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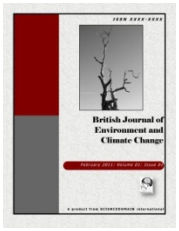

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Effects of Nitrogen Availability on the Fate of Litter-Carbon and Soil Organic Matter Decomposition

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Authors' contributions

This work was carried out in collaboration between both authors. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine whether addition of inorganic nitrogen (N) directly to maize litter (stalk and leaf) with differing tissue quality impacts litter and soil organic matter (SOM) decomposition. We tested whether N addition leads to 1) faster litter decomposition, 2) less SOM-C decomposition and 3) increased incorporation of organic-C into soil-C fractions thereby increasing C sequestration potential in maize-based systems.
Methodology: We investigated decomposition of two types of maize litter (stalk and leaf) with differing tissue quality both in the field and in a laboratory incubation experiment. In the field, litter was placed on the soil surface and at 10 cm soil depth to investigate the effect of litter burial and N addition on litter decomposition. Litter was harvested at six and twelve month intervals. In the incubation experiment, maize and stalk litter was ground and incorporated into the soil and incubated at 25ºC for 120 days. We measured CO_2-C evolved and employed $\delta^{13}C$ natural abundance differences between litter-C and SOM-C to measure both litter-C and SOM-C decomposition. At the end of the experiment, we examined soil-C storage via soil physical fractionation.
Results: Exogenous N addition to litter had little effect both litter and SOM decomposition in the field and the laboratory except for in the stalk litter treatment where there was an 8% decrease in litter-C loss and a 5% increase in SOM-C loss in the laboratory incubation experiment. N addition to litter increased decomposition of litter in the first 20 days of litter decomposition in the laboratory incubation experiment, but

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reduced litter decomposition rates after day 20. N addition to litter had very little effect on C storage in soil aggregates. In the field, litter placement, and physical litter structure influenced decomposition much more than N inputs. Thus, adding N to litter is not an effective strategy to sequester C in maize-based systems.

Keywords: Carbon sequestration; litter decomposition; soil organic matter; nitrogen mining; exogenous nitrogen addition.

1. INTRODUCTION

Agroecosystems, like natural ecosystems, have two large pools of carbon (C) post-harvest: 1) soil organic matter (SOM)-C and 2) litter-C. The litter-C pool is divided between above and belowground litter, and is largely untouched in no-till systems. Post-harvest the litter-C pool can represent 20-23% of ecosystem C and is a highly dynamic and ephemeral C pool [1]. In order to attain long-term carbon sequestration, litter-C must be physically and chemically protected and incorporated into part of SOM-C. Therefore, understanding decomposition patterns and the ultimate fate of litter-C is necessary in order to estimate the dynamics and capacity for carbon sequestration within the ecosystem. While successful techniques for managing agricultural systems for increased crop productivity and yield such as irrigation and nutrient addition are well known, management of the decomposition process in order to increase sequestration of litter-C into SOM-C or the maintenance of SOM-C has proved more difficult [2-5]. Studies have addressed how N addition to crops leads to increased productivity and crop N status as well as SOM-C changes over time [4-6]; yet more studies need to evaluate how exogenous-N addition directly to litter can affect both litter-C decomposition and SOM dynamics in maize based systems [7-9].

Maize-based agroecosystems are characterized by high exogenous inorganic N inputs from fertilizer and large inputs of litter with high C/N ratio and low lignin content. Litter inputs, such as these, provide ample amounts of labile-C, but very little N, which creates a N limiting environment for decomposers [10-15]. This severe N limitation could potentially lead to a phenomenon called “microbial nitrogen mining” whereby microbes use labile-C to access recalcitrant SOM to acquire needed N [16-18]. These microbial nitrogen mining effects could lead to no net C gain or even net C loss if litter-C inputs lead to increased SOM-C decomposition [19-21].

While the effects of inorganic-N additions on litter decomposition are often inconsistent [22-26], even less is known about how inorganic N additions affect SOM decomposition or the stabilization of litter-C as SOM-C [4,9,16]. Further, while we know that litter quality such as the C/N ratio, soluble concentrations, and lignin concentrations of litter can be used as a predictor of litter decomposition rates [27-29], we have very little knowledge of how initial tissue quality of litter could affect litter-C stabilization as SOM-C and SOM decomposition. As such, we investigated the impact of inorganic N addition on the decomposition of maize litter (stalk and leaf) with different initial tissue quality in both a field experiment and a laboratory incubation experiment. Because litter placement (surface applied or buried) has been shown to effect decomposition rates [30], in the field experiment, we also investigated the potential effect of litter placement and N addition on decomposition. In the laboratory incubation experiment, we examined how the addition of exogenous N to litter with differing initial tissue quality affects the decomposition of SOM, and the stabilization of litter-C into SOM.

Because maize litter has a much higher C/N ratio (C/N~40-100) than organic matter in soil (C/N~10) and because microbes require both C and N, we hypothesized that the microbial community accesses N from SOM-N pool, in order to utilize litter-C for energy. The addition of an easily usable N source such as an inorganic-N fertilizer will supplement microbial demand for N, thereby reducing the need for SOM-N. Thus, exogenous N addition may lead to faster litter decomposition and decreased SOM decomposition. The soil used in this study had been consistently cropped with wheat for 30+ years. By using this soil we could differentiate between microbial decomposition of litter (C4 $\delta^{13}C$ signature) and SOM-C (C3 $\delta^{13}C$ signature) while simultaneously monitoring total litter and soil organic matter pools. We tested whether N addition leads to 1) faster litter decomposition in both the incubation and the field, 2) less SOM-C decomposition, and 3) increased incorporation of organic-C into soil-C fractions thereby increasing C sequestration potential in maize-based systems.

2. MATERIALS AND METHODS

2.1 Field Methods

In the fall of 2006, stalk and leaf litter was collected from maize plants in an irrigated agricultural field at the University of Nebraska Agricultural Research and Development Center near Mead, NE. Six mature plants were harvested by hand near areas designated as intensive measurement zones (IMZs) just before grain harvest in October of 2006. (Detailed methods about IMZ location selection within each field are reported in Verma [31]; Kochsiek [32]. The aboveground portion of the each plant sampled was separated into leaves and stalks and oven dried at 60ºC to constant mass. A subsample of the dried litter ground in a Wiley mini-mill with a 40 mesh (2 mm) screen (Thomas Scientific, Swedesboro, NJ) and analyzed for total C and N content on a Costech 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA.). A 32% solution of urea-ammonium nitrate (UAN) was applied to half of the dried stalk and leaf litter at a rate of 5 g N/m^2 yr. UAN solution was applied in the laboratory with a hand-held sprayer with a known spray rate. Litter was laid on a lab bench in a single layer and UAN solution was broadcast over the top of the litter with a hand-held sprayer at a rate of 5 g N/m^2. UAN solution was allowed to dry completely before the litter was packed into litter bags. Sixteen replicate litter bags were prepared for both fertilized and unfertilized stalk and leaf litter for a total of 64 litter bags per IMZ. Each litter bag was 20 cm x 20 cm with a mesh size of 1 mm and 5-10 g of plant tissue was packed per litter bag [33]. These 64 litter bags per IMZ were placed near the six IMZ locations within the irrigated continuous maize field where litter was originally harvested for a total 384 litter bags in the field. Half of the litter bags for fertilized and unfertilized leaf and stalk litter were placed on the soil surface (n=8), while the other half were buried at 10 cm soil depth (n=8). We then harvested half of the litter bags (n=4) for each depth, litter type, and nitrogen addition at six and twelve month intervals and the six IMZ locations within the field (n=24 for the entire field for each type and each harvest). After the bags were harvested they were oven dried at 60ºC to a constant mass, weighed, ground in a Wiley mini-mill with a 40 mesh (2 mm) screen (Thomas Scientific, Swedesboro, NJ), and analyzed for total C/N on a Costech 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA.). After total C/N analysis, ash content was determined by burning a sample at 475ºC in a muffle furnace and used to correct mass loss data for ash content.

2.2 Laboratory Incubation Methods

Soil was sampled at the High Plains Agricultural Laboratory in Sidney, Nebraska in a site consistently cropped with wheat for over 30 years. The soil type at this site is categorized as

Pachic Haplustoll with a soil texture of 25% clay, 35% silt and 40% sand [34]. Ten random soil samples were taken at 0-10 cm depth in plots that had received tillage. Soil was brought back to the lab and stored at 4ºC until use.

Maize litter was harvested from Mead, Nebraska in a no-till irrigated continuous maize field at the end of the growing season just before harvest. Litter was separated into leaf and stalk material, oven dried at 60ºC, ground to 2mm and analyzed for total C and N in the same manner as previously mentioned. We also estimated initial carbon quality with the Ankom 200/220 Fiber Analyzer (Ankom Technology, Macedon, NY), which is a common technique used to determine forage digestibility [35,36]. This technique uses a sequential extraction to determine the amount of soluble, hemicellulose, cellulose and lignin fractions within each sample. These classifications do not represent strictly identical chemical compounds, but rather groups of similar compounds with similar resistance to decomposition. The data for tissue fractions analysis are presented as the four fractions (soluble, hemicellulose, cellulose and lignin) totaling 100% of the plant tissue carbon quality. Therefore, any increase in one fraction leads to an equivalent decrease in the other fractions. Maize leaf (C/N~40) and stalk litter (C/N~102) were used because they have similar tissue fractions, but significantly different initial C/N (Table 1).

Soil was homogenized, sieved through a 2 mm mesh, and larger organic fragments such as root and litter were removed by hand. The experimental soil was amended with two factors, litter and N addition, with six experimental treatments: 1) No litter (soil alone), 2) No litter with N addition, 3) Leaf litter 4) Leaf litter + N addition 5) Stalk litter 6) Stalk litter + N addition. Each experimental unit (1 specimen cup) received 40 g of soil. Each treatment was replicated eight times for a total of 48 samples. All treatments with litter additions received 0.2805 ± 0.0002 g C which was equivalent to 375 g C/m^2 annual aboveground productivity of leaves and stems combined. Ground litter was mixed with the soil to facilitate more rapid decomposition and treatment effects due to limited incubation time. Each N addition treatment received 3.7 mg ammonium nitrate ($NH_4{}^+NO_3{}^-$) per 40 g soil which is equivalent to a nitrogen addition rate of 5 g N/ m^2. Each experimental unit was set to a bulk-density of 1 g/cm^3 and 60% water-filled pore space was maintained throughout the experiment [37]. All experimental units were incubated in the dark for 120 days at 25ºC. Incubation time of 120 days at 25ºC was equivalent to approximately two thermal years and was chosen so as to allow enough time for adequate decomposition of litter.

Each experimental unit remained open to the atmosphere during the incubation except during sampling periods to avoid O_2 limitation. CO_2 emissions were sampled (n= 6 per treatment) on days 1, 5, 10, 15, 20, 35, 50, 75, 90, and 120 days. During sampling, the experimental units were enclosed in mason jars, and CO_2 was cleared from each jar by pumping CO_2 free air through the jar. Twenty-four hours after CO_2 clearing, headspace was sampled and the CO_2 concentration measured on a Shimadzu gas chromatograph-17A (version 3) with an electron capture detector and a Porapak Q column. Delta ^{13}C of the headspace samples was also taken on days 5, 15, 35, 75 and 120 by sampling 12 ml of headspace gas and transferring it to an evacuated exetainer (LABCO, UK) and analyzed at the UC Davis Stable Isotope Facility using a SerCon Cryoprep TGII trace gas concentration system interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). In all treatment combinations we decomposed C_4 plant material on C_3 soil because C_3 and C_4 plants differ in discrimination of $^{13}C/^{12}C$, the soil carbon will have a more negative $\delta^{13}C$ than the plant material (Table 1). By using the differentiation between the two signatures we could determine the amount of CO_2 respired carbon that had originated from soil organic matter and from litter decomposition.

Table 1. Quality and $\delta^{13}C$ of soil and litter additions. Shown are the mean ± 1 S.E. N=3 for $\delta^{13}C$ means and n=6 for all other plant tissue quality measurements

	% soluble	% hemi-cellulose	% cellulose	% lignin	C	N	C/N	C/N + N addition	$\delta^{13}C$
Stalk	30.8±1.2	24.3±0.4	38.6±0.9	6.22±0.3	44.1±0.1	0.43±0.03	102.6	43.6	-11.8±0.01
Leaf	28.7±0.7	31.0±0.4	33.9±0.7	6.40±0.3	41.9±0.1	1.01±0.42	41.4	26.8	-12.8±0.13
Soil	-	-	-	-	2.1±0.02	0.20±0.001	10.5		-22.1±0.63

At the end of the 120 day experiment, soil was physically fractionated (n= 8 per treatment) into four aggregate size classes: large macroaggregates (>2000 μm), small macroaggregates (250-2000 μm), microaggregates (53-250 μm), and silt and clay (<53 μm) [38,39]. Each sample was immersed in room temperature water for five minutes on the largest sieve. The sieve was then moved up and down three cm for two minutes, poured into the next smaller sieve, and repeated [38,39]. Each size class was separated, oven dried at 60ºC to a constant mass, weighed, and analyzed for total C and N, organic C, and $\delta^{13}C$ (n=8 for each fraction in each treatment; n=192 total). Total C and N as well as organic C were measured at the Ecosystem Analysis Laboratory in Lincoln, NE on a Costech 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA.). Organic C for each fraction was determined using a 1 M H_3PO_4 digest to remove soil inorganic C. We added 0.45 ml of 1 M H_3PO_4 to 20 mg of soil to remove inorganic C and then organic C was determined on a Costech 4010 elemental analyzer. Typically, HCl is used to remove soil inorganic C, but this interferes with C analysis on the Costech 4010 elemental analyzer so we modified the method to use H_3PO_4. Delta ^{13}C was determined at the UC Davis Stable Isotope Facility with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Peedee belemite was used as a standard for $\delta^{13}C$ measurements where,

$$\delta^{13}C\ (‰) = (((^{13}C/^{12}C\ \text{sample}) / (^{13}C/^{12}C\ \text{standard})) -1)*1000$$

2.3 Statistical Analysis

2.3.1 Field experiment

All statistics were performed using SPSS v.19. Litter decomposition in the field was determined after six and twelve months of decomposition. The effect of harvest, litter placement (soil surface or 10 cm soil depth), tissue type (stalks or leaves) and nitrogen addition (0 or 5 g N/m^2 UAN) were determined using a four-way ANOVA. When harvest proved highly significant we analyzed both harvests separately with three-way ANOVA with litter placement, tissue type, and nitrogen addition as the main effects.

2.3.2 Incubation experiment

Cumulative respiration rates were determined by fitting linear regressions to the first 10 days of respiration rates for each treatment and then for days 10-120 we fit three parameter exponential decay functions for each treatment. We then used these fitted lines to determine the amount of CO_2-C respired for every day of the experiment. These amounts were then summed for the 120-day experiment to determine cumulative amounts of CO_2-C respired. This data should be interpreted with caution as CO_2-C respired changes rapidly over time and our measurements only capture small time periods. These cumulative differences in total (litter and SOM), SOM, and litter CO_2-C respired were determined using two-way ANOVA with addition (no addition, leaf, or stalk litter) and nitrogen (0 or 5 g N/m^2) as the main effects. To determine the amount of litter and SOM decomposition we used a two source mixing model [40,41] where the contribution of litter to the δ13C of the CO_2-C respired was calculated as:

$$\%\ [\text{Fraction of respired-C from litter}] = (\delta^{13}CO_2{}^{t} - \delta^{13}CO_2{}^{s})/ (\delta^{13}CO_2{}^{\ell} - \delta^{13}CO_2{}^{s}) \quad (1)$$

Where % fraction of respired-C from litter ($CO_2{}^{\ell}/ CO_2{}^{t}$) is the fraction of litter derived CO_2 respired over the total CO_2 respired (litter derived + soil derived); $\delta^{13}CO_2{}^{t}$ represents the

isotopic composition of sampled air CO_2; $\delta^{13}CO_2^s$ is the isotopic composition of the CO_2 respired by the control soil; $\delta^{13}CO_2^\ell$ is the isotopic composition of litter derived CO_2. The main assumption is that no isotopic fractionation was associated with respiration ($\delta^{13}CO_2^\ell$ is the $\delta^{13}C$ signature of litter and $\delta^{13}CO_2^s$ is the $\delta^{13}C$ signature of the soil). We could then calculate the percent of the CO_2-C respired in each sample that originated from soil organic matter decomposition. By multiplying the total CO_2-C respired on each sampling day by the percent of litter and soil respiration determined from the two-source mixing model, we could determine the amount of CO_2-C respired from soil and litter, respectively. We then fit regressions as stated above to calculate the cumulative CO_2-C respired from litter and soil.

To determine the effect of inorganic nitrogen addition on total, SOM, and litter respiration rates, we subtracted the unfertilized treatments from the fertilized treatment at each sampling day (n= 6 per treatment). Non-overlapping 95% confidence intervals in a repeated measures analysis of variance (ANOVA) with day and treatment as the main effects were used to determine significant differences from zero. If the assumption of sphericity was violated in any of the repeated measures ANOVAs performed, we used the greenhouse-geisser correction. This test modification applied a correction factor to the degrees of freedom making the F-ratio more conservative. This correction never changed the overall significance of the test.

2.3.3 Soil fractionation

Differences in the percent of total mass of each soil fraction were analyzed using one way ANOVAs with treatment as the main effect. The amount of organic-C and $\delta^{13}C$ for all soil fractions at the end of the 120-day incubation was first analyzed with a two-way ANOVA with addition and nitrogen as the main effects. We then analyzed each fraction separately in the same manner to determine significant differences in both amount of organic-C and $\delta^{13}C$ within each soil fraction.

3. RESULTS AND DISCUSSION

3.1 Litter Decomposition

3.1.1 Field Litter Decomposition

In the field, leaf litter decomposed significantly faster than the stalk litter and litter burial increased litter-C loss anywhere between 5-30% depending upon treatment (Fig. 1 and Table 2). Litter burial has been shown to increase decomposition of litter in many other studies [42-46]. This increase has primarily been attributed to optimal abiotic factors such as temperature and moisture [44,46] as well as delayed decomposer colonization in surface residue because of the physical separation of litter and soil [42].

During the first six months of decomposition, which was a winter period, there was substantial amounts of litter-C loss, especially for leaf litter which lost approximately 20% for litter placed at the surface and two-fold more for leaf litter placed at 10 cm compared to stalk litter which only lost 15% and 20% for litter placed at the surface and 10 cm, respectively. Physical processes, such as fragmentation of the litter due to interception of precipitation, compressive forces of soil on buried litter, and freeze-thaw dynamics can all lead to the physical break down of litter [33,47-49]. Thus, while microbial activity is, of course, essential for the decomposition process, and decomposition has been shown to occur at temperatures

around 0ºC, [50] early stages of field decomposition, particularly during the winter months, are also strongly influenced by the structure of the litter.

Yet, nitrogen addition had no effect on litter decomposition in the field at any harvest time, litter type, or litter placement. There was, however, a significant litter and nitrogen interaction when all harvests were included in the analysis ($f_{1,363}$=7.27; p=0.007) and this difference was driven by differences seen after twelve months of decomposition, where nitrogen addition decreased leaf litter-C loss, but increased stalk litter-C loss for litter placed at the soil surface (Fig. 1 and Table 2). Many studies have found similar results [22,26,51-54] and this lack of significance could be due to similar effects as seen in the incubation study with the enhanced loss of easily usable substrates early in the decomposition process and decreased loss of recalcitrant substrates late in the decomposition process [7,9]. Due to the fact that we sampled our litter bags at six month intervals, we were not able to detect changes in decomposition that might have occurred on shorter time scales. Any future experiments addressing this topic should harvest litter bags more frequently and for multiple years.

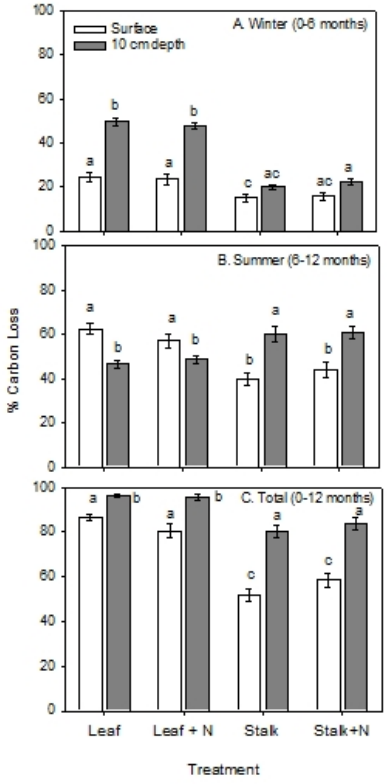

Fig. 1. Percent carbon loss of litter in situ. Shown are the mean ± 1 S.E. for litter at the soil surface and 10 cm soil depth for the A) first 6 months which is from November to May, B) 6-12 months representing decomposition from May to November, and C) 0-12 months representing the total amount of %C loss over the entire one year period. Summer decomposition (6-12 months) was determined by subtracting the % C loss at six months from the total % C loss for the entire year. Letters denote treatment differences and were determined with a one-way ANOVA where P<0.05 of a LSD posthoc comparison

Table 2. The effect of litter placement (surface or 10 cm depth), litter type (leaf or stalk) and nitrogen addition (0 and 5 g N/m^2 UAN) on carbon loss *in situ*. Shown are the *d.f.*, *f*, and *p*-values from three separate ANOVAs. Because harvest was highly significant in the four way ANOVA with harvest, depth, litter, and nitrogen with the six and twelve month harvests included ($f_{1,363}$=279.2; p<0.0001), we separated the data by harvest and ran three-way ANOVAs for winter (0- 6-month harvest), summer (6-12 month harvest), and total (0-12 month harvest). In all analyses shown, significant differences were determined where P<0.05 in a LSD post-hoc comparison. Summer decomposition (6-12 months) was determined by subtracting % C loss at 6 months from % C loss at 12 months

Main Effect	Winter (0-6 months)			Summer (6-12 months)			Total (0-12 months)		
	d.f.	*f*	*P*	*d.f.*	*F*	*P*	*d.f.*	*f*	*p*
Litter Placement (P)	1, 79	153.29	**<0.0001**	1, 86	3.24	0.074	1, 184	137.18	**<0.0001**
Litter Type (T)	1, 79	213.01	**<0.0001**	1, 86	1.58	0.210	1, 184	161.30	**<0.0001**
Nitrogen (N)	1, 79	0.02	0.891	1, 86	0.08	0.782	1, 184	0.30	0.586
P*T	1, 79	58.76	**<0.0001**	1, 86	64.56	**<0.0001**	1, 184	18.73	**<0.0001**
P*N	1, 79	0.35	0.852	1, 86	0.31	0.579	1, 184	0.12	0.731
T*N	1, 79	1.13	0.289	1, 86	1.35	0.247	1, 184	6.67	**0.011**
P*T*N	1, 79	0.22	0.642	1, 86	1.93	0.167	1, 184	1.79	0.183

3.1.2 Incubation litter decomposition

In our laboratory incubation experiment, we found that, at least in the short-term (1-10 days), nitrogen additions increased litter-C loss for both leaf litter and stalk litter by 3% and 8% respectively (Fig. 2C and 3C), with stalk litter having a more sustained increase in litter decomposition in the fertilized treatment for the first 20 days. The additional N reduced the C/N ratio of stalk litter from 102.6 to 43.6 and leaf litter from 41.4 to 26.8 (Table 1) and the leaf litter C/N ratio with added N (26.8) is near the optimal level for microbial activity [8]. However, both stalk and leaf litter saw increased decomposition in the short-term, which indicates that N addition at this rate supplied needed N to the microbial community, most likely until the easily usable substrates were utilized [55]. After day 20, we saw a negative effect of added N on litter-C loss, especially for the stalk litter. We only observed increases in litter-C loss for the first 5-20 days depending upon litter type, which is shorter than the 30-60 days seen with rice decomposition [9], but very similar to another maize decomposition study [7]. This pattern of increased decomposition early in the decomposition process and then a negative effect in the late stages of decomposition is in agreement with many previous studies [22,24,56-59] as added exogenous N has been shown to stimulate the loss of easily usable substrates and decrease decomposition of recalcitrant substrates such as lignin. The inhibitory effects on N in late stages of decomposition has been attributed to the depression of enzyme production by lignin decomposers [22,56] or a general shift in the decomposer community to organisms with enhanced N efficiency [60].

Stalk litter decomposed significantly faster than leaf litter in the laboratory incubation, which was the opposite pattern found in the field decomposition study. These contradictory results may have occurred because the litter for the laboratory incubation was ground and incorporated into the soil, while in the field, natural litter structure was maintained, and whole tissue was placed in litter bags and put into the field. The litter was ground in the laboratory incubation to increase the availability of the litter to decomposers and ensure treatment

effects over the 120-day period. Many studies have shown that fragmentation and incorporation of litter into the soil enhances decomposition rates because of enhanced decomposer access to litter when it is fragmented [7,61,62]. When litter is left intact stalk litter tends to have a tough outer sheath around the stalk, whereas the interior, where the main nutrient and water transport take place, was much more porous and spongy. Our initial tissue quality analysis also showed that stalk litter has higher soluble and lower hemicellulose concentrations than leaf litter which could lead to increased decomposition when ground (Table 1). By grinding the stalk litter and incorporating it into the soil, we were allowing for direct decomposer access to the more easily usable portions of the stalk litter that could be degraded rapidly and that in the field would have been protected by the tough outer tissue [63,64]. Our results are in concordance with another recent incubation study where stalk and leaf maize litter were ground. They also showed that stalk litter had more sugar concentration and less hemicellulose which lead to more % C remaining in leaf litter than stalk litter [65].

3.1.3 Total (soil+litter) incubation decomposition

Litter additions, regardless of C/N ratio, increased the total cumulative CO_2-C respired (Fig. 4A and Table 3A). Exogenous N addition had no significant effect on total cumulative CO_2-C respired for any treatment, but there was a significant litter by nitrogen interaction (Table 3). This significant interaction was the result of a small increase in total cumulative CO_2-C respired in the high C/N stalk litter treatment compared to the low C/N leaf litter treatment that had a small decrease in cumulative CO_2-C respired (Fig. 4A).

3.2 Incubation soil organic matter decomposition

We found that the addition of litter, whether stalk or leaf, decreased the total amount of SOM-C loss, but that exogenous N addition had no significant effect on SOM-C loss for any treatment (Fig. 2B and 3B; Table 3). For high C/N ratio stalk tissue, exogenous-N inputs significantly increased SOM decomposition on day 5 and 40 (Fig. 3B), but this was not enough to lead to cumulative increases in SOM-C loss (Fig. 3C; Table 3B). The significant effects of litter addition on SOM-C loss were driven by the differences between the stalk and soil treatments in the first 35 days, as N addition increased SOM-C loss in the stalk treatment and decreased SOM-C loss in the soil only treatment (Fig. 3B.).

This result is in contrast with our prediction that increased exogenous-N availability would lead to decreased SOM-C decomposition. There have been very few direct tests of exogenous-N inputs to litter and how this effects SOM-C decomposition in managed systems [7-9] and much of the literature surrounding this topic is still theoretical in nature [16,18,66]. It does seem though, that with the high C/N ratio stalk litter additions, exogenous N inputs actually exacerbated SOM-C loss instead of alleviating it by supplying microbes with an easily usable N source. It is possible that N addition stimulated communities of microorganisms that not only decomposed fresh, easily degradable litter, but also those that degrade recalcitrant SOM [66].

It should also be noted that our incubation study was maintained at 60% WFPS which is optimal for microbial activity [37], thusly our rates of decomposition are higher than those seen under field conditions with a more variable temperature and moisture regime.

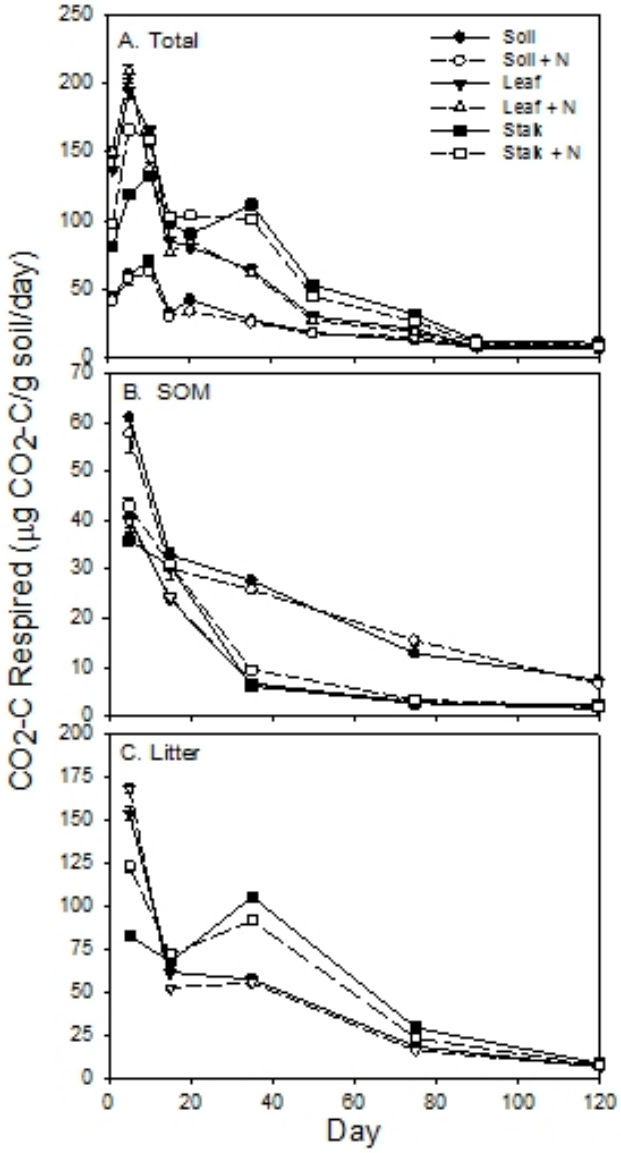

Fig. 2. Rate of CO_2-C respired over the 120 day experiment for A) Total (soil+litter) B) SOM derived and C) litter derived C at each sampling day. Shown are the mean ± 1 S.E. for each treatment at each sampling date (n=6 per treatment at each sampling day)

Table 3. The effect of litter addition and nitrogen on A) cumulative CO_2-C respired, B) cumulative CO_2-C respired from SOM and C) cumulative CO_2-C respired from litter. Shown are the *d.f.*, *f*, and *p*-values from a two-way ANOVA with addition and nitrogen as the main effects. Significant differences were determined where P<0.05 in a LSD post-hoc comparison.

A. Cumulative CO_2-C respired			
Main effect	***d.f.***	***f***	***p***
Litter	2, 30	1082.88	**<0.0001**
Nitrogen	1, 30	0.22	0.645
Litter* Nitrogen	2, 30	5.22	**0.011**
B. Cumulative CO_2-C respired from SOM			
Main effect	***d.f.***	***f***	***p***
Litter	2, 30	36.00	**<0.0001**
Nitrogen	1, 30	1.74	0.192
Litter*Nitrogen	2, 30	1.83	0.178
C. Cumulative CO_2-C respired from Litter			
Main effect	***d.f.***	***f***	***p***
Litter	1, 20	178.06	**<0.0001**
Nitrogen	1, 20	11.09	**0.003**
Litter*Nitrogen	1, 20	2.35	0.141

3.3 Incubation soil fractionation

There were no treatment differences in the percent of total mass of each fractionated sample in each of the four size classes (Table 4A). We presented the data in this manner because each treatment had a different total mass. Each experimental unit originally received 40 g of soil, and the litter additions were scaled by % C. Therefore, the total mass of each experimental unit depended upon the type of litter added.

Generally, we found that N addition had very little effect on C storage in any treatment. However, litter addition, regardless of litter type increased organic-C incorporation and had less negative $\delta^{13}C$ values, which indicates some litter-C incorporation (Table 4C and 5B). Nitrogen only had significant effects in the 250-2000 µm and 53-250 µm size classes. In the 250-2000 µm size class, N addition increased organic-C in the soil and leaf treatments, but decreased organic-C in the stalk treatment while in the 53-250 µm size class it decreased organic-C in all treatments (Table 4B and 5A).

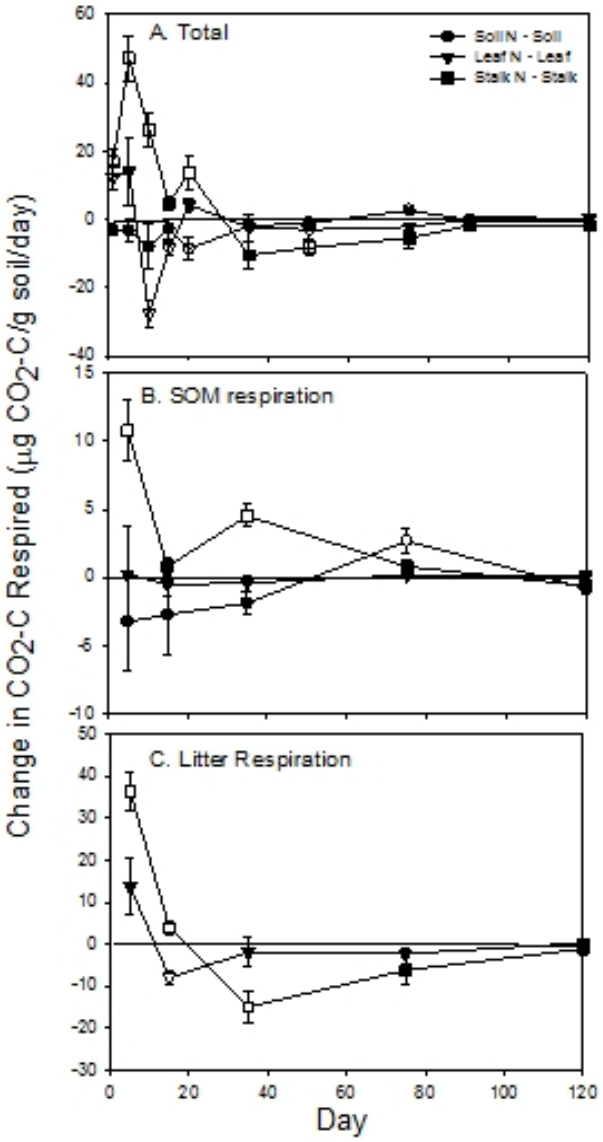

Fig. 3. The effect of exogenous N addition on respiration rates for each treatment over the 120 day incubation experiment. We subtracted the unfertilized treatment from the fertilized treatment to determine the change in respiration rate for A) total (litter + soil) B) soil and C) litter at each sampling day. Open symbols show indicates treatments that are significantly different from zero while closed symbols denote non-significant differences. Significant differences were determined with non-overlapping 95% confidence intervals in repeated measures ANOVA

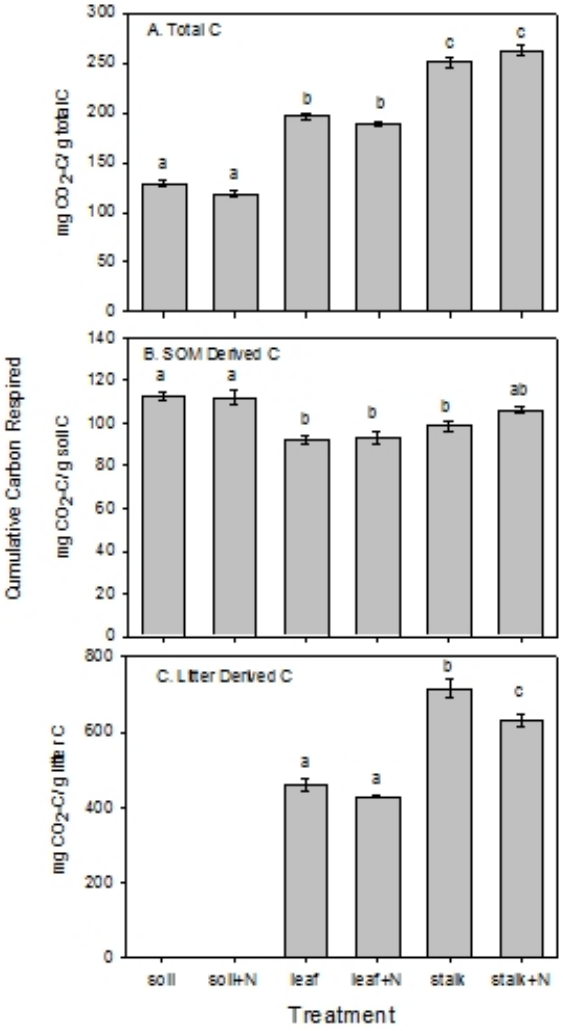

Fig. 4. Cumulative CO_2-C respired over the 120 day incubation experiment for A) Total (litter + soil), B) SOM derived, and C) litter derived C. To calculate the cumulative amount of CO_2-C respired derived from litter and SOM, we used a two-source mixing model (Rubino et al., 2010) to calculate the amount of litter and SOM derived CO_2-C at each time point. We then fit regressions between each sampling date for both litter and SOM derived CO_2-C separately and used these fitted lines to calculate the cumulative amount of CO_2-C respired. Shown are the mean ± 1 S.E for each treatment. Different letters denote significant differences among treatments where P<0.05 of a LSD post hoc (n=6) in a one-way ANOVA

Table 4. Soil physical fractionation after 120-day incubation. The mean ± 1 S.E. are shown for A) percent of total mass, B) amount of organic-C (mg organic-C/g soil), and C) the $\delta^{13}C$ for each soil fraction in each treatment. There were no treatment differences in the % of total mass of each fraction in each treatment ($F_{5,167}$=0.008; p=1.0) so only the mean for each fraction is shown.

Soil fraction (µm)	>2000	250-2000	53-250	<53
A. Percent of total mass				
Mean	6.28±0.44	39.74±0.74	25.24±0.69	28.77±0.66
B. Organic-C (mg C/g soil)				
Soil	17.60±1.10	19.58±1.29	16.89±0.78	17.68±0.54
Soil + N	20.63±0.19	21.96±0.43	12.84±0.34	19.88±0.81
Leaf	20.13±1.21	19.15±1.23	18.34±1.06	19.40±1.01
Leaf + N	21.80±0.74	23.69±0.57	15.87±0.66	18.03±0.41
Stalk	22.18±0.24	23.00±0.61	17.25±0.75	20.62±1.15
Stalk + N	19.97±1.16	22.70±0.55	16.41±0.64	20.00±1.14
C. $\delta^{13}C$ signature				
Soil	-21.99±0.11	-22.19±0.04	-22.70±0.09	-21.17±0.12
Soil + N	-22.00±0.05	-22.37±0.07	-22.90±0.07	-21.23±0.11
Leaf	-21.19±0.09	-21.08±0.09	-21.61±0.05	-20.47±0.05
Leaf + N	-21.01±0.13	-21.12±0.03	-21.54±0.04	-20.61±0.16
Stalk	-20.87±0.10	-20.93±0.06	-21.38±0.05	-20.52±0.19
Stalk + N	-20.86±0.04	-21.06±0.08	-21.48±0.08	-20.62±0.19

The amount of organic-C in each fraction was variable and was more affected by litter additions than by exogenous N additions (Table 4B and 5A). There was a small increase in the amount of organic-C in the litter additions as compared to the soil only treatment, but this was not consistent across soil aggregate size classes (Table 4B). The fertilized leaf treatment had increases in organic C in the large macroaggregate (>2000µm) and a significant increase in the small macroaggregate fraction (250-2000µm), but then decreases in both the microaggregate (53-250µm) and mineral associated (<53µm) fractions. The stalk tissue had no significant changes in organic-C storage in any fraction with a trend for decreased organic-C with exogenous N addition in the large macroaggregate fraction (>2000µm). The small and generally insignificant trends that we saw for exogenous N addition increasing macroaggregate formation could be due to the initial stimulation of the soil microbial community as we saw higher respiration rates for all litter treatments in the first 10 days. These macroaggregates are bound by microbial polysaccharides or easily decomposable substrates in the early stages of decomposition and therefore tend to be unstable and transient [67]. Microaggregate fractions (53-250 µm and < 53 µm), on the other hand, represent fractions in which long-term stabilization of carbon occurs. They tend to be formed by more recalcitrant compounds, forming organo-mineral complexes, which are highly stable. Thus, for the leaf tissue, where we saw nitrogen addition leading to an increase in macroaggregate formation but a decrease in microaggregate formation, this may not translate to long-term C sequestration as macroaggregates tend to be unstable. Because maize litter has relatively low amounts of lignin, maize based systems may not have the high amounts of recalcitrant compounds to form nucleation sites for microaggregate formation.

Table 5. The effect of litter addition and nitrogen on A) organic carbon in each fraction (mg organic-C/g soil) and B) $\delta^{13}C$ of each soil fraction. Shown are the *d.f.*, *f*, and *p*-values from a two-way ANOVA with addition and nitrogen as the main effects. Significant differences were determined where P<0.05 in a LSD post-hoc comparison.

A. Organic C (mg OrganicC/gsoil)

Main effect	***d.f.***	**>2000 µm**		**250-2000 µm**		**53-250 µm**		**<53 µm**	
		f	***p***	***f***	***p***	***f***	***p***	***f***	***p***
Litter (L)	2, 42	3.13	**0.054**	3.10	0.056	5.51	**0.008**	2.05	0.142
Nitrogen (N)	1, 42	1.33	0.256	10.00	**0.003**	16.66	**<0.0001**	0.01	0.925
L* N	2, 42	4.77	**0.014**	4.02	**0.025**	2.40	0.103	2.23	0.120
B. $\delta^{13}C$ signature									
Main effect	***d.f.***	**>2000 µm**		**250-2000 µm**		**53-250 µm**		**<53 µm**	
		f	***p***	***f***	***p***	***f***	***p***	***f***	***p***
Litter (L)	2, 42	80.53	**<0.0001**	250.03	**<0.0001**	273.49	**<0.0001**	13.17	**<0.0001**
Nitrogen (N)	1, 42	0.58	0.452	5.25	**0.027**	2.29	0.138	0.74	0.396
L* N	2, 42	0.63	0.539	0.64	0.531	2.06	0.140	0.04	0.965

4. CONCLUSION

N addition had very little effect on litter decomposition in the field regardless of litter placement. It also had small effects on litter and SOM decomposition in the laboratory incubation, but only in the first 20 days of the experiment and then there was reduced decomposition in later stages of decomposition leading to non-significant cumulative differences. We saw no evidence for the maintenance of SOM or increased organic-C stabilization in soil due to exogenous N additions and thus the use of N inputs to litter post-harvest is not a viable option to increase C sequestration in these systems.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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