertilized treatment. Litter mass remaining after one year of field decomposition was greater with wheat litter (37%) than with corn litter (23%), but was not influenced by N fertilizer rate. In litter, N fertilization led to increases in the activities of two hydrolase enzymes involved in simple carbohydrate metabolism (β-D-cellobiohydrolase and β-1,4-glucosidase) and periodic increases in one related to N metabolism (β-1,4-N-acetylglucosaminidase), but had no effects on enzymes regulating the breakdown of aromatic compounds (phenol oxidase), or on enzymes measured in the soil. N fertilization also decreased arthropod densities in decomposing litter. We found contrasting effects of N fertilizer on processes regulating decomposition, but altogether our results were consistent with a limited or nil role for N fertilization in accelerating litter and soil C turnover, and thus do not support N fertilization as a contributor to depletion of C stocks in agricultural soils.

1. Introduction

Litter decomposition regulates soil organic matter (SOM) dynamics and nutrient cycling, yet the influence of agricultural litter quality and N availability on litter remains contentious. For example, recent long-term field experiments (e.g. Khan et al., 2007; Mulvaney et al., 2009) and new insights into the priming effect highlight the uncertain relationships between fertilizer use, increased plant residue inputs and SOM accumulation (Conde et al., 2005; Cong et al., 2012). Indeed, Khan et al. (2007) found that inorganic N fertilizer use in the Morrow Plots in Illinois corresponded with both increases in plant productivity and declines in SOM. Other studies have shown that synthetic N application in agricultural systems can increase soil CO_2 emissions and soil C loss (Al-Kaisi et al., 2008), but results are mixed and reports show accumulation (Reay et al., 2008; Al-Kaisi et al., 2008; Poirier et al., 2009; Ladha et al., 2011), loss (Hofmann et al., 2009; Khan et al., 2007; Mulvaney et al., 2009) or no change in soil C (Halvorson et al., 2002; Liang et al., 2012).

One explanation for such inconsistency is that N fertilization may fundamentally alter litter decomposition dynamics at the same time that it increases litter C inputs. In forest and grassland ecosystems, studies have shown changes in soil communities, enzymatic activities, and litter decomposition rates following simulated N enrichment (Deforest et al., 2004; Knorr et al., 2005; Garland et al., 2012). N generally inhibits oxidase enzymes and litter lignin decomposition, while accelerating the activity of hydrolases and the degradation of plant litter-derived carbohydrates (Carreiro et al., 2000; Sinsabaugh et al., 2002; Frey et al., 2004; Hofmann et al., 2009; Tiemann and Billings, 2011). Thus, the specific effects of N fertilization in forests appear to depend on initial litter quality, but in agricultural systems the biological mechanisms underlying soil and litter C responses to N availability remain uncertain.

Given the widespread and expanding use of inorganic N fertilizer and the uncertainties surrounding its effects on litter

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decomposition and SOM dynamics, our objectives were to: (1) investigate the effects of initial litter quality and N fertilizer application rates on litter decomposition rates; (2) examine the relationships between litter decomposition dynamics and litter biological processes; and (3) determine whether soil C pools with short to intermediate turnover times respond to N fertilizer rate in corn-based grain systems in the Upper Midwest.

2. Materials and methods

2.1. Site description and design

Our research was carried out at the N Fertility Gradient Study established in 2005 at the Michigan State University W.K. Kellogg Biological Station (KBS) Long-Term Ecological Research (LTER) site. The mean annual precipitation at the KBS LTER site is ~890 mm and soils are classified as Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) mixed, mesic, Typic Hapludalfs (Alfisols) developed on glacial outwash.

The study consists of a no-till corn–soy–wheat rotation, with winter wheat produced in 2007, corn in 2008, and soybean in 2009. Experimental plots are 5 m × 30 m, replicated four times, and arranged in a randomized complete block design. Overhead irrigation at the site is used to maintain soil water content at or near optimum levels for crop production. During the course of our study, monthly measurements of gravimetric soil moisture content ranged from 12% to 16% between June and September and increased to 17% in October and November. On 07/28/2008, 2.2 cm of irrigation water was applied, but irrigation was not necessary at any other time.

There are nine different N levels used in the experiment, ranging during the wheat phase from 0 to 180 kg N ha^{-1} y^{-1}, and during the corn phase from 0 to 291 kg N ha^{-1} y^{-1}. We examined three of the nine N fertilization rate treatments, corresponding with 0, 134, and 291 kg N ha^{-1} y^{-1} in corn and 0, 90, and 180 kg N ha^{-1} y^{-1} in wheat. Given our experiment was initiated in 2008 during the corn phase of the rotation, hereafter we refer to the N treatments as 0 N, 134 N, and 291 N. Our rationale for using these rates of N fertilizer was to have experimental treatments with very different levels of soil N availability, representing: (1) N limitation, in which crop N demand exceeds soil N availability (0 N); (2) Best management practices, in which N application rates are based on crop N requirements (134 N); and (3) N excess, in which N availability exceeds crop demand through the season (291 N). Corn (Pioneer 36W66) was planted on May 16 2008 at a rate of 69,000 seeds ha^{-1}. Nitrogen fertilizer was applied during May and June of 2008. In May, N was injected subsurface at 34 kg N ha^{-1} in all plots except for the 0 kg N ha^{-1} treatment; in June, fertilizer was applied at 0, 100 or 257 kg N ha^{-1} as 28% urea-ammonium-N using subsurface, side-dress injection when corn was at a height of 10–25 cm.

2.2. Litterbags and soil sampling

Standing dead corn plants and wheat straw were collected in fall 2007 from nearby, non-experimental sites managed using Michigan State University Best Management Practices. Wheat and corn were selected because they represent litter types with different initial chemistries (e.g., C:N ratios of 111 ± 5.0 in wheat and 60.9 ± 4.9 in corn), but also because they represent the majority of litter C entering SOM pools of field cropping systems in the Northern Central U.S. Moreover, we used litter sourced from the same field, rather than using residues grown under different levels of N application, to isolate N effects on decomposition processes independent of potential changes in litter quality. Air dried corn leaves and stems or wheat straw were cut into 2–4 cm pieces and homogenized. Corn or wheat litter (7 g) were placed into 18 cm × 18 cm nylon mesh litter bags with a mesh size of 1.4 mm and secured to the soil surface on June 17, 2008. Each plot received six litter bags containing each type of litter (12 bags total per plot) in order to sample six times over the following 12 months. The placement of the litterbags on the soil surface mimics the deposition of aboveground residues in no-till systems, which typically accumulate as surface litter. In spring 2009, litterbags remained on the soil when 80 kg K ha^{-1} liquid fertilizer was broadcast over the plots on May 23 prior to soybean planting. Given the possible effects of soil-injected N on soil processes and their feedbacks to litter decomposition, we also made intensive measurements of soil responses to different fertilizer rates. At sampling, we collected 10 soil cores per plot to a depth of 10 cm and combined them into a single representative soil sample. Soil cores from each plot were sieved to 6 mm, mixed and stored at 4 °C until analyses. Soil and litter bags were collected for physical and biological analyses during the growing season in 2008 (July, August, and September) and early in 2009 (May and June) and transported to the lab on ice where they were sub-sampled. Soil samples used to determine inorganic N concentrations were collected monthly between June and November in 2008.

2.3. Litter and soil C and N dynamics

Litter decomposition rate was determined by mass loss on five dates between July 2008 and July 2009 using the method described in Wickings et al. (2012). Any roots attached to the litter bags were carefully removed and litter was air dried. The mass of the air dried litter was recorded and a 0.5 g sub-sample of the ground litter was incinerated at 500 °C for 4 h in a muffle furnace to determine the ash content of the sample. All masses were converted to a percentage of ash-free dry mass remaining. Light fraction organic matter (LF) was separated from soil by density fractionation using sodium polytungstate (NaPT; $d = 1.7$ g cm^{-3}; Grandy and Robertson, 2007). Total C and N in whole soils and in LF were analyzed after grinding using an elemental analyzer (Costech ECS 4010, Costech Analytical Technologies, Inc, Valencia, CA). We measured permanganate oxidizable carbon (POXC) spectrophotometrically using small modifications of the method by Weil et al. (2003) described in recent publications (Culman et al., 2012; http://lter.kbs.msu.edu/protocols/133). Soil inorganic N concentrations were determined monthly between June and November 2008 in 1 N KCl soil extracts analyzed colorimetrically (SmartChem 140, Westco Scientific, Danbury, CT; McSwiney et al., 2010).

A laboratory incubation experiment was set up to measure potentially mineralizable C (PMC) using soil CO_2 flux as described previously (Grandy and Robertson, 2007). We placed 20 g of air-dried soil into 60 ml serum vials and brought the soil moisture content up to 60% of the water holding capacity. The vials were tightly capped with rubber septa and water was added as needed during the incubation period of 114 d to keep moisture levels constant. On each of 42 sampling dates over a 125 d period, three headspace samples were drawn over 60 min and CO_2 concentrations determined with an infrared gas analyzer (Li-Cor 820). Potential C mineralization kinetics were calculated for each sample using a 2-pool, first-order kinetics model: $C_{min} = C_1 e^{k1^* t} + C_2 e^{k2^* t}$, where C_1, k_1 are parameters of the active pool and C_2, k_2 are parameters for the slow pool.

2.4. Enzyme activities and microarthropods

The activities of four extracellular enzymes involved in C and nutrient cycling were determined following well established methods (e.g. Saiya-Cork et al., 2002; Grandy et al., 2007;

Table 1
Soil inorganic N concentrations in response to three N fertilizer rates (0, 134 and 291 kg N ha^{-1} y^{-1}) in 2008 at the KBS LTER N Fertility Gradient Study.[a,b]

	Treatment	June (μg N g^{-1} soil)	July (μg N g^{-1} soil)	August (μg N g^{-1} soil)	September (μg N g^{-1} soil)	October (μg N g^{-1} soil)	November (μg N g^{-1} soil)
NH_4^+-N	0 N	3.14 (0.34)	2.02 (0.01)	3.61 (0.54)	3.95 (0.56)	4.04 (0.55)	4.06 (0.22)
	134 N	5.01 (0.93)	24.03 (8.01)	2.67 (0.26)	3.67 (0.07)	3.94 (0.39)	3.24 (0.38)
	291 N	4.37 (0.97)	79.60 (14.62)	5.72 (1.47)	3.28 (0.03)	3.72 (0.34)	2.99 (0.46)
NO_3^--N	0 N	7.04 (1.41)	2.22 (0.45)	3.33 (0.32)	2.73 (0.23)	2.90 (0.08)	4.07 (0.14)
	134 N	16.73 (4.34)	16.81 (3.75)	3.24 (0.14)	3.07 (0.08)	3.11 (0.11)	4.44 (0.19)
	291 N	18.61 (3.37)	24.94 (2.97)	24.76 (7.91)	10.14 (0.43)	3.55 (0.21)	6.43 (0.65)

[a] ANOVA indicated a significant N fertilizer rate by time interaction ($P = 0.0001$) for both NH_4^+-N and NO_3^--N.
[b] Numbers are means followed by standard errors.

Wickings et al., 2012). The activity of three hydrolytic enzymes (β-1,4-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG), and β-D-cellobiohydrolase (CBH)) and one oxidative enzyme (phenol oxidase; PHENOX) were assessed using 96-well microplates and enzyme-specific substrates. Enzyme activities were analyzed using litter collected in 2008 on June 17, July 09, August 05, September 03, and September 29 and in 2009 on June 09. Enzyme activities were analyzed using soil collected July 10, August 11, October 13, and November 21, 2008.

Arthropods were extracted from litter bags using Berlese funnels (Bioquip Inc., Rancho Dominguez, CA) over a 5 d period during which funnel temperatures were increased from ~22–50 °C. Arthropods were extracted into 90% ethanol and were separated into major taxonomic groups (oribatida, mesostigmata, prostigmata, collembolan families, diplopoda, chilopoda, enchytraeid worms, and insect orders and families) using a dissecting microscope. Arthropod densities were determined as the number of individuals g^{-1} ash-free dry litter using ash content determined by combustion of a 0.5 g subsample of litter at 500 °C.

2.5. Statistical analysis

Analysis of variance on soil and litter variables was conducted in PROC MIXED (SAS Institute Inc., 2002). Normality of the residuals was checked using normal probability and box plots. Data were log transformed where deviations from normality were observed. The homogeneity of variances was checked using Levine's test. Soil variables with multiple sampling points (i.e. enzymes, LF, inorganic N, POXC) were analyzed as repeated measures in the PROC MIXED. Variance–covariance structure for the repeated measures was selected on AIC and BIC criteria. The results are reported statistically significant at $\alpha = 0.05$.

3. Results and discussion

Consistent with earlier N gradient studies (McSwiney et al., 2010), fertilization strongly influenced total soil inorganic N levels and provided an opportunity to compare litter decomposition and SOM within a N-limited (0 N) and N-saturated (291 N) environment (Table 1). On all but one sampling date, NH_4^+-N concentrations ranged from 2 to 6 μg N g^{-1} soil, but in July concentrations were 79.60 in 291 N, 24.03 in 134 N, and 2.02 μg N g^{-1} in 0 N. NO_3^--N concentrations were also higher in the fertilized treatments and in both June and July approached 25 μg N g^{-1} in 291 N.

3.1. Soil C and N dynamics

Total soil C was not altered by N fertilizer doses over the short time frame of this experiment (0 N: 11.05 ± 0.47; 134 N: 11.60 ± 0.86; 291 N: 10.63 ± 0.60 g kg^{-1}), but potential soil respiration rates and PMC pools determined by laboratory incubations showed consistently greater cumulative respiration rates in unfertilized soils relative to fertilized soils (Fig. 1 and Supplement Table 1). Compared to 0 N, potential soil respiration was reduced in 291 N

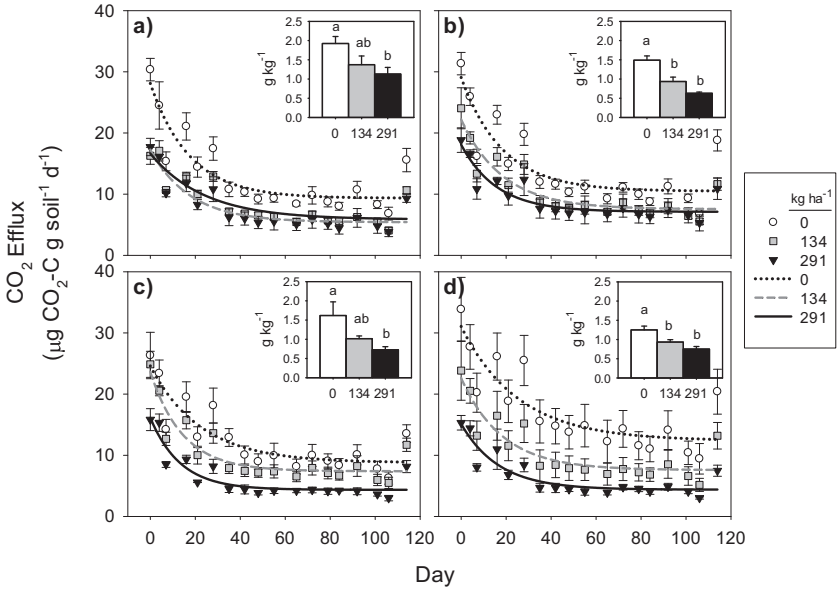

Fig. 1. Soil carbon mineralization rates in 125 d laboratory incubations from sites receiving 0, 134, or 291 kg N ha y^{-1}. Data are reported as response curves (main graphs) and cumulative soil CO_2 emissions (insets). Samples were collected on (a) June 17, (b) July 09, (c) August 05, and (d) September 03, 2008. Modeling the response curves showed significant differences in pools with fast-to-intermediate turnover times (see Supplement Table 2).

by 42% in June, 58% in July, 55% in August, and 39% in September. While other studies have found N fertilization decreases PMC (Ladd et al., 1994; Smolander et al., 1994; Al-Kaisi et al., 2008; Liu and Greaver, 2010; Chen et al., 2012), the mechanisms underlying these negative N fertilization effects on microbial activity and PMC likely vary across sites. N fertilization could lower soil pH, which could increase aluminum toxicity and reduce cation availability (Liu and Greaver, 2010), but we did not detect any differences in pH among sites (0 N: 5.78; 134 N: 5.72; 291 N: 5.70). N fertilization may also play a role in the formation of precursors involved in SOM stabilization reactions (Haider et al., 1975; Skene et al., 1997; Jokic et al., 2004) but the importance of these reactions in field-scale SOM dynamics have not been well established (Schmidt et al., 2012).

Excess N may also reduce SOM mineralization by alleviating the microbial demand for N bound in SOM (Al-Kaisi et al., 2008; Phillips et al., 2012) or by altering microbial biomass and community structure. For example, Treseder (2008) reported in a global meta analysis that microbial biomass declined by an average of 15% following N fertilization, and declines in soil respiration rates were correlated with these changes in soil microbial biomass. In another synthesis, Liu and Greaver (2010) found that N addition reduced microbial biomass C by 20% and microbial respiration rates by 8%. Moreover, Ramirez et al. (2010) showed that N fertilization increased *Gammaproteobacteria* and *Actinobacteria* abundance while decreasing *Cyanobacteria*, *Nitrospira*, and *Acidobacteria* abundance at our sites, providing an additional, microbial community-level mechanism for changes in potential soil respiration rates.

Although more effort is needed to understand the declines in potential soil respiration that are reported here and elsewhere (e.g. Treseder et al., 2007; Ramirez et al., 2010), we did not find that these declines are associated with changes in total SOM, LF, or POX C pool sizes (Supplement Table 2) or in soil enzyme activities (Supplement Table 3), none of which responded to N. More likely, changes in microbial biomass, perhaps coupled with changes in microbial community structure, altered potential respiration rates following N fertilization. Whether or not these community- and process-level dynamics will influence long-term soil C stocks needs to be monitored, but we did not observe any changes in two soil C pools (LF and POX C) that serve as early indicators of potential future changes in total soil C. We did observe trends ($P < 0.1$) toward greater soil LF C and N concentrations with N fertilization, which point to possible shifts in the chemistry of plant litter inputs or in the microbial processing of these inputs.

3.2. Litter decomposition dynamics

Before decomposing in the field, the C/N ratio of wheat (111 ± 5.0) was higher than corn (60.9 ± 4.9). Reflecting these differences in tissue chemistry, corn litter (23% mass remaining after one year) decomposed significantly faster than wheat litter (37% mass remaining; $P < 0.001$; Fig. 2 and Supplement Table 4), but N fertilizer rate did not influence decomposition rates in either litter type. Consistent with rates of mass loss and C/N ratios, enzyme activities tended to be higher in corn than wheat litter (Fig. 3), and CBH activity was significantly higher (28%) in corn than in wheat litter ($P = 0.02$). Densities of detrito-fungivores, including oribatid mites ($P = 0.03$) and millipedes ($P = 0.02$), as well as predatory mesostigmatid mites ($P = 0.03$), were also greater in corn litter than in wheat litter.

Previous studies show that N additions frequently increase the activity of hydrolase enzymes and the decomposition rate of carbohydrates, while decreasing phenol oxidase activity and the decomposition rate of lignin and its derivatives (Frey et al., 2004; Zak et al., 2008). For example, in a recent meta-analysis, Whittinghill et al. (2012) found that exogenous N inputs increased

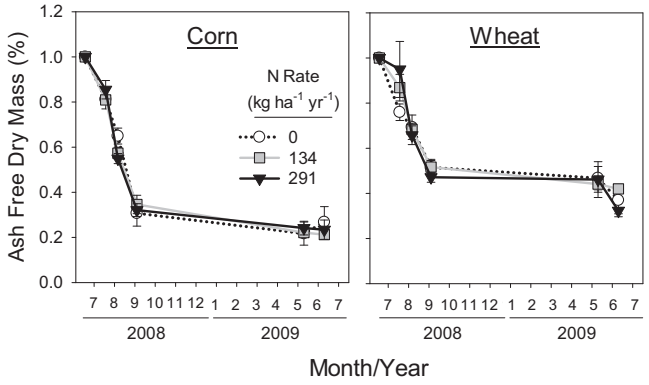

Fig. 2. Corn and wheat litter mass loss responses to N fertilizer rate. ANOVA indicated significant differences ($P < 0.0001$) between litter types with higher mass remaining in wheat litter but no effects of N fertilizer rate.

cellulose decomposition by 9% but decreased the decomposition rates of lignin by 30%. As a result, N fertilization should be most influential in the early stages of litter decomposition when cellulose is abundant, and in litter with initially low lignin:polysaccharide ratios (Waldrop and Firestone, 2004; Treseder, 2008). Consistent with this, the cellulose-degrading enzymes CBH and BG were significantly increased by N fertilizer and the N-cycling enzyme NAG exhibited significant nitrogen by time interactions (Fig. 3 and Supplement Table 5). The cellulose-degrading enzyme BG was also increased by N fertilizer use, providing additional evidence for N-accelerated activities of enzymes involved in the decomposition of labile substrates.

The role of soil nitrogen availability in structuring arthropod communities is poorly understood, and the effects of N amendment

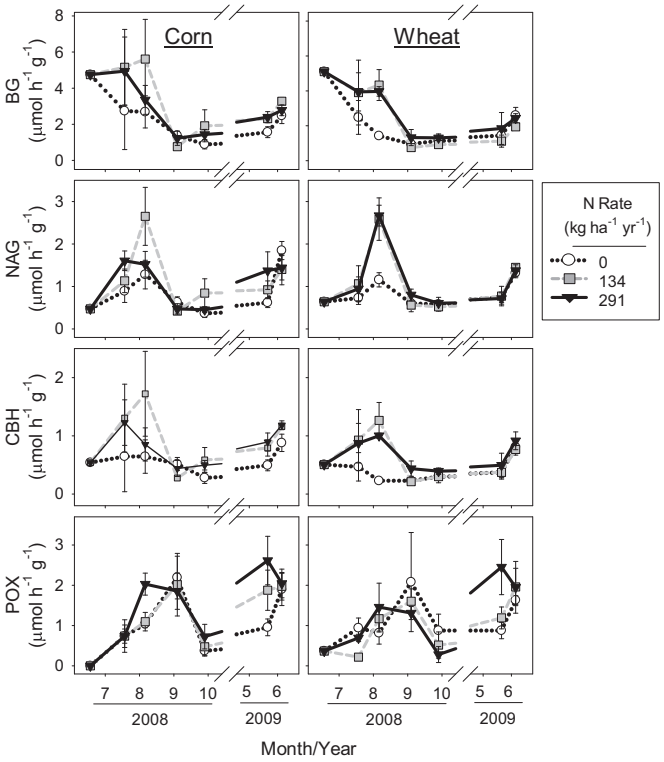

Fig. 3. Enzyme activities on wheat and corn litter in response to N fertilizer rate. Enzymes are β-glucosidase (BG), N-acetyl-β-D-glucosaminidase (NAG), β-D-cellobiohydrolase (CBH), and phenol oxidase (PHENOX). ANOVA indicated a significant N effect ($P < 0.05$; see Supplement) for CBH and BG and a significant N by time interaction for NAG.

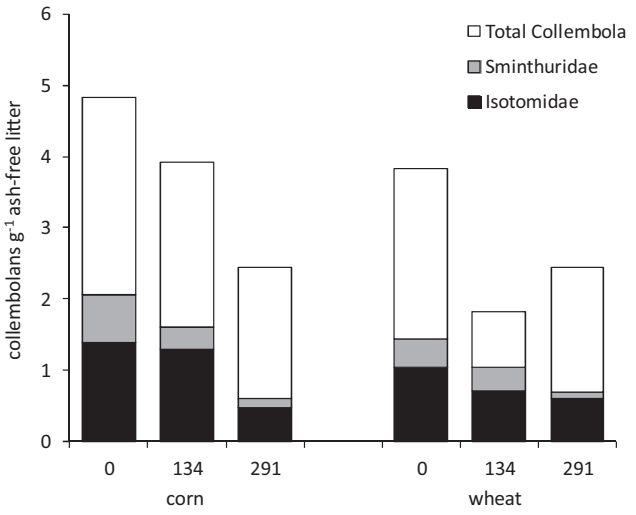

Fig. 4. Densities of litter-colonizing collembolans (total collembola, and families Isotomidae and Sminthuridae) in response to N fertilizer rate. Total collembolans (0 N > 134 N/291 N, $P = 0.02$), Isotomidae (0 N > 291 N, $P = 0.05$), Sminthuridae (0 N > 291 N, $P = 0.01$).

on arthropod density and diversity are highly variable (Jandl et al., 2003; Cole et al., 2008; Barbercheck et al., 2009; Cao et al., 2011). In one of the first studies to assess the effects of N on litter colonizing arthropods in a no-till agroecosystem, we observed N fertilizer rate had no effect on some litter taxa while decreasing densities of total collembolans, including the collembolan families Isotomidae and Sminthuridae (Fig. 4). This finding may reflect either a decrease in total soil collembolan densities or, alternatively, an increase in soil resource availability (i.e. increased root tissue quality, or shifts fungal biomass or community composition), thus, decreasing the importance of litter as a resource for collembolans (Frey et al., 2004; Ngosong et al., 2011).

Several phenomena related to our experimental design and the unique attributes of agricultural systems may explain why N fertilizer rate did not influence decomposition rates. First, our fertilizer N was soil-injected and thus not applied directly to the litter. We did this to be consistent with no-till management practices in the region but it is possible that surface-applied N would have more strongly influenced enzyme expression and decomposition rate. Second, some studies have suggested that N-accelerated litter decomposition in agricultural systems may be due to changes in the C/N ratio of litter inputs (Russell et al., 2009). We were unable to address this possibility because our study used the same wheat and corn litter in each of the N treatments in order to target soil biological responses to N independent of changes in litter quality. Third, differences in decomposer communities may have been more strongly expressed in either very high quality litter (e.g. litter possessing a C:N ratio <15–20) or very low quality litter (e.g. litter possessing a C:N ratio >75 or inhibitory compounds) compared to the corn and wheat residues that we examined (Wickings et al., 2012). Finally, N fertilization may have little impact on decomposition dynamics in agricultural systems because of the overriding effects of factors other than N on microbial community structure and function. Such factors could include the simplification of the plant communities, altered resource inputs, and soil disturbance ubiquitous in agricultural systems.

4. Conclusions

Consistent with our results suggesting that N fertilization is not likely to accelerate litter decomposition rates or deplete soil C stocks, Gregorich et al. (1996) used natural abundance methods to demonstrate that the half-life of corn-derived C in continuous corn cropping systems was the same irrespective of N fertilization rate. The authors concluded that fertilization increases crop residue production, without accelerating its decomposition rate, resulting in increased SOM concentrations. Many other studies have shown that N fertilization increases SOM content by increasing crop residue inputs (e.g. Sainju et al., 2006; Christopher and Lal, 2007; Kaur et al., 2008; Banger et al., 2010). The long-term coupling of these increased litter inputs to declines in soil respiration rates should *increase* soil organic matter concentrations. In support of this argument, Ramirez et al. (2012) argued that their observed N-induced declines in respiration rates, microbial biomass and enzyme activities, and altered microbial community structure in 28 different North American soils should increase soil C sequestration.

We are quick to acknowledge that short-term, process-level studies such as the one we present here may not always reflect long-term dynamics, and future efforts to understand N effects on decomposition and SOM dynamics need to encompass a range of temporal and spatial scales. Nonetheless, when the results of our study are interpreted along with others examining long-term soil C responses to N amendments (e.g. Gregorich et al., 1996; Banger et al., 2010), there is little evidence that N fertilizer-induced changes in soil biological processes, per se, will accelerate litter decomposition or SOM losses in corn-based cropping systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2013.04.020.

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