THESIS

FROM LITTER DECOMPOSITION TO SOIL ORGANIC MATTER FORMATION: USING STABLE ISOTOPES TO DETERMINE THE FATE OF CARBON AND NITROGEN

Submitted by

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Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2014

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ABSTRACT

FROM LITTER DECOMPOSITION TO SOIL ORGANIC MATTER FORMATION: USING STABLE ISOTOPES TO DETERMINE THE FATE OF CARBON AND NITROGEN

Litter decomposition releases the energy and nutrients fixed during photosynthesis into the atmosphere and soil. In the soil, carbon and nitrogen from the litter can be stabilized in soil organic matter pools, which globally represent large pools of both carbon (C) and nitrogen (N). Soil organic matter pools are heterogeneous, the product of different stabilization processes and will stabilize C and N for periods of time ranging from years to millennia. A thorough mechanistic understanding of the fate of above-ground litter C and N is essential to understand how climate change could affect both carbon sequestration and soil health.

This research studied the fate of litter derived organic matter. Isotopically labeled litter was used in a field incubation to trace litter derived C and N into different SOM pools and soil depths over the course of 3 years. Additionally, naphthalene was used to suppress microarthropods to determine the impact of mesofauna on the fate of litter derived N. In the laboratory, soil from the field experiment was incubated for 150 to determine how different SOM pools contributed to respiration and leaching.

Microarthropods do not increase overall N mineralization rates, but do influence the fate of litter derived N. When present, microarthropods increased the amount of litter derived N in the light fractions, suggesting that microarthropods increase litter fragmentation. Surprisingly, litter derived organic matter does not contribute to respiration and leaching equally, suggesting

that leaching and respiration are not directly related. Litter derived OM behaves differently than older OM present in the soil, with the newer litter derived C and N being more readily lost from SOM pools. This result supports the onion layering model suggested by Sollins (Sollins et al. 2006). In order to create more accurate models, microarthropods and the onion layering model should be included in future C and N dynamic studies.

ACKNOWLEDGEMENTS

Firstly, I want to express my deepest gratitude to Dr. Francesca Cotrufo. It has been my delight to be her student these past three years, and I have grown tremendously under her tutelage. Her high expectations have pushed me beyond what I believed to be my limits, and only in retrospect can I see the great impact she has left in my life. Francesca, it is from the depth of my heart that I express the gratitude for all the support, both as my mentor and as my friend. Grazie di cuore. To the other members of my masters committee, Dr. Joe von Fisher and Dr. Mark Paschke, thank you for your support and guidance, this thesis would not be here if not for your time and energy. I would be amiss if I did not address Dr. Jenny Soong's assistance in the lab, with writing, and as a wonderful friend. I need to thank Dan Reuss and Colin Pinney for helping me with every step of data collection, and for all the patience they displayed in teaching me laboratory techniques. I also want to thank the entire Cotrufo lab group for all the assistance in the laboratory and critical feedback in all my presentations. Additionally, I want to thank the entire community of NREL that has supported and accepted my eccentricities with open arms. Y'all are making leaving very difficult for me. To all my friends, both here in Colorado and back in Texas, thank you for sanity-saving talks and constructive conversations. Finally, a big thank you to my mom, dad, sisters and extended family members, for years of support and for always believing in me, even when I didn't. To everyone who helped me, I have this to say:

"I did it!"

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1. INTRODUCTION

Litter decomposition is the process by which energy and nutrients fixed during photosynthesis are released into the atmosphere and soil. Traditionally, litter mass loss had been the focus of decomposition studies, with little consideration for the ultimate fate of the mass once it has been lost from the litter (Zhang *et al.* 2008). Mass is lost from the litter in three processes catabolism, leaching, and fragmentation, with the latter two generating soil organic matter (SOM) (Cotrufo *et al.* 2009). Globally, SOM represents a large pool of both carbon (C) and nitrogen (N), which is increasingly important, as global climate change alters the global C and N cycles (Jobbagy & Jackson 2000; Schulten & Schnitzer 1998; Schlesinger 2013; Lal 2004; Davidson & Janssen 2006). Understanding how litter decomposition contributes to SOM formation is crucial both for preserving soil health and ecosystem stability (Grandy & Neff, 2008; Cameron, *et al.* 2013).

Litter decomposition can be broken down into different phases, with the first phase lasting from one to two years (Lutzow *et al.* 2006). In this phase, leaching of dissolved organic matter (DOM) is the largest loss of mass from the litter (Lutzow *et al.* 2006). This DOM is either sorbed directly onto mineral surfaces, or utilized by soil microbes, who transform the OM and their products become associated with silt and clay particles in the soil (Lutzow *et al.* 2006; Grandy & Neff 2008). In the later phase of decomposition, mass loss is dominated by litter physically broking down, and small litter fragments enter the soil, where they can be separated as the SOM light fraction and are utilized by soil microbes within a decadal time frame (Six *et al.* 2002). Soil organic matter is heterogeneous, with different levels of stabilization. For example, different SOM pools have C turnover rates ranging from years to millennia (von Lützow *et al.* 2007), and

represent the interactions of intrinsic recalcitrance, physical stabilization, chemical stabilization and inhibition of microbial activity (Trumbore, 2009).

Litter decomposition and SOM formation are driven by bacteria and fungi (Paul 2007). Their activity, and controls on soil community composition, influence N release or storage in the soil (Mooshammer *et al.* 2014). In addition to bacterial and fungal controls, soil mesofauna may influence microbial composition and community activity through top-down controls (Wall *et al.* 2008; Hattenschwiler *et al.* 2005). It is important to empirically and directly test the effects of soil mesofauna on N stabilization in order to determine if soil mesofauna need to be included in future soil N management practices.

Grasslands contain an estimated 30% of the world's total soil carbon, despite only covering less than one fifth of the Earth's land surface (Anderson 1991; Grieser *et al.* 2006). In the tallgrass prairie, production is limited by water as well as light and nutrients (Knapp & Seastedt 1986; Schimel *et al.* 1991; Seastedt *et al.* 1991), and the system is N limited (Knapp *et al.* 1998). Thus, litter decomposition is key to N availability in this system, and contributes to its storage into SOM. Because of grassland's importance in the global C cycle, and the limited N present in the system the tallgrass prairie of central U.S.A. is an ideal system to measure C and N dynamics.

These observations motivated the following studies, in which the fate of litter derived (LD) N were investigated. Specifically, we asked:

1. Do soil microarthropods increase litter-N contributions to SON and increase N stabilization?

We also investigated how LD OM already in the soil was lost through respiration and leaching. Specifically, we asked:

2. How is fresh LD OM released as respiration and leachate?
3. How does LD OM transfer between primary SOM pools?

In the following chapters I will address these specific questions through a field study in a tallgrass prairie ecosystem (1), and a laboratory incubation (2 & 3).

2. INVESTIGATING THE FATE OF ABOVE-GROUND LITTER NITROGEN INTO BULK SOIL, SOIL ORGANIC MATTER POOLS AND VEGETATION IN A TALLGRASS PRAIRIE BY THE USE OF ^{15}N ENRICHMENT

2.1 INTRODUCTION

Litter nitrogen (N) dynamics are fundamental to soil N availability and plant growth. While we understand and are able to predict litter N dynamics (Parton *et al.*, 2007), we know very little about the fate of N and the contribution of above-ground litter N to soil organic N (SON) pools and N availability for plant growth. Understanding these contributions is ecologically important, as the decomposition of litter is the process by which plant N is returned to the soil. Additionally, losses of N from the soil/plant system not only reduce soil fertility and plant yield but can also create adverse impacts on the environment (Cameron *et al.* 2013). Soil organic matter (SOM) is a vital resource for humanity and represents a carbon (C) sink, with turnover rates as lengthy as centuries to millennia (Sollins *et al.* 2006; Wiedemeier *et al.* 2012; Grandy & Neff, 2008). SOM has a C:N ratio much lower than the plant litter from which it is formed, thus the retention of N during litter decomposition is integral to the ability of soil to preserve SOM and sequester C. As such, the factors that affect litter decomposition and SON formation are also important to SOM persistence and C storage.

Litter decomposition is driven by three processes: fragmentation, leaching and catabolism (Swift *et al.* 1979). Fragmentation is both a biotic, facilitated by the soil fauna (Coleman *et al.* 2004; Bardgett 2005), and an abiotic process. During fragmentation, litter is reduced into smaller fragments that move down the soil profile, where they can be isolated as the SOM light fraction (LF). Leaching, the loss of water soluble substrates from the litter layer into the soil, is

responsible for fluxes of dissolved organic N (DON) from the standing litter to the mineral soil. Leaching depends on the concentration of soluble compounds in the litter and, thus, occurs largely in the early phase of litter decay (Swift *et al.* 1979). Catabolism is the process by which saprotrophic organisms, mostly bacteria and fungi, whose populations are regulated by microfauna, use the dead litter constituents for their growth and activity. As a product of catabolism, N is released in a mineral form (e.g. $NH4^{+}$) or used for microbial biomass and microbial metabolites. Both the mineral N and the organic N compounds of plant and microbial origin are released into the soil, the first being available for plant and microbial uptake, while the second contributes to the formation of SON (Paul 2007). Litter decomposition is a lengthy process generally requiring years to decades for completion (Parton *et al.* 2007). However, N losses are not uniform throughout the decomposition. According to Parton *et al.* (2007) litter nitrogen is lost from the litter after a period ranging from three months to almost two years in temperate grassland (Parton *et al.* 2007). Simultaneously, exogenous litter N can be immobilized from the soil to augment endogenous N, when this is present at low concentrations (<1.02%) limiting microbial catabolism (Parton *et al.* 2007). The endogenous N mineralization and exogenous N immobilization have been found to balance each other during the first two years of decomposition, maintaining the litter N pool stable, while increasing litter N concentration (Berg 1988; Zeller 1998; Cotrufo *et al.* 2000). Afterwards, litter N tends to mineralize at the same rate as litter mass loss (Berg 1988; Zeller 1998; Cotrufo *et al.* 2000). Over the course of decomposition, %N influence the rate of decomposition (Berg 1988), thus both the endogenous (% of initial N) but also the availability of exogenous N can be important determinant of litter decay. Additionally, differentiating between the dynamics of endogenous and exogenous N during litter decomposition is key to elucidate the “true” contribution of new litter N inputs to

soil formation, and determine the dependence of litter microbial catabolism on soil available N. In the soil, N is present in four major forms, organic matter, soil organisms and microorganisms, ammonium ions held by clay and organic matter, and mineral N forms (Cameron *et al.* 2013). The gains, losses and transformations of N within the soil/plant system affect the availability of N to plants and the transfer of N into the wider environment (Cameron *et al.* 2013). Almost 95% of total soil N is closely associated with SOM (Schulten & Schnitzer 1998). Soil organic matter can be separated into physically distinct fractions, such as a light fraction (LF; <1.85 g cm^{-3}), and a heavy fraction (>1.85 g cm^{-3}). The heavy fraction can be further separated into a sand, a silt and a clay-sized fraction. These fractions are characterized by progressively higher concentrations of N as compared to C (i.e., by progressively lower C:N ratios), going from the LF to the clay fraction (Christensen 2001). Thus they are believed to correspond to progressively higher level of decomposition and microbial transformation (Grandy & Neff, 2008). These fractions also differ in the stabilization mechanisms and time of persistence in soil. The LF is primarily stabilized by chemical recalcitrance, and persists for time periods in the order or decades. Silt and the clay fractions, by contrast, are stabilized through chemical bonding to the minerals and can persist for centuries to millennia (von Lützow *et al.* 2007). Studying the dynamics of litter-derived N incorporation into these soil fractions can therefore elucidate the mechanism of SON formation and its persistence.

Soil microarthropods have been shown to accelerate early stages of litter decomposition when climate is not limiting (Wall *et al.*, 2008), and can affect concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) in three ways: first by feeding on microbes that mineralize, nitrify and/or denitrify; second, by transporting and dispersing the microbes within the soil, thereby stimulating microbial growth and activities; and third by increasing the surface area of substrates

by shredding of litter which facilitates microbial colonization on the substrates (Petersen 7 Luxton, 1982; Seastedt, 1984; Verhoef & Brussaard, 1990; Gessner *et al.*, 2010). These interactions between microbes and soil fauna are important with respect to N-mineralization: nearly 30% of N mineralization in soil can be due to the presence and activity of soil fauna (Verhoef & Brussaard, 1990), despite the fact that they only encompass a weight of 2.5% of the total soil microbial biomass (Moore *et al.*, 1988). Thus, soil fauna can play a pivotal role on litter N dynamics and the subsequent retention of N in SOM, which requires investigation.

Stable isotope enrichment has been shown to be the best approach to trace the fate of element (e.g., C and N) into the environment (Sollins *et al.* 2006).The advantage of using stable isotope enrichments is that the environment under investigation can be left unaltered. By substituting a native leaf litter with a ^{15}N labelled litter, the input of N to the soil from the decomposing litter can be assessed (Bird *et al.* 2008). The change in soil isotope composition is measured as it becomes enriched in litter-derived N. This change in isotope composition is proportional to the amount of N derived from the litter and this amount can be quantified applying a two sources mixing model (Balesdent *et al.* 1987). This method can be applied to the bulk soil as well as to SOM fractions and specific compounds, as demonstrated with carbon (Del Galdo *et al.* 2003; Rubino *et al.* 2008), thus giving the opportunity to accurately trace litter derived N into the soil.

The goals of this experiment were to: a) quantify N dynamics during above-ground-litter decomposition, distinctly for the endogenous and the exogenous N, b) determine the contribution of above-ground litter N to different SON pools, and c) determine the extent to which above-ground litter N is recycled through plant uptake or lost from the soil in tallgrass prairie. Additionally, we aimed to determine if litter and soil microarthropods affect the above

processes. Given the relatively high N content of our litter, we expected N to be lost throughout the decomposition process, with no immobilization of exogenous N uptake. We hypothesized that early litter derived (LD) N contributions to SON pools are primarily in the clay sized fraction, since this could stabilized DON due to leaching. Later, with the beginning of fragmentation we expected to retrieve more LD N in the LF. We also expected that as decomposition progressed, LD N would start accumulating in the silt and sand sized fractions (Six *et al.* 2002). We hypothesized that a significant amount of LD N will be rapidly recycled into plant tissues, due to N being a limiting nutrient in the system (Crain *et al.* 2012; Knapp *et al.* 1998). We hypothesized that microarthropods will increase litter mass loss, and will increase litter N mineralization rates, thus favoring LD N plant uptake. Microarthropods will also alter the incorporation of SON, increasing the amount of LD N in the LF due to increased fragmentation.

To achieve those goals we incubated isotopically labeled *Andropogon gerardii* above-ground litter in a tallgrass prairie, and applied a microarthropod suppression treatment using naphthalene (Cotrufo *et al.*, 2014). We then followed the dynamics of litter N during decomposition, and traced it into the soil, SOM fraction and vegetation for three years.

2.2 MATERIALS AND METHODS

2.2.1 Experimental site

This experiment was conducted at the Konza Prairie long-term ecological research site in northeastern Kansas, USA The site is a tallgrass prairie, dominated by *Andropogon gerardii*. Climate at the site is temperate-continental, with a mean annual temperature of 12.8°C and an

average annual precipitation of 835 mm. The soil is silty–clay–loam Mollisol, with 4% C and 0.32% N across the top 20 cm depth and presents the characteristic of the lowland soils at the site. The experimental area used for the study was burned annually from 1972 to 2000, at which time, annual burning treatments ceased. The site was burned accidentally once again in 2008. Detailed site descriptions can be found in Knapp *et al.* (1998).

2.2.2 Experimental design

In order to test the effect of microarthropods on litter mass loss and SON pools, a fully randomized, split-split plot block experiment was designed, with one split being control and a naphthalene treatment to suppress microarthropods (hereafter referred to as microarthropod suppression (MS) treatment). The second split was isotopically enriched litter or bare soil, to utilize the mixing model (described below).

The field experiment consisting of 80 PVC collars (20 cm diameter and 10 cm tall) was established at Konza. This design allowed for five harvests: 6 months, 12 months, 18 months, 24 months, and 36 months with four replicate blocks at each harvest. In each replicate, there were four PVC collars inserted in the ground to 5 cm, randomly assigned to one of the five harvesting dates. All collars were inserted on June 1st 2010, when the native litter was removed. After removing the native litter, half of the collars received naphthalene, (14.8g/month) to suppress microarthropods (Cotrufo *et al.* 2013). Monthly naphthalene additions continued throughout the experiment. Half of both the control and the MS treatments received 18.4 g of isotopically enriched *Andropogon gerardii* above-ground plant material, and the other half was left bare to serve as a control i.e., the background end-member in the isotopic mixing model (see data analyses below). The isotopically enriched litter (δ^{13}C Value: 2122.96 ‰, Atom%: 3.38, δ^{15}N

Value: 10308.70 ‰, Atom%: 3.99) was generated at the Colorado State University Greenhouse, using the continuous labeling chamber described in Soong *et al.* (2014).

2.2.3 Soil and litter sampling and analyses

On May 1st 2011, October 8th 2011, April 14th 2012, September 29th 2012, and September 25th 2013, soil from within one control and one MS treatment PVC collar was collected in each of the four replicates blocks. The remaining litter in each collar was collected, and stored in pre-labeled plastic bags. After taking a soil core, the soil within the collar was gently excavated with the use of hand shovel by incremental depths (0–2; 2–5; 5–10 and 10–20 cm) and the soil collected from each layer was stored separately in pre-labeled plastic bags. All soil samples were stored with ice in coolers before being brought to the laboratory the following day. There they were stored at 4°C until they were processed within two weeks of collection.

The litter samples were oven dried at 60°C weighed, and a subsample was run on the Carlo Erba NA1500 coupled to a VG Isochrom continuous flow IRMS, Isoprime Inc.) for %N and ^{15}N. A subsample was incinerated in a muffle furnace at 600°C for 5 hours to determine the % ash of the samples. All soil samples were sieved to 2 mm, air dried and stored separate by replicate at room temperature, until farther analyses.

A subsample of each soil from the 0-2 an 2-5 cm depth was fractionated, by density and size using a method modified from Marzaioli *et al.* (2010) and Denef *et al.*, (2013). Soil was fractionated by density into light fraction, (LF; < 1.85 g cm^{-3}), and a heavy fraction, which was farther separated by size into a sand sized (53-2000 µm), and a finer mineral fraction, subsequently separated by centrifugation into sand and clay. Five grams of soil was added to 25 ml of 1.85 g cm-3 Sodium Polytungstate (SPT) with twelve glass beads, and shaken for 6 hours

to disperse macro aggregates. After centrifugation at 1100 g for 60 minutes at 20 degrees Celsius a pellet of heavy SOM (HF) was formed, leaving the LF suspended. The LF was aspirated off on to 20 µm nylon filter via a Millipore glass filter unit. After repeated rinses to remove residual SPT, the HF pellet was dispersed and sieved on a 53 µm filter, separating the sand sized fraction (> 53 m) from the finer silt and clay fraction. This finer fraction was then separated into a silt and a clay fraction then using centrifugation (200 g for 3.4 minutes at 20C), which pelleted the silt and left the clay suspended. The suspended clay was aspirated off, and the clay pellet was dispersed and recovered with DI water.

Each bulk soil and SOM fraction was oven dried at 60° C, pulverized and analyzed on an Elemental Analyzer Isotope Ratio Mass Spectrometer (EA-IRMS). For the first four harvests we used a Carlo Erba NA1500 coupled to a VG Isochrom continuous flow IRMS (Isoprime Inc.) (observed precision: 0.2‰ for C and 0.3‰ for N), while for the final we used a Costech ESC 4010 (Costech Inc.) coupled to a Thermo Delta V Advantage continuous flow IRMS, (Thermo-Fisher Corp)) (observed precision: 0.2‰ for C and 0.3‰ for N) for %C, %N, $\delta^{13}C$ and $\delta^{15}N$. Instruments were inter-calibrated before analyses.

Additionally at the beginning of the experiment four soil cores were randomly collected from the experimental area and separated into 4 depth layer (0–2; 2–5; 5–10 and 10–20 cm). In the lab, the soil samples were sieved to remove eventual root or stones, oven dried at 105°C, and then weighed to determine the bulk density of the soil.

2.2.4 Plant sampling and analyses

On October 8th 2011 (H2) September 29th 2012 (H4) and September 25th 2013 (H5) *A. gerardii* plants were sampled along four orthogonal transects at 0cm, 15cm and 30cm distance

from the sampled PVC collars with the enriched litter. After identifying an individual plant, it was dug gently from the ground to a depth of 20 cm, and both roots and shoots were collected and stored in paper bags. Bags were transported and stored at room temperature, until the above and below-ground plant matter was divided. Additionally, at each sampling time four *A. gerardii* plant samples were collected as described above but outside of the experimental area to be used as control plants for the mixing model.

In the laboratory, each plant was divided into above-ground and belowground plant matter, cleaned of residual soil, over dried at 60°C, pulverized, and analyzed for %C, %N, $\delta^{13}C$ and $\delta^{15}N$, by EA-IRMS as described above.

2.2.5 Data analysis

To measure the amount of initial litter N (endogenous N) still present in the litter at each harvest, the mixing model (Fry, 2006; Eq.1) was used to determine the contribution of endogenous N ($f_{\text{endogenous N}}$) to the total litter N, and this was then multiplied by the amount of N (mg) in the litter at each harvest. For this mixing model, the end members were the initial litter $\delta^{15}N$ and the 0-2cm bulk soil $\delta^{15}N$, on the assumption that the exogenous N uptake would derive from there.

Equation 1

$$f_{endogenous\ N} = \frac{(\delta_{litter\ remaining} - \delta_{soil})}{(\delta_{initial\ litter} - \delta_{soil})}$$

The N immobilized from the soil (exogenous N) was determined by subtracting the endogenous N from the total litter N.

The litter derived N contribution (flitter) to the bulk soil and SOM pools at each harvest was also calculated applying the isotope mixing model (Eq. 2). In this case, as end-members the 15N of the corresponding bulk soil or SOM pool determined by the overall average of all bare collars (n=20) and the 15N of the initial litter were used:

Equation 2

$$f_{litter} = \frac{(\delta_{sample} - \delta_{control})}{(\delta_{litter} - \delta_{control})}$$

To determine the total amount of litter derived N incorporated in the bulk soil at depth and within each fraction, first each average bulk soil and SOM fraction N pool size was determined for the area of the collar (20 cm diameter) using the average %N and % fraction measured across all samples (n=80) and bulk density values determined for each depth interval at the beginning of the experiment (n=4). Then the flitter values were multiplied to the corresponding pool amount. Similarly, the mixing model was used to calculate litter N contribution to both above and below ground plant matter. Here δ sample is the δ15N value of the plant biomass, δ control is the δ15N value of the control plant biomass and δ litter is the initial δ15N value of the *A. gerardii* litter added in the field. To determine the total amount of litter derived N incorporated by the plants, above-ground and below-ground estimates of plant biomass were used (400 mg/m2 for above-ground and 425 mg/m2 for below-ground (La Pierre, et. al 2011)) to convert the mg of LD N/ gram of plant biomass to mg of LD N/m2. To determine the area sampled, the sampling distance was used as the radius of the area, for the two circles separately, and the inner circle (radius 15 cm) subtracted from the area of the larger circle (radius 30cm) when this was calculated.

A general linear mixed model was used to determine a) the effect of time and MS treatment on litter mass and exogenous and endogenous N, and litter derived N in plants and b)

the effect of time, depth, and MS treatment on litter derived N pools. The effect of block and the two way interaction between the effects were included as categorical random effects with standard variance components. The approximation by Kenward and Roger (1997) was used for f-tests and post hoc t-test. To determine the simple effects of MS within time, a post-hoc pairwise t-test with a Tukey adjustment to control type 1 error was used. All general linear mixed models were generated using SAS® software version 9.3. In all cases, type III tests of fixed effects were used. We report least means and standard errors. Treatments were considered significantly different at a 0.05 level unless otherwise noted.

2.3 RESULTS

2.3.1 Litter mass loss and N dynamics

Both the control and MS treatments lost over half of litter mass within one year of the incubation, and lost the remaining mass by the third year of the experiment (figure 1). There was both a time and MS effect on litter mass remaining (table 1), specifically the control litter had less mass remaining after six months than the MS litter (p=0.0238) (figure 1). Both the control and MS treatments lost over half of their endogenous N within one year, and lost all N by the third year of the experiment (figure 2). Exogenous N had entered the litter as early as six months after the start of the incubation (figure 2). There was an effect of time on litter N, but there was no effect of the MS treatment on the exogenous N dynamics (figure 2) (table 1). Both the control and MS treatments had a higher correlation between endogenous N and mass remaining than total N and mass remaining (figure 3).

Table 1: Results of the general linear mixed models of the effect of harvest (harv) MS (fauna) soil depth (depth) and transect (transect) on the different variable measured. LD stands for litter derived.

Value	Effect	P-value
Soil		
LD N in bulk soil	harv	0.0001
	faun	0.8848
	harv*faun	0.3549
	depth	<.0001
	harv*depth	<.0001
	faun*depth	0.9992
	harv*faun*depth	0.853
LD N in light fraction	harv	0.0002
	faun	0.0021
	harv*faun	0.198
	depth	<.0001
	harv*depth	0.0009
	faun*depth	0.0589
	harv*faun*depth	0.5249
LD N in sand sized fraction	harv	<.0001
	faun	0.0608
	harv*faun	0.0205
	depth	<.0001
	harv*depth	0.066
	faun*depth	0.2455
	harv*faun*depth	0.2242
LD N in silt sized fraction	harv	0.0005
	faun	0.0755
	harv*faun	0.2063
	depth	<.0001
	harv*depth	0.3281
	faun*depth	0.0823
	harv*faun*depth	0.7088
LD N in clay sized fraction	harv	0.0049
	faun	0.0345
	harv*faun	0.5665
	depth	<.0001
	harv*depth	0.6118
	faun*depth	0.3122
	harv*faun*depth	0.8154

Value	Effect	P-value
Litter		
Mass remaining	harv	<.0001
	faun	0.0217
	harv*faun	0.3262
Endogenous N	harv	<.0001
	faun	0.526
	harv*faun	0.4401
Exogenous N	harv	<.0001
	faun	0.6647
	harv*faun	0.8198
Plant Biomass		
LD N in plant biomass	harv	0.0003
	faun	0.4064
	harv*faun	0.8766
	transect	0.2746
	harv*transect	0.8956
	faun*transect	0.154
	harv*faun*transect	0.0911

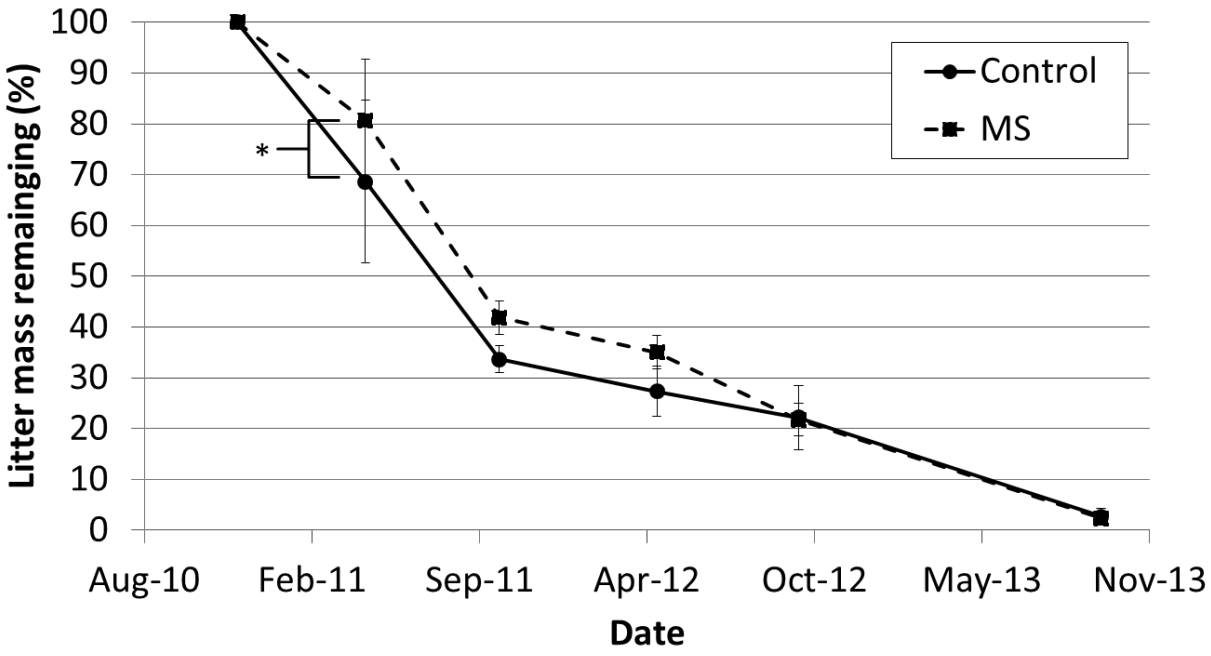

Figure 1: Percentage of litter mass remaining for the *A. gerardii* labeled litter over the three years of field incubation. Full circles represent control treatment, and dashed squares represent microarthropod suppression (MS). Data are average (n=4) with error bars showing SE. Stars reflect significant differences between treatments ($p < 0.05$)

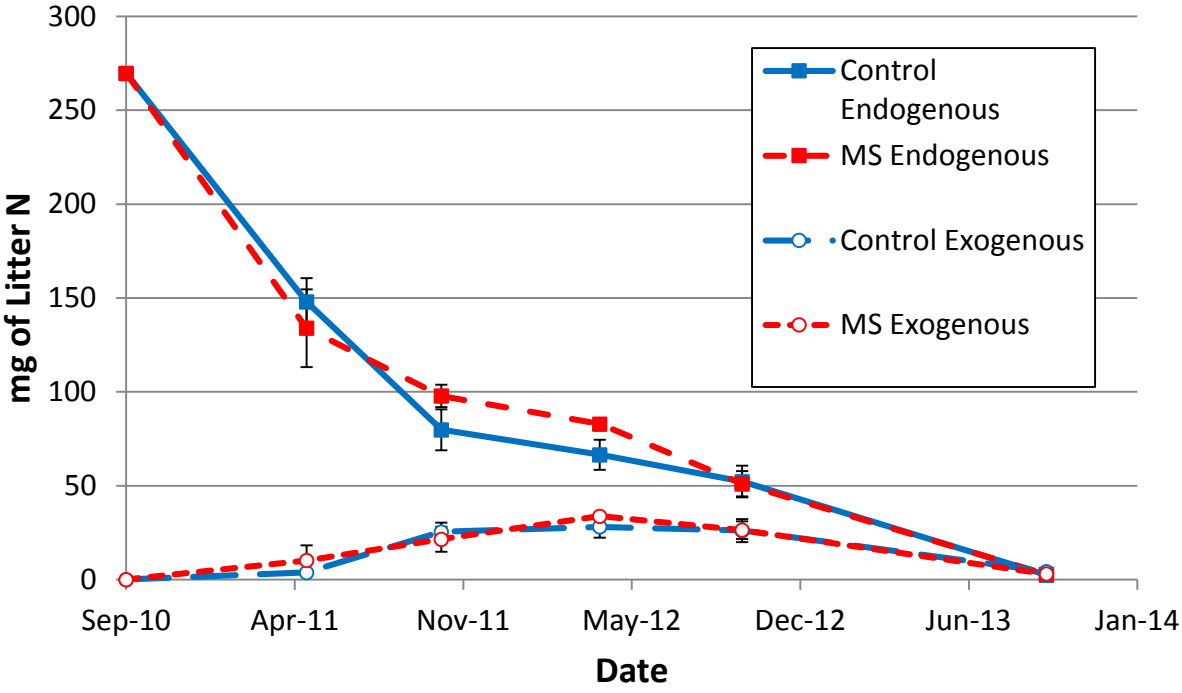

Figure 2: Dynamics of the amount of exogenous and endogenous litter N in the *A.gerardii* litter over the three years of incubation. Full blue squares represent endogenous N present in the control treatment, open blue circles represent exogenous N present in the control treatment, and full red squares represent endogenous N present in the microarthropod suppression (MS) treatment, open red circles represent exogenous N present in the MS treatment. Data are average (n=4) with error bars showing SE.

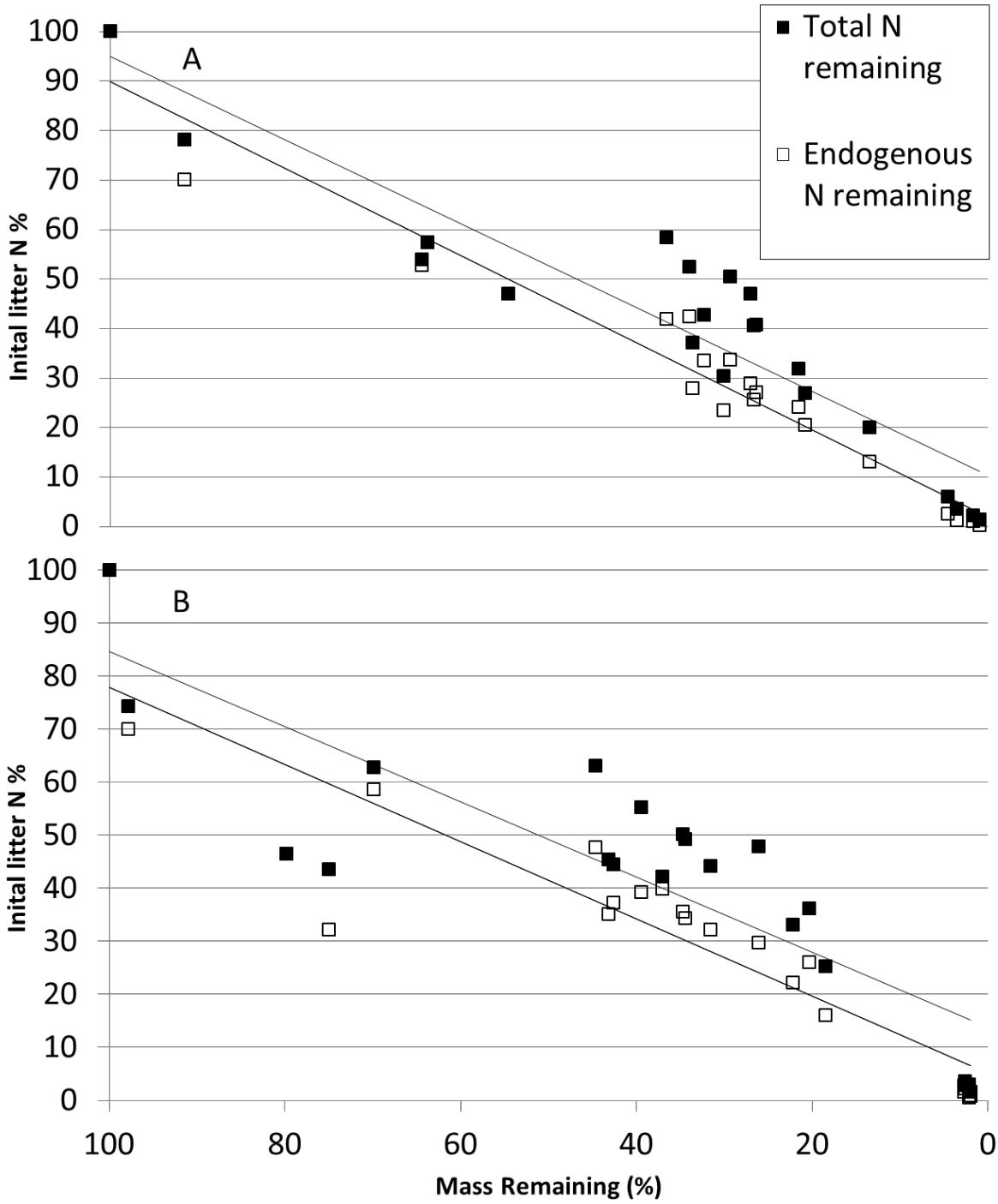

Figure 3: Percentage total litter N (y=0.0085x+0.104, r^2=0.85 and endogenous litter N (y=0.0088x+0.0189, r^2=0.9489) as a function of the litter mass remaining, for (A) the control (y=0.0071x+0.1374, r^2=0.7352) and the (B) microarthropods suppression treatments (y=0.0073x+0.0504, r^2=0.8231). Data are individual readings.

2.3.2 Soil N pools and litter derived N in soil

Due to the random variation in %N, and % fraction across all sampled soils, these values were averaged by depth and fraction across the five harvest times (n=80) to determine the N pool size of bulk soil and SOM pools (table 2). Similarly, due to the random variation in the $\delta^{15}N$ values in the background soil, these values were also averaged by depth and fraction across the five harvest times (n=20), and data are presented in table 2. These values were used, as described in the method section above, to calculate LD N pools.

The amount of LD N recovered in the soil profile increased over time (figure 4) (table 1). LD N was found in all sampled depths after six months, with more LD N present in the shallower soil layers (figure 4; (table 1). Treatments did not differ in the LD N recovered in soil, with the exception of the eighteen months harvest, when there was more LD N present in the 0-2 depth of the control soil than the MS 0-2 soil (p=0.0403) (figure 4). By fractionating the top soil layers (i.e., 0-2 and 2-5 cm) in their primary SOM fractions, some interesting dynamics emerged (Figure 5). Litter derived N was recovered in all fractions as early as after six months of litter incubation, when the majority of the LD N was recovered in the mineral SOM fractions (silt and clay). Except for the clay fraction, in all fractions the LD N pool increased with time. By the end of the incubation the majority of the LD N was retrieved in the LF (Figure 5). With respect to the MS treatments, at intermittent harvests the MS suppression reduced the recovery of LD N in the SOM fractions, in particular in the top soil layers, but without much of a time consistency (Figure 5).

Table 2: Percentage C, %N, %fraction (n=80) and background δ15N (n=20) values of the soil used for this experiment. Data are averages ± standard errors

Soil fraction	Soil Depth (cm)	Average of % Carbon	Average of % Nitrogen	Average of % Fraction	Average of δ ^{13}C bare	Average of δ ^{15}N bare
	0-2	5.2±0.1	0.40±0.00	100	-16.20±0.32	1.71±0.20
	2-5	4.4±0.0	0.27±0.00	100	-14.91±0.10	2.00±0.21
	5-10	3.8±0.0	0.35±0.00	100	-13.99±0.07	2.92±0.21
BULK	10-20	3.3±0.1	0.31±0.00	100	-13.25±0.07	3.75±0.22
	0-2	27.4±0.5	1.37±0.02	4.460.17	-18.45±0.31	-0.71±0.27
LF	2-5	25.3±0.5	1.19±0.02	2.74±0.11	-17.66±0.19	-1.08±0.18
	0-2	6.4±0.3	0.40±0.02	10.14±0.40	-16.35±0.18	-0.50±0.12
SAND	2-5	5.5±0.3	0.33±0.01	9.11±0.30	-15.76±0.13	-0.08±0.18
	0-2	2.7±0.1	0.23±0.01	70.63±0.48	-15.28±0.11	1.07±0.18
SILT	2-5	2.4±0.1	0.21±0.01	73.30±0.39	-13.83±0.51	1.74±0.18
	0-2	4.3±0.1	0.41±0.02	14.77±0.35	-15.29±0.31	3.63±0.13
CLAY	2-5	4.3±0.1	0.42±0.02	14.85±0.31	-14.34±0.08	4.08±0.20

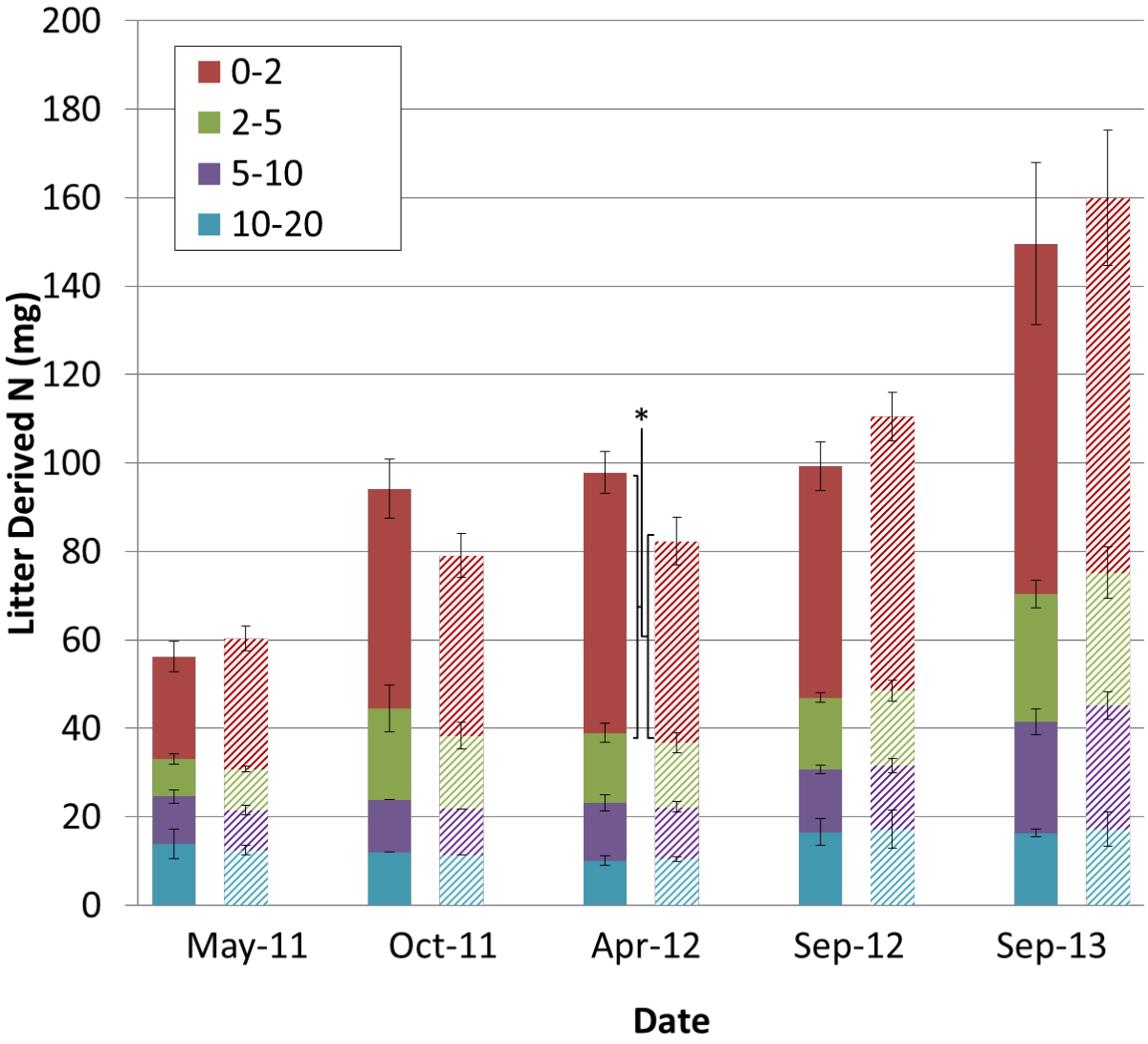

Figure 4: The total amount of litter derived N recovered in the soil profile to a depth of 20 cm over the three years of the labeled litter incubation experiment. Solid colors represent control treatment and dashed colors represent microarthropod suppression (MS) treatment. Data are average (n=4) with error bars showing SE. Stars reflect significant differences between treatments ($p < 0.05$)

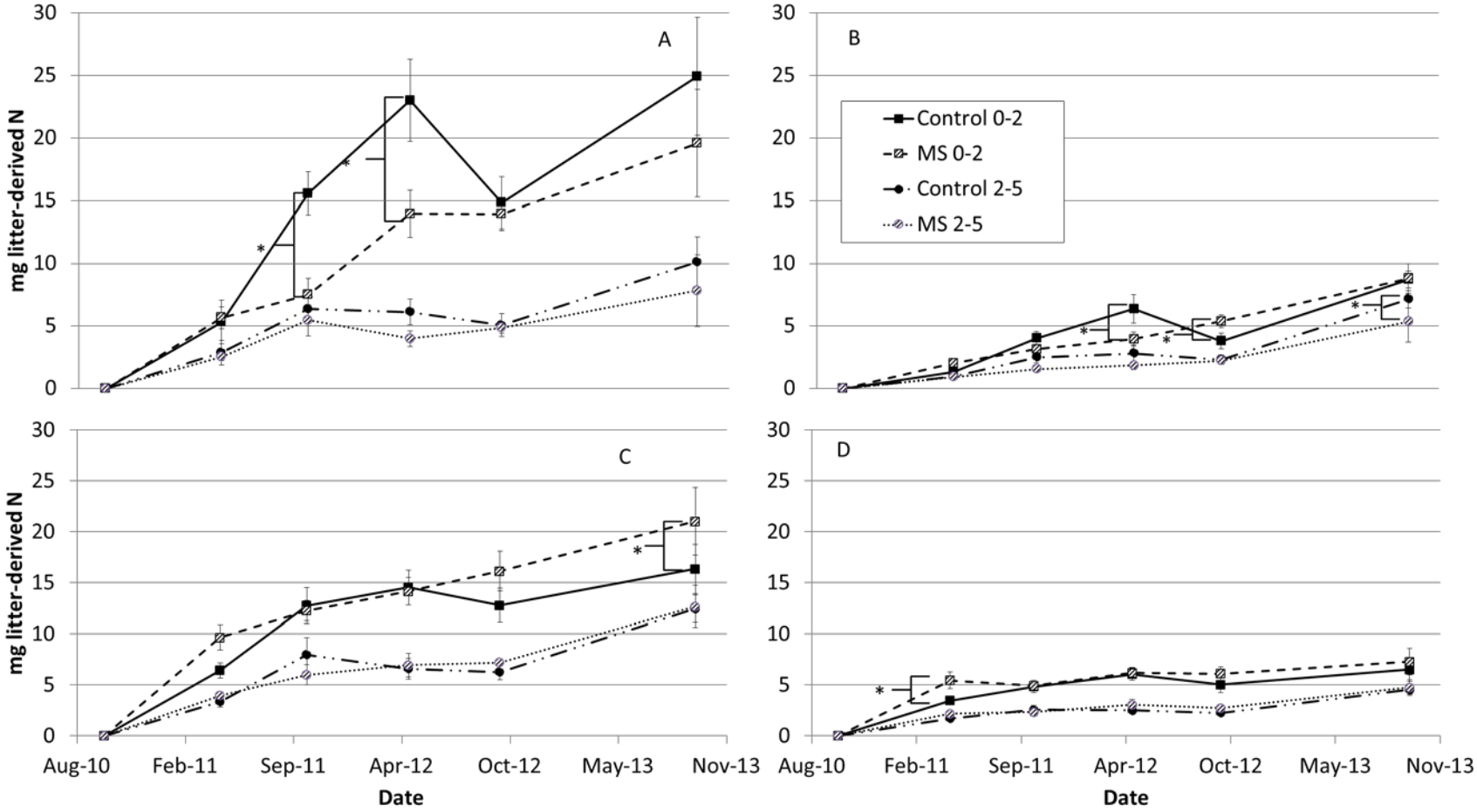

Figure 5: Dynamics of the litter derived N pool in (A) the light fraction, (B) the sand sized fraction; (C) the silt sized fraction and (D) the clay sized fraction, over the three years of the field labeled litter incubation. Squares and circles represent the 0-2 and 2-5 cm depth layer, respectively. Dashed and full symbols represent the microarthropod suppression (MS) and control treatments, respectively. Data are averages (n=4) with error bars showing SE. Stars reflect significant differences between treatments ($p < 0.05$).

2.3.3 Plant biomass and full litter derived N recovery

Litter derived N was recovered in plant biomass after only one year from the incubation of the labeled litter, and its amount increased over time (figure 6; table 1). After the three years of incubation, the soil and plant biomass retained cumulatively approximately 65% of the initial litter N, with the majority (56%) being however recovered in the soil. Approximately 35% of the initial litter N was lost from the system, which did not increase over time (figures 6).

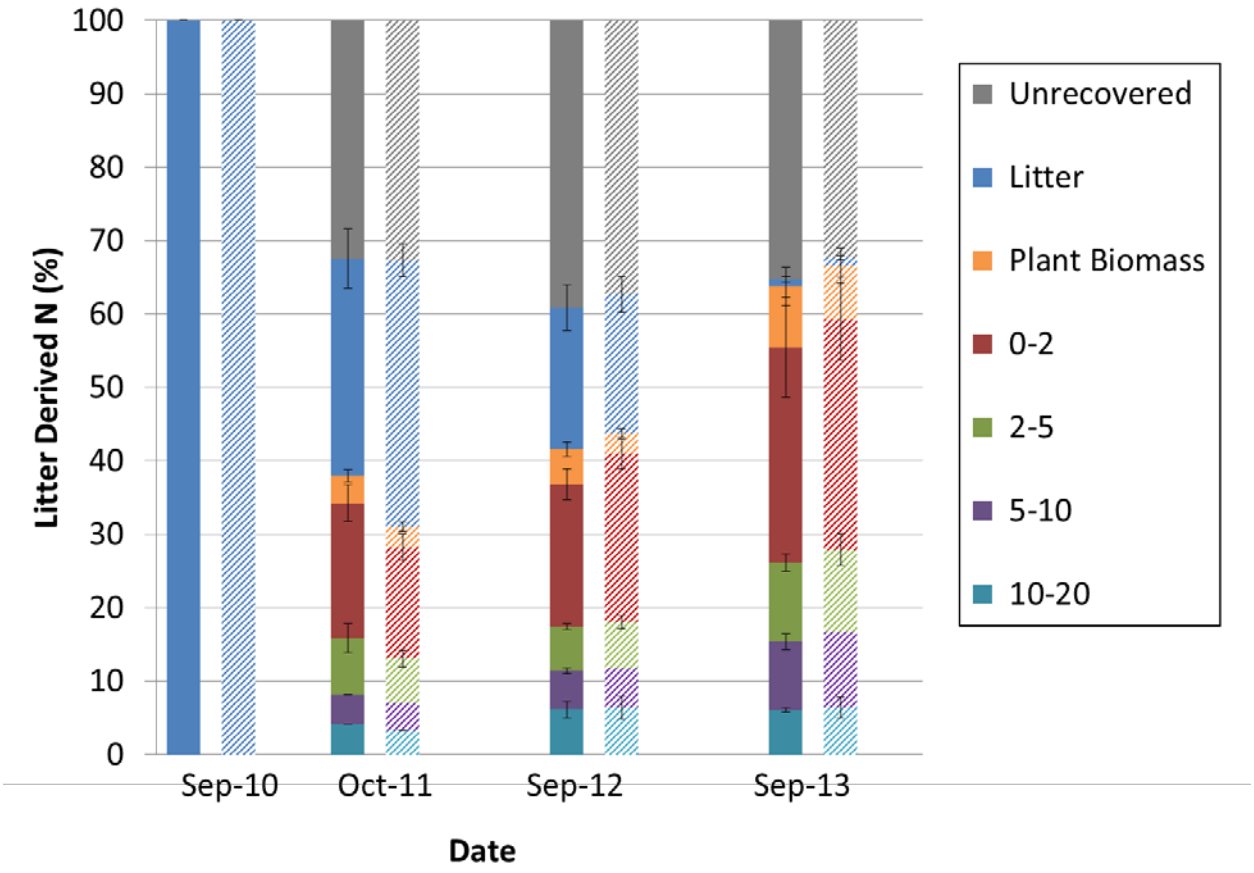

Figure 6: Relative amounts of initial litter N recovered in the soil to a depth of 20 cm, litter and plant biomass over the three years of the labeled litter incubation. Solid colors represent control treatment and dashed colors represent microarthropod suppression treatment. Data are average (n=4) with error bars showing SE.

2.4 DISCUSSION

The use of a highly ^{15}N labeled litter allowed for accurate tracing of litter derived N in the litter layer, mineral soil, and plant biomass over the course of the three year field incubation. This was demonstrated to be a long enough time period to allow for complete decomposition of our litter and thus accurate budgeting of the fate of the N contained in it. In fact there was no litter remaining at the end of the experiment on the grassland floor. Most decomposition experiments conducted using litter bags retrieve a fraction of mass remaining even after 10 years of field incubation (Adair *et al.*, 2008). With our bag-free method, we demonstrated that litter decomposition does not necessarily reach an asymptotic value, as proposed by Berg *et al.* (1996), rather, the litter residues are incorporated into the mineral soil throughout the decomposition process. Interestingly, the year three harvest had a large amount of LD N present in the LF, suggesting that this final stage of decomposition is driven by fractionation. This would explain why litter bag studies, which inhibit fractionation, do not reach total mass loss (Hobbie *et al.* 2012, Preston *et al.* 2013).

The rate of litter N loss was consistent throughout the experiment with the rate of mass loss, suggesting that N was not preferentially retained in the litter. Parton *et al.* (2007) showed that litter N retention is expected for litter with an initial N concentration of less than 1.02%. Because the %N was higher in our litter (1.47%), we expected to see very little immobilization (Parton *et al.* 2007), which is exactly what we observed. However, we also did not expect to see exogenous N incorporation. Even if at low levels of remaining litter N, we did see some exogenous N being incorporated during the intermediate stage of decay, suggesting that this is a common process probably linked to the fungal hyphae growing from the soil into the litter and not necessarily due to litter N limitation. At the site, A. gerardii native litter has a lower %N

than the labeled litter we produced (Soong *et al.*, submitted), thus slower decay rate and higher exogenous N incorporation may be expected in native litter.

Microarthropods increased early mass loss exactly as expected (Wall *et al.* 2008), but did not affect litter N dynamics in our experiment, probably because decomposition was not N limited in our litter (Parton *et al.* 2007). Microarthropods have been observed to increase decomposition rates in temperate grasslands, probably through fragmentation (Wall *et al.* 2008), which explains why there was less N in the LF early in the experiment when microarthropods were excluded. The lack of an effect of microarthropods on litter N dynamics may be due to the high %N in our litter, (1.47% N in our litter, 0.60%N in the background litter) which exceeded decomposers N demand (Parton *et al.* 2007).

In the soil, LD N was found as early as six months in all of the fractions. Surprisingly the mineral fractions (silt and clay) received most of their LD N in the early stage of decomposition before the incorporation of litter fragments in the LF peaked. This early dynamic of LD N incorporation in SOM fraction is somewhat different from what conventionally thought: that litter enters the soil as litter fragments (LF) and that from there, progressive decomposition and microbial transformation generates the mineral fractions (Grandy & Neff, 2008). The observed early incorporation of LD N on minerals suggests a different mechanism and possibly that DOM leaching of water soluble litter components may be the source of the initial LD N on mineral fractions. High DOM leaching was observed from *A. geradii* litter in laboratory incubation (Soong *et al.*, submitted). The litter dynamics of LD N incorporation in SOM fractions are more consistent with the conventional model of SOM stabilization (Six *et al.* 2002), in which decomposition of the LF, the LD N enters the sand sized fraction, and finally stabilizes in minerally associated fractions. In our study, microarthropods affected LD N incorporation in

SOM mostly by depressing fragmentation and the incorporation of the LF. This is consistent with the general understanding that microarthropods control the amount of fragmentation, possibly by physically breaking the litter and moving the fragments below-ground (Hättenschwiler *et al.* 2005). Interestingly, without the losses from fragmentation, the LD N was higher in the silt and clay sized fractions, which could be due to increases in leaching. So although microarthropods do not increase total litter N losses, they do alter the pathways through which the N enters the soil.

Litter N was recycled quickly into the plant biomass. Litter derived N was found in plant biomass as early as one year from the start of litter incubation and the amount increased over the three years. We are unable to determine the species of N the plants absorbed, or if the plants were short circuiting the soil N cycle, but we did confirm that plant biomass is a pool that receives and retains early LD N inputs (Neff *et al.*, 2003; Yu *et al.* 2002). At our site, between plant biomass and soil, retention of litter N was around 65%. Percentage recovery did not change over time, suggesting that the 35% unrecovered N was lost from the system during the early stages of decomposition. We did not measure N2O emissions, or other source of N losses, but, considering that the grassland that our study took place in was N limited, the high recovery is expected (Craine *et al.* 2006). The effect of microarthropods on plant uptake could be masked by the litter being unusually high in N, resulting in decomposers mineralizing more N regardless of microarthropod presence, or soil microbes altering their N uses efficiency to retain the additional N mineralized with microarthropod presence (Mooshammer *et al.* 2014).

2.5 CONCLUSIONS

Our study confirmed that stable isotope enrichment is a powerful method to trace N dynamics in the natural environment. At our mesic tallgrass prairie characterized by a fine-

texture soil litter N recycling demonstrated to be relatively open, with 65% of the litter N remaining in the plant-soil system. Surprisingly early stages of litter decomposition contributed to clay fraction SON formation, while later stages produced light fraction. These SON dynamics require confirmation in other ecosystem, and eventually to revisit conventional model of SOM formation. Microarthropods were shown to be a significant factor of litter and SON dynamics, mostly by accelerating litter mass loss, likely through fragmentation and incorporation of LF in the top mineral soil.

3. TRACING THE FATE OF ABOVEGROUND LITTER-DERIVED CARBON AND NITROGEN THROUGH SOIL PHYSICAL FRACTIONS, RESPIRATION AND DISSOLVED ORGANIC MATTER LEACHATES

3.1 INTRODUCTION

The atmospheric C pool is continuously increasing due to anthropogenic activities (IPCC 2014). Yet, there are around 600 Pg of carbon (C) in the atmosphere, while over three times that amount is stored in the largest terrestrial pool: soils (1500-2400 PgC) (Ciais *et al.*, 2013). Atmospheric and terrestrial carbon fluxes are currently in disequilibrium, with more C entering the terrestrial ecosystem through photosynthesis(108.9 PgC/y) than carbon entering the atmosphere through respiration (1.72 PgC/y), globally making the terrestrial ecosystem a C sink (Ciais *et al.* 2013). Soil organic matter (SOM) is the largest reservoir of C in terrestrial ecosystems, in addition to being closely associated with almost 95% of total soil N (Jobbagy & Jackson 2000; Schulten & Schnitzer 1998). In addition to C and N storage, SOM is an important component of the soil: it is the source of plant nutrients, stabilizes soil structure, and plays a central role in soil surface-atmosphere exchange of greenhouse gases (Balesdent *et al.*, 2000; Smith *et al.*, 2001; Grandy & Robertson, 2006). Thus, it is important to understand how C and N move through the SOM system.

Soil organic matter can be separated into physically distinct fractions, such as a light fraction (LF; <1.85 g cm^{-3}), and a heavy fraction (>1.85 g cm^{-3}). The heavy fraction can be further separated into a sand, a silt and a clay-sized fraction. These fractions are characterized by progressively higher concentrations of N as compared to C (i.e., by progressively lower C:N ratios), going from the LF to the clay fraction (Christensen 2001). Thus they are believed to

correspond to progressively higher level of decomposition and microbial transformation (Grandy &Neff, 2008). These fractions also differ in the stabilization mechanisms and time of persistence in soil.

Soil organic matter can be stabilized through four processes: Chemical stabilization, intrinsic recalcitrance, physical stabilization, and inhibition of microbial activity (Trumbore 2009). Chemical stabilization refers to the chemical bonding which links organic matter to mineral surfaces, and prevents decomposition. Intrinsic recalcitrance refers to the fact that some organic compounds are more resistant to decomposition, for example tannins and some lipid compounds (Sollins 2009). Physical stabilization occurs when organic matter becomes associated within aggregates, and microbial activity can be inhibited through freezing temperatures, low O_2 content and water saturation. while The LF is primarily stabilized by chemical recalcitrance, and persists for time periods in the order or decades. Silt and the clay fractions, by contrast, are stabilized through chemical bonding to the minerals and can persist for centuries to millennia (von Lützow *et al.* 2007). Studying the dynamics of litter-derived C and N incorporation into these soil fractions can therefore elucidate the mechanism of SOM formation and its persistence.

The accepted OM path in soil is that from plant litter (LF), OM moves directly or through microbial processing to the sand-sized free OM and to minerally associated OM, with losses via respiration (Six *et al.* 2002.) This understanding of the OM path is mostly derived from the chemical properties (e.g. C:N ratio) of the fractions (Grandy & Neff, 2008). In addition to SOM pools, C and N can become dissolved organic matter (DOM), which is often readily available to soil microbes and moves C and N deeper into soils. (Marschner & Bredow, 2002). Dissolved organic matter represents an additional pool of C and N in the soil, and is extremely

important to soil microbes (Marschner & Bredow, 2002). In this experiment, we propose to empirically measure the flow of C and N through SOM fractions with an isotope enrichment study. Empirically understanding how these SOM fractions differentially stabilize C and N, as well as generate DOM and respiration is key to accurately quantify total SOM stabilization.

The objectives of this experiment were to quantify and understand how aboveground litter-derived organic matter (a) is released as CO_2, and DOM leaching, and (b) transfers between primary SOM pools. The specific hypotheses tested were (a) DOC leaching and respiration are both products of SOM decomposition and thus draw from the same carbon pools in the soil, b) litter-derived OM in soil is more labile (e.g. decompose faster) when derived from fresh litter at the early stage of decomposition, and before it becomes stabilized in minerally associated fractions, and c) once stabilized in mineral fractions such as silt and clay, the litter-derived OM will be more resistant to further decomposition (loss of C and N) than OM in the light fraction or associated with sand.

To achieve those goals we incubated isotopically labeled soil for 150 days, and regularly collected respiration and leachate. At the beginning and conclusion of the incubation a sample of the soil was fractioned to investigate how the SOM fractions changed.

3.2 MATERIALS AND METHODS

3.2.1 Production and collection of the ^{13}C and ^{15}N enriched soil

In order to trace the fate of litter-derived C and N in soil and SOM fractions, we used soils which had received ^{13}C and ^{15}N enriched above ground litter inputs in the field. This enriched litter decomposition experiment was conducted at the Konza Prairie long-term ecological research site in northeastern Kansas, U.S.A. The site is a tall grass prairie, dominated

by *Andropogon gerardii*. Climate at the site is temperate-continental, with a mean annual temperature of 12.8◦C and an average annual precipitation of 835 mm. The soil is silty–clay–loam Mollisol, with 4% C and 0.32% N and presents the characteristic of the lowland soils at the site (Knapp *et al.*, 1998). The experimental area used for the study was burned annually from 1972 to 2000, when burning treatments ceased, and was burned accidentally once again in 2008. Detailed site descriptions can be found in Knapp *et al.* (1998).

3.2.2 Field experimental design

At Konza, a field experiment was established as described in Chapter 2. For the purpose of this study we used the soils collected at two harvests: 12 months and 24 months, with four replicate blocks at each harvest. In each replicate, there were two PVC collars inserted in the ground to 5 cm, randomly assigned to one of the harvesting dates. All collars were inserted on June 1st 2010, when the native litter was removed. On September 29th, 2010, one of the collars in each replicate received 18.4 g of isotopically enriched *Andropogon gerardii* above-ground plant material, and the other collar was left bare to serve as a control (i.e., the background end-member in the isotopic mixing model, see data analyses below). The isotopically enriched litter (δ^{13}C Value: 2122.96 ‰, Atom%: 3.38037, δ^{15}N Value: 10308.70 ‰, Atom%: 3.9917) was generated at the Colorado State University Greenhouse, using the continuous labeling chamber described in Soong *et al.* 2014.

3.2.3 Soil sampling and analyses

On October 8th 2011 (12 months or Y1), and September 29th 2012 (24 months or Y2), soil from within one PVC collar for each treatment (i.e., litter and control) was collected in each of the four replicate blocks. The soil within the collar was gently excavated with the use of hand

shovel by incremental depths (0–2; 2–5; 5–10 and 10–20 cm) and the soil collected from each layer was stored separately in pre-labeled plastic bags. All equipment was sterilized with 70% ethanol before each sample. All soil samples were stored with ice in coolers before being brought to the laboratory the following day. There they were stored at 4◦C until they were processed within two weeks of collection. All soil samples were sieved to 2 mm, air dried and stored separate by replicate at room temperature.

3.2.4 Experimental design

The laboratory experiment consisted of four replicates of the 2-5 cm depth enriched soil samples from Y1 and Y2, and of four control soils, two taken from Y1 and two from Y2. The experimental set-up was designed to allow for repeated gas samplings and leachate extractions on the same soil sample, and follows the method used by Zheng *et al.* 2012. Each soil sample (40 grams) was placed on top of a Whatman grade GF/C 1.2 mm filter, within filter unit (150 mL Falcon Filter Model number 7102, Becton Dickinson Labware). These were then placed in 1.89 L air-tight glass jars with lids containing septa for gas sampling. Deionized water was used to bring the soil to 60% water holding capacity (WHC), which was previously determined on the soil using the method described in Linn and Doran (1984). The 60% WHC was maintained by adding DI water at each leachate collection time. Soils were incubated in a constant temperature room at 25°C in the dark for 150 days.

3.2.5 CO_2 sampling and measurement

Soil respiration was measured on Days 1, 4, 11, 18, 32, 46, 60, 81, 112, and 150 of the incubation by determining the CO_2 concentration accumulated in the jar headspace during the

time elapsed between the last sampling. After mixing the jar's headspace, a 2-mL subsample was manually injected into an infrared gas analyzer (IRGA, model LI6252, LICOR.) Calibration with an external standard curve determined CO_2 concentration.

On the same dates, 0.75 ml of headspace air was also injected into a VG Optima IRMS (Isoprime Inc., Manchester, UK) with microgas injector and equilibration block to determine the $\delta^{13}C$ value of the CO_2 (observed precision: 0.3‰). After gas sampling and leachate extraction (see below) jars were flushed for 20 minutes with CO_2 free air and re-incubated in the constant temperature room (Zheng *et al.* 2012).

3.2.6 Soil leachates extraction and analysis

On Days 1, 11, 32, 46, 60, 81, 112, and 150 after gas sampling was completed, the filter units containing the soil were removed from the jars, and flushed with 100 ml of a C and N free extraction solution: (modified method from Nosshi *et al.* 2007): 4.0 mmol L^{-1} $CaCl_2$, 2.0 mmol L^{-1} KH_2PO_4, 1.0 mmol L^{-1} K_2SO_4, 1.0 mmol L^{-1} $MgSO_4$, 25 mmol L^{-1} H_3BO_3, 2.0 mmol L^{-1} $MnSO_4$, 2.0 mmol L^{-1} $ZnSO_4$, 0.5 mmol L^{-1} $CuSO_4$, and 0.5 mmol L^{-1} Na_2MoO_4. This solution was slowly and gently added into extraction cups, allowed to equilibrate for 0.5 h, and extracted using a 59-kPa vacuum for 15 min or until the flow of solution through the cup ceased. Sample cup weights were recorded before and after extraction to assure constant soil moisture. The extraction was placed into 50-mL plastic vials and then placed in a freezer until C and N analysis. Samples of the leachate were lyophilized and analyzed on an Elemental Analyzer Isotope Ratio Mass Spectrometer (EA-IRMS, Costech ESC 4010 (Costech Inc.) coupled to a Thermo Delta V Advantage continuous flow IRMS, (Thermo-Fisher Corp))(observed precision: 0.2‰ for C and 0.3‰ for N) for %C, %N, $\delta^{13}C$ and $\delta^{15}N$.

3.2.7 Soil organic matter fractionation and elemental and isotopic analysis

After gas sampling and leachate extraction on day 150, the soils were removed from the filter units and air dried. These soil (day 150), as well as the soils prior to incubation (day 0), were then fractionated, by density and size using a method modified from Marzaioli et al (2010) and Denef *et al.*, (2013). Soil was fractionated into four fractions: a light fraction, (LF; < 1.85 g cm^{-3}), and an heavy fraction, which was farther separated by size into a sand sized (53-2000 µm), and a finer mineral fraction, subsequently separated by centrifugation into silt and clay sized OM fraction. For each sample, 5 grams of soil was added to 25 ml of 1.85 g cm^{-3} Sodium Polytungstate (SPT) with twelve glass beads, and shaken for 6 hours to disperse macro aggregates. After centrifugation for 60 minutes at 20 degrees Celsius at 2500 rmp, a pellet of heavy SOM (HF > 1.85 g cm^{-3}) was formed, leaving the LF suspended. The LF was aspirated off on to 20 µm nylon filter via a Millipore glass filter unit. After repeated rinses to remove residual SPT, the HF pellet was dispersed and sieved on a 53 µm filter, separating the sand sized fraction (> 53 µm) from the finer silt and clay fraction. This finer fraction was then separated into a silt and a clay fraction using centrifugation (3.4 minutes at 20C and 1000RPM), which pelleted the silt and left the clay suspended. The suspended clay was aspirated off, and the silt pellet was dispersed and recovered with DI water. Each bulk soil and SOM fraction was oven dried at 60 °C, pulverized and analyzed for δ13C, δ15N, %C and %N on an Elemental Analyzer Isotope Ratio Mass Spectrometer. The day 0 samples were run on a Carlo Erba NA1500 coupled to a VG Isochrom continuous flow IRMS, Isoprime Inc. (observed precision: 0.2‰ for C and 0.3‰ for N) and the day 150 samples were run on the Costech ESC 4010 as described above, after having inter-calibrated the two instruments.

3.2.8 Data analysis

The litter-derived C and N contribution to the C and N released via DOM leaching , respiration (for C only) or remaining in SOM fractions was assessed by the isotope mixing model (Fry, 2006), using the control soils and the initial enriched litter as end members, following:

Equation 3

$$\boldsymbol{f_{litter}} = \frac{(\boldsymbol{\delta_{sample}} - \boldsymbol{\delta_{control}})}{(\boldsymbol{\delta_{litter}} - \boldsymbol{\delta_{control}})}$$

where f_{litter} is the relative proportion of litter derived C or N in a CO_2, DOM or SOM sample; δ sample is the $\delta^{13}C$ value of the CO_2, or the $\delta^{13}C$ or $\delta^{15}N$ value of the DOM bulk soil, or SOM fraction, δ control is the $\delta^{13}C$ or $\delta^{15}N$ value of the respective control and δ litter is the initial $\delta^{13}C$ or $\delta^{15}N$ value of the *A. gerardii* litter added in the field. Litter derived pool sizes were obtained by multiplying the f_{litter} values for the respective total C or N pool (e.g., mg C-CO_2, -DOC, etc.).

The effect of year from litter addition in the field (from hereon called “year” and abbreviate to Y) and time of laboratory incubation (from hereon called “time”) on all data was tested by means of a general linear mixed model. The effect of replicate and the two way interaction between replicate and harvest were included as categorical random effects with standard variance components. The approximation by Kenward and Roger (1997) was used for f-tests and post hoc t-test. To determine the simple effects of experimental time within time in the field, a post-hoc pairwise t-test with a Tukey adjustment to control type 1 error was used. All general linear mixed models were generated using SAS® software version 9.3. In all cases, type

III tests of fixed effects were used. We report least means and standard errors. Treatments were considered significantly different at a 0.05 level unless otherwise noted.

3.3 RESULTS

3.3.1 Soil respiration

The Y1 and Y2 soils cumulative respiration showed declined exponentially during the incubation time, just as expected (Fig. 7A). The Y1 soil respired 22 mg C CO_2 over the course of the 150 days, which was 12% more than the Y2 soil (18 mg C CO_2). This difference was statistically significant (Table 3). In particular, Y1 soils had significantly more respiration than Y2 at days 11(p =0.0075), 18 (p =0.0021), 32(p =0.0005), 46 (p =0.0002), 60 (p =0.0001), 81 (p <0.0001), 112 (p <0.0001), and 150 (p =0.0001) (Fig. 7A). Both the Y1 and Y2 respiration fluxes were enriched in ^{13}C well above the background respiration enrichment (Fig. 7B) (Table 3). Y1 respiration was significantly more enriched at day 4 (p=0.0465) and 11 (p=0.0494) than Y2. At day 150, the Y1 respiration (27.03‰ ± 2.25) was as enriched as the day 0 Y2 soil (25.07‰ ± 5.47) (Fig. 7B). The background $\delta^{13}C$-CO_2 value was not affected by time of incubation, and remained consistently around 17‰, which allowed for a robust mixing model (Fig. 7B). Using the mixing model, we calculated the fraction of litter-derived C-CO_2 which, multiplied by the emitted CO_2 gave the litter-derived C-CO_2 (Fig. 7C). Y1 respiration had significantly more litter-derived C than Y2 at day 1 (p = 0.0337) (Fig. 7C). There was a time effect on litter-derived C CO_2 for Y1, (p <0.0001) but not for Y2 (p =0.2621) soils (Fig. 7C) (Table 3).

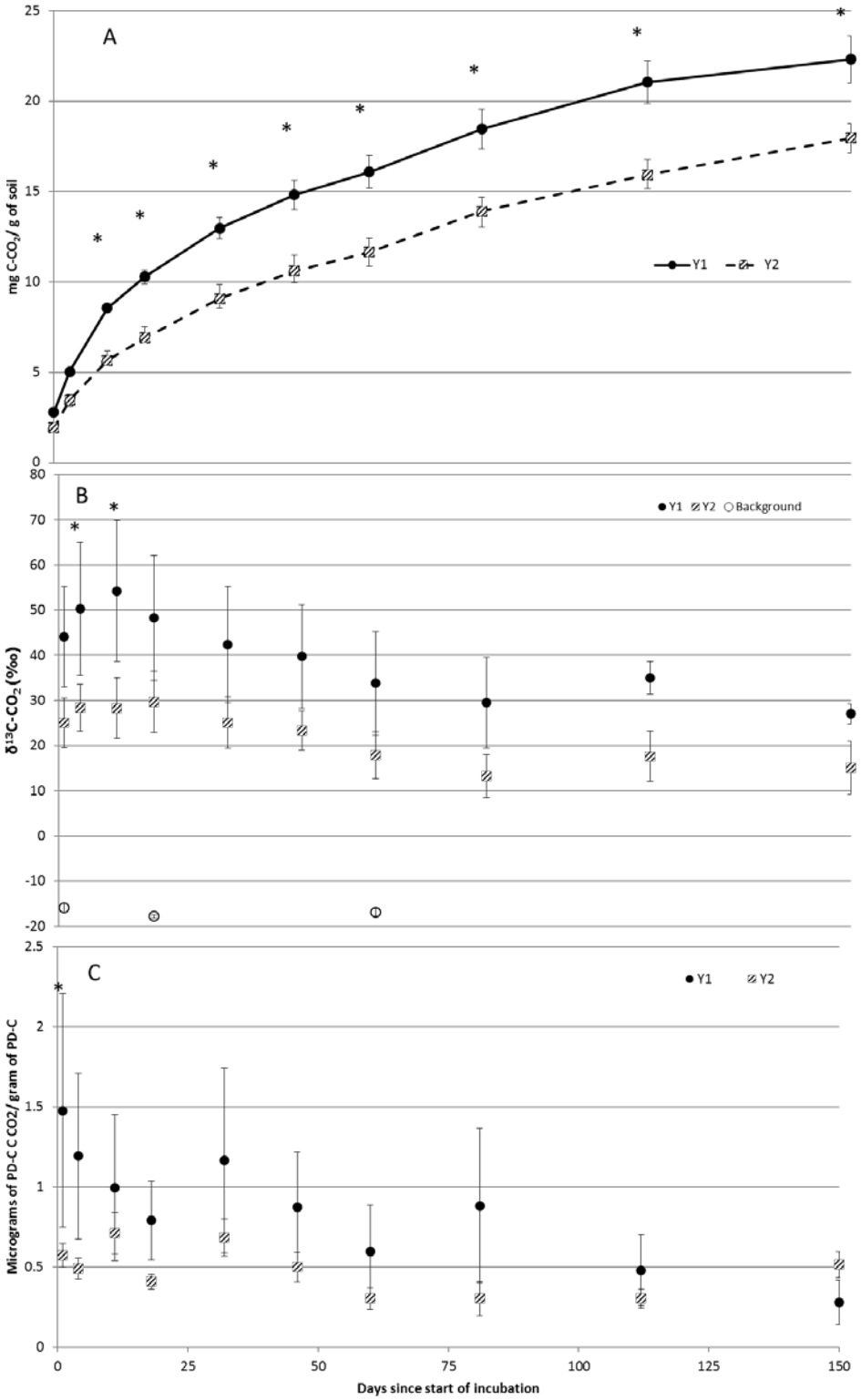

Figure 7: A) Mean cumulative CO_2 respiration (mgC CO_2 per gram soil). B) Mean δ ^{13}C values of soil respiration throughout the experiment. C) Mean litter-derived carbon (LD) respiration throughout the experiment. Solid circles represent Y1, and dashed squares represent Y2. Data are average (n=4) with error bars show SE. Stars reflect significant differences between treatments ($p < 0.05$)

Table 3: Results of the general linear mixed models of the effect of time (time) and treatment (year)

Value	Effect	P-value
Respiration		
Cumulative C CO_2	time	0.0071
	year	<.0001
	time*year	<.0001
$\delta^{13}C$ CO_2	time	0.0018
	year	<.0001
	time*year	<.0001
LD C CO_2	time	0.3638
	year	<.0001
	time*year	0.0946
Leachate		
Cumulative C	time	<.0001
	year	0.1835
	time*year	0.071
$\delta^{13}C$	time	0.0009
	year	0.0017
	time*year	0.0934
LD C	time	<.0001
	year	0.1294
	time*year	0.0002
Cumulative N	time	<.0001
	year	0.767
	time*year	0.2224
$\delta^{15}N$	time	0.0196
	year	0.0003
	time*year	0.1393
LD N	time	<.0001
	year	0.1085
	time*year	0.3246

Value	Effect	P-value
Soil		
Δ $\delta^{13}C$ in bulk soil	year	0.4094
Δ $\delta^{13}C$ in light fraction	year	0.293
Δ $\delta^{13}C$ in sand sized fraction	year	0.0427
Δ $\delta^{13}C$ in silt sized fraction	year	0.4423
Δ $\delta^{13}C$ in clay sized fraction	year	0.0584
Δ LD C in bulk soil	year	0.3312
Δ LD C in light fraction	year	0.4342
Δ LD C in sand sized fraction	year	0.0091
Δ LD C in silt sized fraction	year	0.0287
Δ LD C in clay sized fraction	year	0.0836
Δ $\delta^{15}N$ in bulk soil	year	0.2792
Δ $\delta^{15}N$ in light fraction	year	0.0977
Δ $\delta^{15}N$ in sand sized fraction	year	0.1959
Δ $\delta^{15}N$ in silt sized fraction	year	0.1869
Δ $\delta^{15}N$ in clay sized fraction	year	0.733
Δ LD N in bulk soil	year	0.2642
Δ LD N in light fraction	year	0.3531
Δ LD N in sand sized fraction	year	0.0612
Δ LD N in silt sized fraction	year	0.0354
Δ LD N in clay sized fraction	year	0.6658

3.3.2 Leachate

For both Y1 and Y2 soils, DOC losses were two orders of magnitude smaller than C losses through respiration (Fig. 7A, Fig. 8A). The Y1 soil lost 0.202 mg C/gram of soil through DOC leaching over the course of the 150 days, which was 25% more than the Y2 soil (0.152 mg C), and in particular, Y1 soils had significantly more DOC in the leachate than Y2 at days, 32(p =0.0290), 46 (p =0.0237), 60 (p =0.0124), 81 (p =0.0192), 112 (p =0.0297), and 150 (p =0.0559) (Fig. 8A). However, overall the treatment difference result was not statistically significant (Table 3). Both the Y1 and Y2 leachate were enriched in ^{13}C, well above the background leachate enrichment (Fig. 8B) (Table 3). Y1 leachate was significantly more enriched at day 1 (p=0.0115) (Fig. 8B). The background $\delta^{13}C$ value was not affected by time of incubation, and remained consistently around -17‰, which allowed for a robust mixing model (Fig. 8B). Using the mixing model, and the DOC pools we calculated the micrograms of litter-derived C in leachate (Fig. 8C). There was a time effect on litter-derived C in leachate for both Y1 (p=0.0003) and Y2 (p <0.0001), with the flux decreasing through time (Fig. 8C) (Table 3).

Similarly to C, the Y1 soil lost 0.132 mg N/ gram of soil through leaching over the course of the 150 days, which was 15% more than the Y2 soil (0.112 mg N/gram of soil) (Fig. 9A). In particular, Y1 soils had significantly more N in the leachate (TN) than Y2 at days, 46 (p =0.0492), 60 (p =0.0164), 81 (p =0.0237), and 112 (p =0.0403), (Fig 9A). However, overall the treatment difference result was not statistically significant (Table 3). Both the Y1 and Y2 TN fluxes were enriched in ^{15}N well above the background TN values (Fig. 9B) (Table 3). The background δ^{15} N-TN value was not affected by time of incubation, and remained consistently around 0‰, which allowed for a robust mixing model (Fig. 9B). Using the mixing model, and the TN pool size we calculated the micrograms of litter-derived N in leachate (Fig. 9C). Y1

leachate had significantly more LD-N than Y2 at day 11 (p = 0.0103) and 32 (p=0.0458) (Fig. 9C). There was a time effect on LD-N in leachate for both Y1 (p <0.0001) and Y2 (p =0.0024) (Fig. 9C) (Table 3).

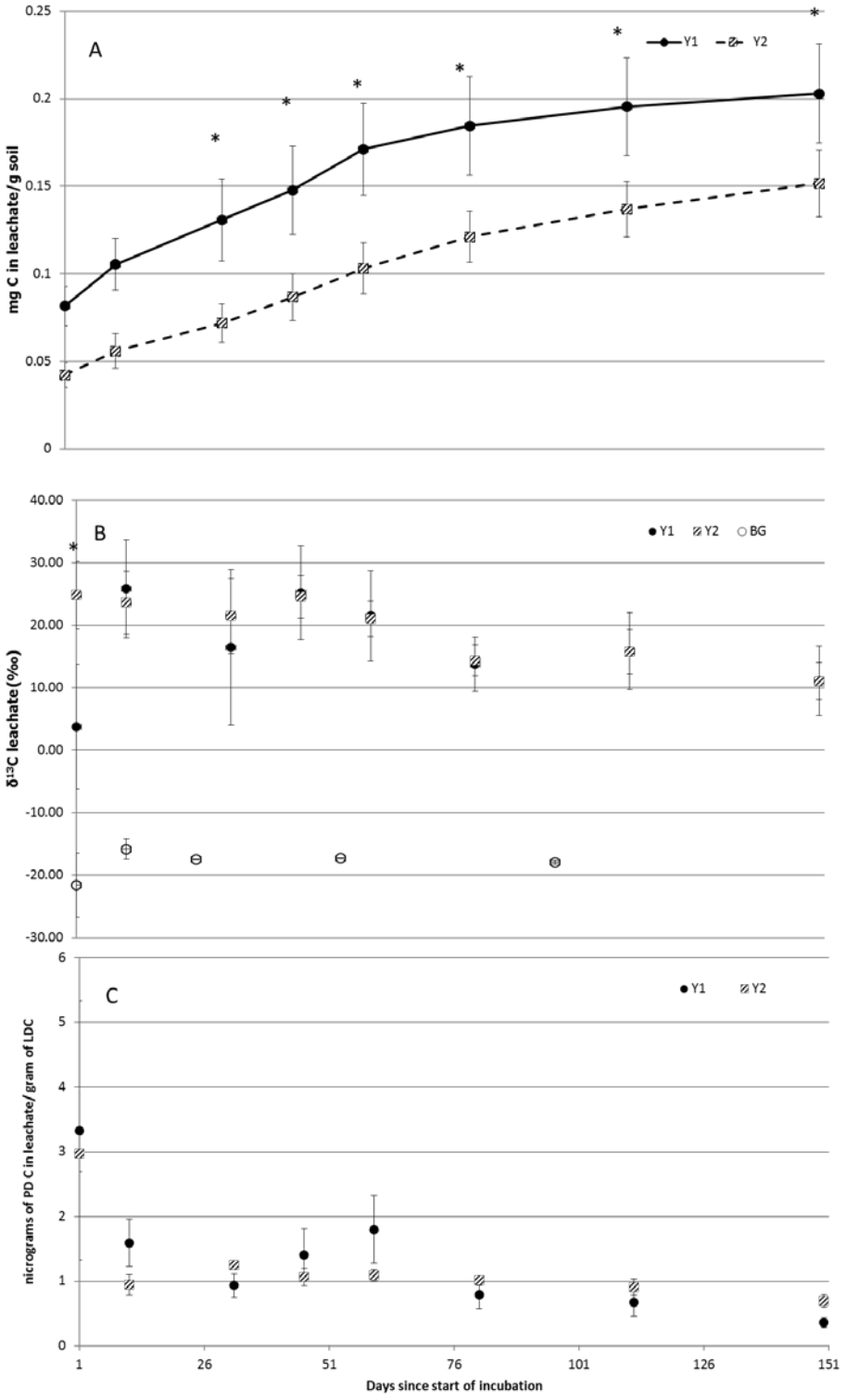

Figure 8: A) Mean cumulative C in leachate (mg C per gram soil). B) Mean δ ^{13}C values of soil leachate throughout the experiment. C) Mean litter-derived C in the DOM leachate throughout the experiment. Solid circles represent Y1, and dashed squares represent Y2. Data are average (n=4) with error bars show SE. Stars reflect significant differences between treatments ($p < 0.05$)

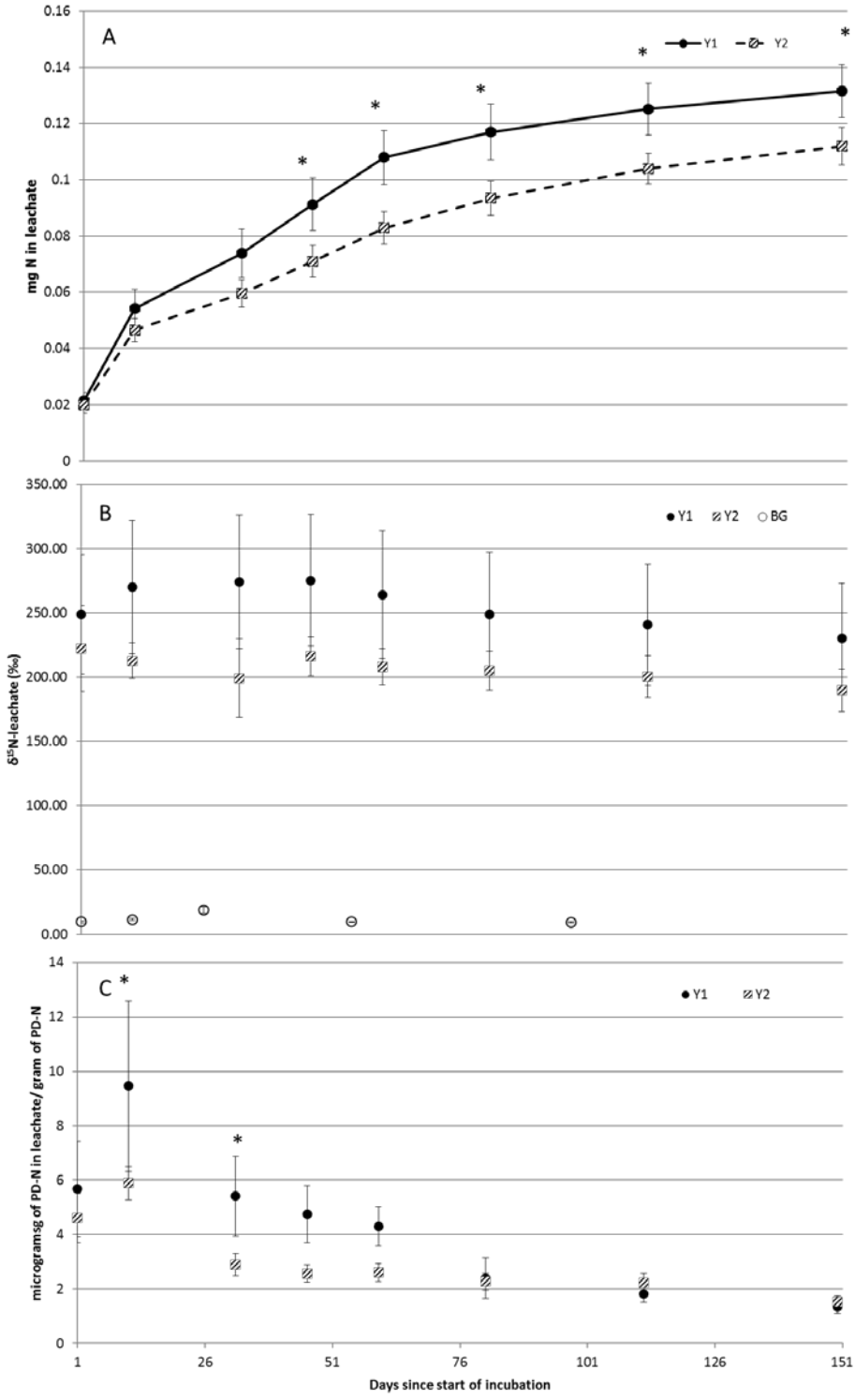

Figure 9: A) Mean cumulative N in leachate (mg N per gram soil). B) Mean δ ^{15}N values of soil leachate throughout the experiment. C) Mean litter-derived N in the leached DOM throughout the experiment. Solid circles represent Y1, and dashed squares represent Y2. Data are average (n=4) with error bars show SE. Stars reflect significant differences between treatments ($p < 0.05$)

3.2.4 Litter-derived C and N in soil and SOM fractions

Amounts of litter derived C and N in the bulk soil and SOM fractions were calculated using the mixing model, and the fraction pool size calculated in the field study (see chapter 2; Equation 1, Table 2). The silt fraction was dominant in our soils (Table 4). All four fractions as well as bulk values showed ^{13}C and ^{15}N enrichment, which was enough to differentiate them from background levels, and allowed for use of the mixing model to determine mg of litter-derived-C and N both at the beginning and end of the incubation (Table 4).

Y1 soil lost ^{13}C enrichment (a negative value on the graph) from all fractions and bulk soil, but this lost was statistically significant only for the clay sized fractions (p =0.0075) (Fig. 10A) Y2 soil gained ^{13}C enrichment in all fractions except the clay, but this gain was only statistically significant in the sand sized fraction (p=0.0417), which was also statistically different from the Y1 sand sized fraction (p=0.0427) (Table 3) (Fig. 10A) Y1 soil lost litter-derived -C (a negative value on the graph) from all fractions and bulk soil, but this lost was statistically significant only for the sand (p =0.0295) and silt (p =0.0241) sized fractions (Fig. 10B) Y2 soil did not lose or gain litter-derived-C from any fraction of bulk over the course of the incubation (Fig. 10B). Additionally, Y1 sand and silt sized fraction values were significantly different than Y2 sand ((p =0.0091) and silt (p =0.0287) sized fractions (Fig 10B) (Table 3).

There was no linear relationship between amount of LD-C released through respiration and leachate in either Y1 of Y2 soils (Fig. 11). There is more variability in the Y1 leachate and respiration values (Fig. 11).

Y1 soil lost ^{15}N enrichment (a negative value on the graph) from all fractions and bulk soil, but this lost was not statistically significant. (Fig. 12A) Y2 soil gained ^{15}N enrichment in the

bulk soil, and the sand sized fraction, and lost ^{15}N enrichment in the LF silt and clay sized fractions, but these differences were not statistically significant (Fig. 12A)

Y1 soil lost litter derived-N (a negative value on the graph) from all the fractions except the clay sized fraction, but these losses were only statistically significant in the sand (p =0.0408) and silt (p =0.0116) sized fractions (Fig. 12B). Y2 soil gained litter derived-N in the Bulk soil and LF, and lost litter derived-N in the sand, silt and clay sized fractions, but the differences were only statistically significant in the silt sized fraction (p=0.0462) (Fig. 12C). Additionally, Y1 silt sized fraction values were significantly different than Y2 sized fractions (p =0.0354) (Fig. 12B) (Table 3).

Table 4: %C, %N, %fraction (n=80) and Background $\delta^{13}C$ and $\delta^{15}N$ (n=20) values of the soil used for this experiment. Data are averages ± standard errorsTable 3: Main soil, soil fractions properties and litter-derived C and N in the two soils (Y1 and Y2) at the beginning and after 150 days of incubation. Means with standard errors (n=4) are reported.

Harvest	Day	Soil Properties						Litter Derived C and N			
		Fraction	% fraction	% Carbon	% Nitrogen	Delta 13C	Delta 15 N	% litter derived C	litter derived C (mg/ g soil)	% litter derived N	litter derived N (mg/ g soil)
Y1	0	BULK	100.00	4.4±0.0	0.27±0.00	-0.62±4.53	91.69±23.22	0.67±0.21	0.3±0.09	0.9±0.23	0.024±0.0061
Y1	0	LF	2.74±0.11	25.3±0.5	1.19±0.02	16.86±9.37	231.10±50.13	1.62±0.44	0.1±0.03	2.3±0.49	0.007±0.0016
Y1	0	SAND	9.11±0.030	5.5±0.3	0.33±0.01	-3.21±2.53	96.94±17.44	0.59±0.12	0.0±0.01	0.9±0.17	0.003±0.0005
Y1	0	SILT	73.30±0.39	2.4±0.1	0.21±0.01	-4.00±2.23	62.75±12.62	0.46±0.11	0.1±0.02	0.6±0.12	0.009±0.0019
Y1	0	CLAY	14.85±0.31	4.3±0.1	0.42±0.02	-5.87±1.26	52.46±7.94	0.40±0.06	0.0±0.00	0.5±0.08	0.003±0.0005
Y2	0	BULK	100.00	4.4±0.0	0.27±0.00	-2.45±0.81	72.98±4.39	0.59±0.40	0.3±0.02	0.7±0.04	0.019±0.0012
Y2	0	LF	2.74±0.11	25.3±0.5	1.19±0.02	1.17±5.83	183.62±33.05	0.88±0.27	0.1±0.02	1.8±0.32	0.006±0.0010
Y2	0	SAND	9.11±0.30	5.5±0.3	0.33±0.01	-6.80±1.94	89.82±14.96	0.42±0.09	0.0±0.00	0.9±0.15	0.003±0.0004
Y2	0	SILT	73.30±0.39	2.4±0.1	0.21±0.01	-5.86±1.05	49.76±5.68	0.37±0.50	0.1±0.01	0.5±0.06	0.007±0.0009
Y2	0	CLAY	14.85±0.31	4.3±0.1	0.42±0.02	-6.77±0.66	46.70±4.13	0.36±0.03	0.0±0.00	0.4±0.04	0.003±0.0003
CONTROL	0	BULK	100.00	4.4±0.0	0.27±0.00	-14.91±0.10	2.00±0.21				
CONTROL	0	LF	2.74±0.11	25.3±0.5	1.19±0.02	-17.66±0.19	-1.08±0.18				
CONTROL	0	SAND	9.11±0.30	5.5±0.3	0.33±0.01	-15.76±0.13	-0.08±0.18				
CONTROL	0	SILT	73.30±0.39	2.4±0.1	0.21±0.01	-13.83±0.51	1.79±0.18				
CONTROL	0	CLAY	14.85±0.31	4.3±0.1	0.42±0.02	-14.34±0.80	4.08±0.200				
Y1	150	BULK	100.00	4.3±0.1	0.33±0.02	-4.86±1.78	64.02±8.624	0.49±0.08	0.2±0.03	0.6±0.08	0.020±0.0025
Y1	150	LF	3.01±0.34	28.8±1.0	1.29±0.08	-0.97±3.74	140.87±31.21	0.82±0.18	0.1±0.02	1.3±0.30	0.005±0.0012
Y1	150	SAND	5.81±0.47	3.4±0.6	0.18±0.02	-5.88±1.78	87.95±15.02	0.47±0.08	0.0±0.00	0.8±0.15	0.001±0.0002
Y1	150	SILT	67.08±0.72	0.5±0.1	0.05±0.01	-5.22±2.06	42.76±8.69	0.46±0.10	0.0±0.00	0.4±0.08	0.001±0.0003
Y1	150	CLAY	20.50±0.84	4.7±0.2	0.42±0.01	-12.38±2.13	49.40±9.04	0.13±0.10	0.0±0.01	0.4±0.09	0.004±0.0007
Y2	150	BULK	100.00	4.4±0.1	0.32±0.00	-0.49±3.51	84.31±18.43	0.70±0.17	0.3±0.08	0.8±0.18	0.025±0.0056
Y2	150	LF	2.84±0.87	28.4±1.7	1.37±0.12	1.69±7.34	166.04±39.83	0.95±0.34	0.1±0.07	1.6±0.39	0.007±0.0044
Y2	150	SAND	5.87±0.20	5.8±1.3	0.33±0.07	-0.13±4.06	122.16±25.25	0.74±0.19	0.0±0.01	1.1±0.25	0.002±0.0005
Y2	150	SILT	72.21±1.05	1.0±0.2	0.09±0.01	-5.32±2.42	47.10±9.40	0.45±0.11	0.0±0.01	0.4±0.09	0.002±0.0005
Y2	150	CLAY	18.71±0.19	5.0±0.2	0.47±0.01	-9.04±0.98	45.92±4.96	0.29±0.05	0.0±0.00	0.4±0.05	0.003±0.0004
CONTROL	150	BULK	100.00	4.2±0.2	0.31±0.01	-15.36±0.12	2.12±0.10				
CONTROL	150	LF	2.22±0.64	24.5±1.6	1.21±0.09	-18.54±0.58	-0.48±0.19				
CONTROL	150	SAND	7.31±0.45	3.9±2.0	0.26±0.12	-15.86±0.58	2.99±1.47				
CONTROL	150	SILT	74.48±1.48	1.1±0.1	0.09±0.01	-14.92±0.28	1.73±0.37				
CONTROL	150	CLAY	17.46±1.38	3.9±0.1	0.35±0.00	-15.17±0.17	5.33±0.46				

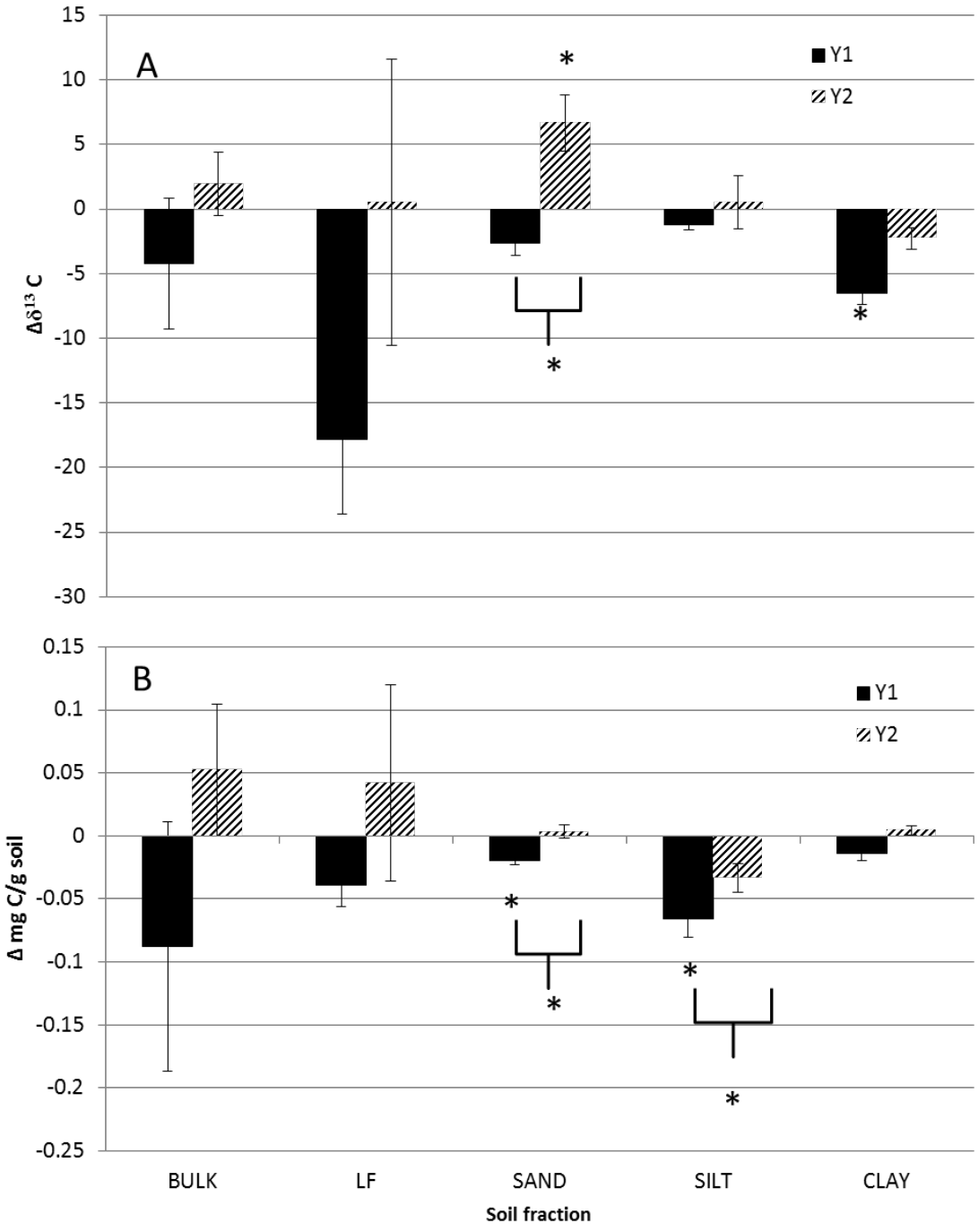

Figure 10: A) Mean $\delta^{13}C$ losses or gains from soil fractions over 150 days. B) Mean litter derived carbon (LD-C) losses or gains from soil fractions over 150 days. The mg of litter derived-C at day 150 was subtracted from the mg of litter derived-C at day 0. Solid bars represent Y1, dashed bars represent Y2. Data are average (n=4) with error bars showing SE. Stars reflect significant differences between treatments (represented with a bracket) and 0 ($p < 0.05$)

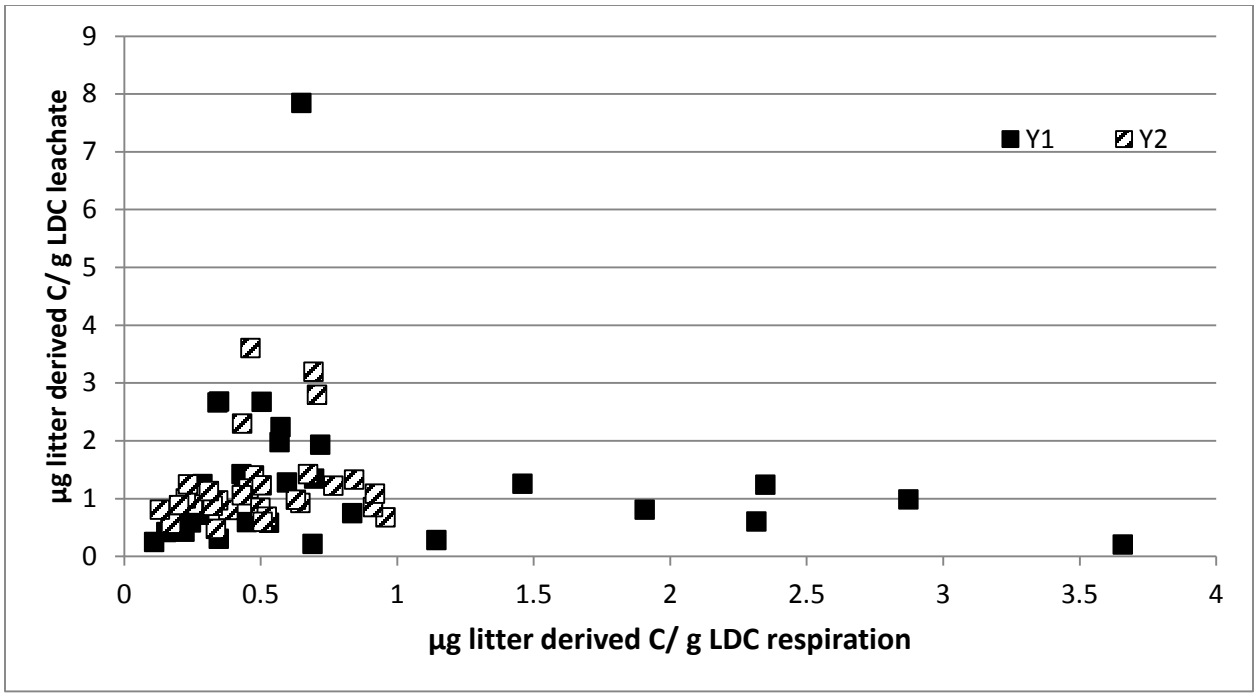

Figure 11: Regression between litter derived C in respiration and leachate. Solid squares represent Y1, dashed bars represent Y2. Points represent individual harvest values. The equation for the linear regression for Y1 y=-0.2062x+1.4713, r^2 value: 0.016, and the equation of the linear regression for Y2 y=0.6165x+0.9281, r^2 value: 0.037 are not shown.

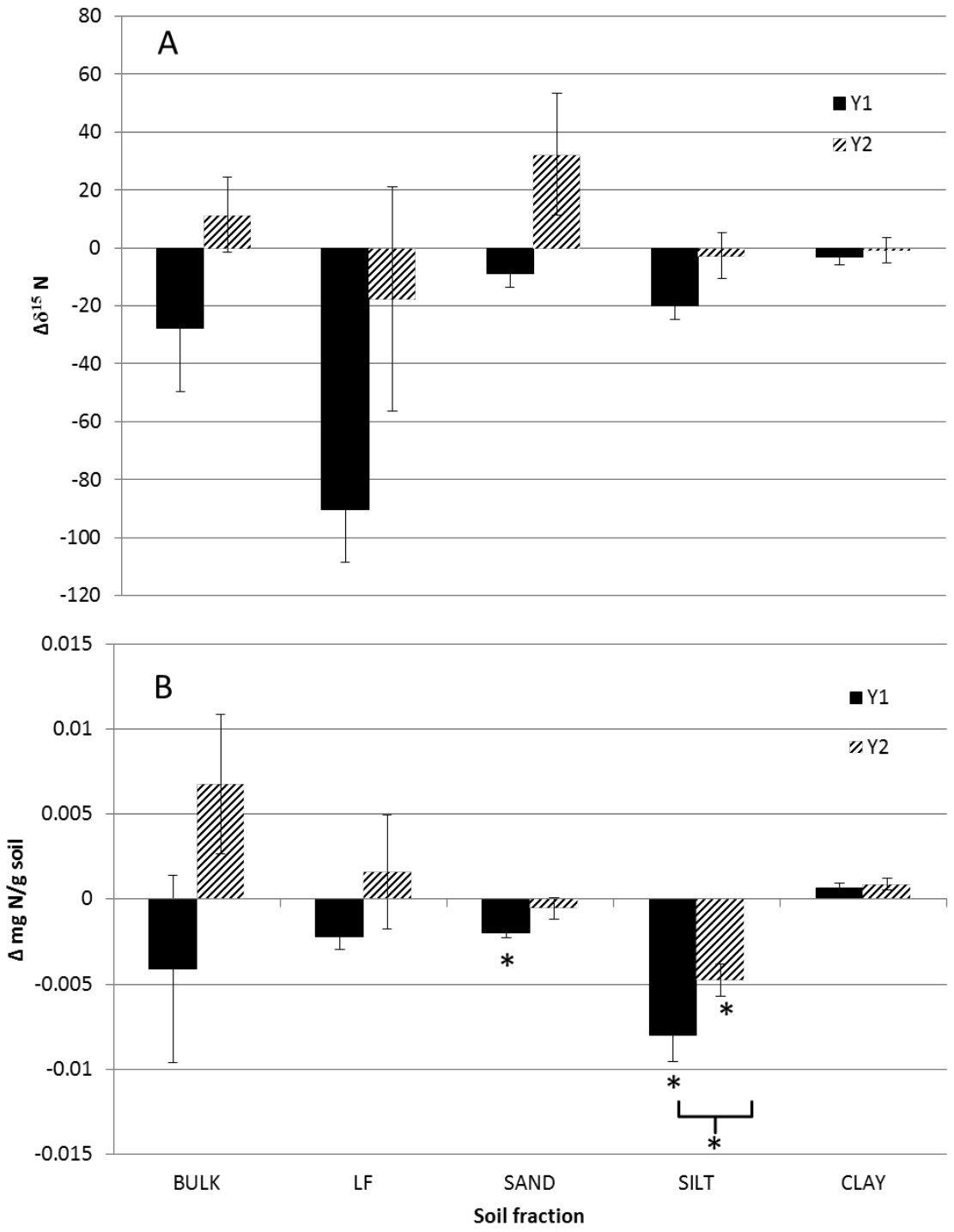

Figure 12: A) Mean $\delta^{15}N$ losses or gains from soil fractions over 150 days. B) Mean litter derived nitrogen (LD-N) losses or gains from soil fractions over 150 days. The mg of PD-N at day 150 was subtracted from the mg of LD-N at day 0. Solid bars represent Y1, dashed bars represent Y2. Data are average (n=4) with error bars showing SE. Stars reflect significant differences between treatments (represented with a bracket) and 0 ($p < 0.05$)

3.4 DISCUSSION

Judging by the lack of a relationship between the respiration and leachate values, there is no direct relationship between carbon released through leachate and lost through respiration. This is surprising, as I was expecting there to be a tight relationship, that fresh inputs would dominate both respiration and leaching. There are aspects of our experimental design that could have destabilized older C, which might account for lack of a respiration/leachate relationship. Fresh inputs and disruption of soil aggregates have been shown to increase the decomposition of older C, and we broke up the soil prior to the incubation (Lundtquist *et al.* 1999; Grandy & Neff, 2008). Additionally, we increased the soil moisture to 60% WHC, which would have altered the amount of water in the system, which is known to increased decomposition of older OM (Wang *et al.* 2014; Grandy & Neff, 2008). We can hypothesize that the carbon that is respired off is probably derived from SOM pools with higher C:N ratios, like the LF,(Kelleher 2006) while the carbon that is lost through DOM leaching is probably derived from water soluble carbon, which would be attached to sand, silt and clay fractions (Kalbitz *et al.* 2000). The $\delta^{13}C$ values of the respiration and leachate seem to support this idea. The LF fraction had by far the highest $\delta^{13}C$ of all the fractions, and the respiration initially had a much higher $\delta^{13}C$ than the leachate.

Carbon was lost from the soil throughout the experiment through both leaching and respiration in both Y1 and Y2 treatments. Interestingly, there was a treatment effect for respiration losses, suggesting that stabilization of C has occurred in the field after one year. This is supported by the lack of C lost from Y2 in bulk soil or SOM fractions. There is difference between the early respiration in Y1 and Y2 soils, suggesting that Y1, or newly incorporated plant derived C (NIPD C) is more labile. Additionally, there is more C lost from Y1 bulk soils and

fractions than Y2, supporting the idea that NIPD becomes stabilized in soils in as little as one year from incorporation.

However, we saw significant losses of NIPD C and N from a mineral fraction (silt sized), suggesting that NIPD OM is not fully stabilized upon entering the mineral fraction. Instead, the onion hypothesis proposed by Sollins *et al.* (2006), which suggests that OM is added to mineral fractions in layers, is supported by these data. The NIPD OM binds to the outermost layer of the silt, and is thus the first C and N lost when those fractions decompose. It would seem that NIPD C and N form these layers more readily on the silt sized OM than on the clay sized OM, considering that NIPD C and N were significantly lost from silt sized, but not clay sized fraction. We cannot determine if this was due to due to stronger binding or additional physical protection in the clay (von Lützow *et al.* 2007). In the greater context of stabilization studies, this hypothesis should be considered, as short term estimates (less than one year) could over estimate the amount of C and N stabilized in soil fractions.

3.5 CONCLUSIONS

In this study, we demonstrated that the products of leaching and respiration draw from different pools, with likely respiration drawing more from fresh inputs, and leaching from older inputs. NIPD C and N are much more labile than older OC and ON. Additionally, NIPD C and N inside soil fractions behave differently than the other C and N in the pool, which supports Sollins' onion hypothesis for mineral stabilization (Sollins *et al.* 2006). The labile nature of NIPD OM must be considered when designing experiments to investigate C and N stabilization: studies must be conducted beyond a single year in order to accurately measure the amount of fresh inputs is stabilized in the long term.

4. CONCLUSIONS

The primary objectives of my thesis was to improve our understanding of how (a) N released during the decomposition of plant litter is stabilized in SOM, or made available for plant uptake and of how (b) fresh litter derived (LD) OM behaves in the soil. My major questions were

1. Do soil microarthropods increase litter-N contributions to SON and increase N stabilization?
2. How is fresh LD OM released as respiration and leachate?
3. How does LD OM transfer between primary SOM pools?

In the field incubation presented in chapter 2, I addressed the 1st question, and determined that microarthropods do not increase overall N mineralization rates, but do influence the SOM pools where LD N is stored. In the presence of soil microarthropods, more LD N is found in the light fraction (LF), which suggests that microarthropods increase fragmentation of litter, and should thus be considered in future N dynamic studies as a driver of the ultimate fate of LD N.

In the laboratory incubation presented in chapter 3, I addressed the 2nd and 3rd questions. I found that LD OM does not contribute to respiration and leaching equally, with more LD C was found in respiration than in leachate. I propose that the respiration and leaching are generated by different fractions of the primary SOM pools, with respiration drawing from higher C:N ratio pools such as LF, and the leachate drawing from silt and clay sized fractions. I found that fresh LD OM behaves differently than older OM present in the different primary SOM pools. This result supports Sollins' onion hypothesis (Sollins *et al.* 2006) for mineral stabilization, where

fresh inputs are less strongly bound to minerals in the soil, and this hypothesis should be considered when designing experiments to investigate the stabilization of C and N in the soil.

In my thesis research, I focused on improving our understanding of the fate of C and N released through decomposition. I have used a mechanistic approach to answer my specific questions. In particular through a combination of isotope enrichement and SOM physical fractionation I elucidated the underlying controls of the fate of C and N in decomposing litter and soil. My approach can be extended to ecosystems outside of the tallgrass prairie, and generally to improve our understanding of the processes of litter decomposition and SOM formation.

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