

## AN ABSTRACT OF THE THESIS OF

Kimberly L. Townsend for the degree of Master of Science in Environmental Sciences presented on December 16, 2013.

Title: The Legacy of Dr. Francis Hole: Examining Soil Organic Carbon after 50 Years of Detrital Manipulation

Abstract approved:

---

Kate J. Lajtha

Models of ecosystem carbon (C) balance generally assume a strong relationship between net primary productivity (NPP), litter inputs, and soil C accumulation, but there is little direct evidence for such a coupled relationship. Using a unique 50-year detrital manipulation experiment in a in a mixed deciduous forest and restored prairie grasslands in Wisconsin, combined with sequential density fractionation, isotopic analysis, and short-term incubation, we examined the effects of detrital inputs and removals on soil C stabilization, destabilization, and quality. Both forested sites showed greater decline in bulk soil C content in litter removal plots (55 and 66% decrease in surface soils compared to controls) compared to litter addition plots (27 and 38% increase in surface soils compared to controls). No accumulation in the mineral fraction C was observed after 50 years of litter addition of the two forest plots, thus increases in the light density fraction pool drove patterns in total C content. Litter removal across both ecosystem types resulted in a decline in both free light

fraction and mineral C content, with an overall 51% (7%) decline in mineral-associated carbon in the intermediate ( $1.85 - 2.4 \text{ g cm}^{-3}$ ) density pool; isotopic data suggest that it was preferentially younger C that was lost. The carbon content response in the  $>2.4 \text{ g cm}^{-3}$  mineral fraction was variable. In contrast to results from other, but younger litter manipulation sites, there was with no evidence of priming even in soils collected after 28 years of treatment. In prairie soils, aboveground litter exclusion had an effect on C levels similar to that of root exclusion, thus we did not see evidence that root-derived C is more critical to soil C sequestration. There was no clear evidence that soil C quality changed in litter addition plots in the forested sites;  $\delta^{13}\text{C}$  and  $\Delta^{14}\text{C}$  values, and incubation estimates of labile C were similar between control and litter addition soils. C quality appeared to change in litter removal plots; soils with litter excluded had  $\Delta^{14}\text{C}$  values indicative of longer mean residence times,  $\delta^{13}\text{C}$  values indicative of loss of fresh plant-derived C, and decreases in all light fraction C pools, although incubation estimates of C quality did not change. In prairie soils,  $\delta^{13}\text{C}$  values suggest a loss of recent C<sub>4</sub>-derived soil C in litter removal plots along with significant increases in mean residence time, especially in plots with removal of roots. Our results suggest surface mineral soils may be more vulnerable to loss than to gain, in association with disturbance, land use change, or perhaps even climate change over century - decadal timescales, and also highlight the need for longer-term experimental manipulations to study soil organic matter dynamics.

© Copyright by Kimberly L Townsend  
December 16, 2013  
All Rights Reserved

The Legacy of Dr. Francis Hole: Examining Soil Organic Carbon after 50 Years of  
Detrital Manipulation

by  
Kimberly L. Townsend

A THESIS

submitted to

Oregon State University

in partial fulfillment of  
the requirements for the  
degree of

Master of Science

Presented December 16, 2013  
Commencement June 2014

Master of Science thesis of Kimberly L Townsend presented on December 16, 2013

APPROVED:

---

Major Professor, representing Environmental Sciences

---

Director of the Environmental Sciences Graduate Program

---

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

---

Kimberly L. Townsend, Author

# TABLE OF CONTENTS

	<u>Page</u>
Chapter 1: Introduction .....	2
Chapter 2: Materials and Methods.....	8
Site description.....	9
Sample collection and handling .....	10
Laboratory incubation for labile carbon.....	11
Acid hydrolysis .....	11
Sequential density fractionation.....	12
$\delta^{13}\text{C}$ Isotopic analysis.....	12
Radiocarbon sample preparation and analysis .....	13
Statistical Analysis.....	14
Results.....	16
Bulk C response to detrital manipulation.....	17
Short-term incubation for labile C .....	17
Acid hydrolysis estimate of recalcitrant C .....	18
Sequential density fractionation.....	18
Patterns of $\delta^{13}\text{C}$ across soils and density fractions .....	19
Radiocarbon in bulk soils and soil fractions .....	20
Chapter 4: Discussion .....	40
Chapter 5: Conclusion.....	47
Bibliography .....	51
Appendix:.....	59

## LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Soil Organic Carbon (SOC) concentrations for the Noe Woods (A) and Wingra Woods (B) sites of the Francis Hole Detrital Input and Removal Treatments in 1984, 1997 and 2006.....	23
2. Cumulative respiration after a 28 day incubation of bulk soil by treatment from Noe Woods (A, B), Wingra Woods (C, D), Curtis Prairie 1 (E, F), and Curtis Prairie 3 (G,H).....	24
3. SOC concentration in density fractions of Noe Woods (A), Wingra Woods (B), Curtis Prairie 1 (C), and Curtis Prairie 3 (D) plots in the Francis Hole Dirt Experiment by treatment.....	25
4. Patterns of $\delta^{13}\text{C}$ by density fraction of soils sampled after 50 years of detrital manipulation from Noe Woods (A), Wingra Woods (B), Curtis Prairie 1 (C) and Curtis Prairie 3 (D), sites in the Francis Hole DIRT Experiment.....	26
5. Patterns of $\delta^{13}\text{C}$ in soil sequential density fractions from the control plots in the Francis Hole DIRT sites plotted in comparison with data from soils studied by Throop et al. (2013) and Sollins et al. (2009).....	27
6. Relationship between $\Delta^{14}\text{C}$ and soil organic carbon concentration of bulk soil for the two Curtis Prairie sites of the Francis Hole DIRT plots.....	27
7. Relationship between mineral fraction C content change (%) and $\Delta^{14}\text{C}$ (‰) across all detrital removal treatments (Forest and Prairie combined).....	28

## LIST OF TABLES

<u>Table</u>		<u>Page</u>
1	Treatment and methods of the Francis Hole Experimental Detrital Input and Removal Treatments (DIRT) plots installed at the Wisconsin Arboretum in 1956.....	29
2	Soil Organic Carbon (SOC) and Soil Organic Nitrogen (SON) concentration and content and bulk density, in forest Francis Hole Experimental DIRT plots at the Wisconsin Arboretum, sampled in 2006 after 50 years of detrital manipulation.....	30
3	Soil Organic Carbon (SOC) and Soil Organic Nitrogen (SON) concentration and content in prairie Francis Hole Experimental DIRT plots at the Wisconsin Arboretum, sampled in 1997 and 2006 after 41 and 50 years of detrital manipulation.....	32
4	Mass recovery, carbon (C) concentration and C recovery by density fraction of soils from Noe Woods, Wingra Woods and the Curtis Prairie sites of the Francis Hole DIRT plots sampled in 2006.....	35
5	Soil Organic Carbon (SOC) and Soil Organic Nitrogen (SON) concentration and content and bulk density, in forest Francis Hole Experimental DIRT plots at the Wisconsin Arboretum, sampled in 2006 after 50 years of detrital manipulation.....	38
6	$\Delta^{14}\text{C}$ per thousand (error) of bulk soil and density fractions for the Francis Hole DIRT plots for Curtis Prairie and Noe Woods.....	39



The Legacy of Dr. Francis Hole: Examining Soil Organic Carbon after 50 Years of  
Detrital Manipulation

## Chapter 1: Introduction

*Oh, I'm hoping for a thousand years at least.*" F. D. Hole's reply when asked how long his Arboretum plots should be maintained after his retirement.

Soil organic matter (SOM) is derived from decomposing plant detritus and microbial materials, modified by biotic and abiotic processes. SOM is a major component of the global C cycle, containing more C than plant biomass and the atmosphere combined (Field and Raupach 2004). SOM constitutes approximately two thirds of the terrestrial C pool and is estimated to be about 2300 Pg C in the surface 3 m (Jobbágy and Jackson 2000). The C flux between soils and the atmosphere is large, with soil respiration creating about 10 times the C emissions due to fossil fuel combustion (Post et al. 1990, Raich and Schlesinger 1992). Annually, about 75 Gt C are added to this pool through inputs of dead biomass and root deposits, but a similar amount is released as CO<sub>2</sub> so the balance is nearly equal (Schlesinger and Andrews 2000). SOM can follow a number of trajectories as it is processed within soil; it can be stored for a period of time, leached to surface waters as dissolved organic matter (DOM), or mineralized by microbes and returned to the atmosphere as CO<sub>2</sub>. Despite the key role of SOM in the global carbon cycle, interactions among the biological, chemical, and physical processes regulating SOM storage, accumulation, stabilization, and turnover are poorly understood (Battin et al. 2009, Foley and Ramankutty 2004, Fang et al. 2005). Given the enormous size of the soil C pool, understanding its sensitivity to management, disturbance, and temperature/moisture regime change is critical. For example, climate changes can be

expected to change both quantity and quality of litter inputs, but the resulting effects on SOM stability and turnover cannot now be predicted accurately. Soils may act as sources or sinks for C depending on environmental conditions and land use.

Understanding the mechanisms that control stabilization and release of C is important for the prediction of effects of climate change and for the development of management strategies to increase C sequestration of soils (Jandl et al. 2007, Lal 2010).

Interest in SOM continues to increase as concern about steadily increasing levels of atmospheric CO<sub>2</sub> grows. Changes in ecosystem net primary productivity (NPP) and thus litterfall are predicted under climate change scenarios (Melillo et al. 1993; King et al. 1997; Raich et al. 2006), but it is not clear if there will be parallel changes in soil organic matter stores, or what the dynamics of C change are. Models of ecosystem C balance generally assume a strong relationship between NPP, litter inputs, and soil C accumulation (Liski et al. 2002, Gottschalk et al. 2012), but there is little direct evidence for such a coupled relationship. Soils likely have a finite capacity to sequester C, and might “saturate” (Chung et al. 2010, Stewart et al. 2009, Six et al. 2002, Mayzelle et al. 2013), effectively decoupling litter inputs and C sequestration; saturation level might be more dependent on climate and soil mineralogy than on quality and quantity of C inputs. By ‘quality’ we refer to the decomposability of SOM (Nadelhoffer et al. 2004). In addition, the addition of both simple and complex organic substrates to soil has been shown to result in increased turnover of native SOM, termed the ‘priming effect’ (Kuzyakov et al. 2000), and thus enhanced microbial respiration in response to additional plant litter inputs could

increase the rate of release of stored soil organic matter, paradoxically decreasing C sequestration in soil. Although it is possible that improvements in crop, forest, and soil management may allow significant amounts of CO<sub>2</sub> to be removed from the atmosphere and sequestered in soil, it is also possible that increased soil respiration rates will unleash a positive feedback in which temperatures rise even faster than now expected (Heimann and Reichstein 2008).

While there has been a great deal of attention given to mineralogical control of SOM chemistry and accumulation (i.e. Torn et al. 1997, Parfitt et al. 2002, Kaiser and Guggenberger 2003, Plante et al. 2006a, Kramer et al. 2012) and to climate/temperature control of SOM stability (i.e. Townsend et al. 1997, Leifeld and Fuhrer 2005, Giardina and Ryan 2000, Wynn et al. 2006, Raich et al. 2006), little attention has been given to the role, if any, of detrital quantity or quality in determining SOM formation and stability. Although there have been a large number of litter composition studies, these studies typically last for 2 to 5 years, (Nadelhoffer et al. 2004) and have focused on relatively rapid cycling of nutrients through the litter layer. Far less is known about the role of plant litter in determining soil organic matter content and function over the decadal timescale.

One opportunity to address this gap is provided by an experiment started by Francis D. Hole at the University of Wisconsin Arboretum in 1950s. The Arboretum, established in the 1930s by the university with a mission to recreate and manage natural ecosystem and a commitment to sustaining it as a long term research site, was an ideal location to implement a long-term study of soil formation. The original impetus was knowledge about soil forming processes and plant-soil interactions to

improve plant community restoration efforts. The interest in control of organic matter accumulation in soils was later included as a core theme of the National Science Foundation's Long-Term Ecological Research (LTER) program (Nadelhoffer et al. 2004).

Francis Hole's experiment was the inspiration for a network of similar experiments set up in forested ecosystems throughout the United States that examine the role of both above and belowground detrital inputs. Inspired by this unique, long-term experiment to study the time course of SOM formation and stabilization, several DIRT sites were subsequently established in both the U.S. and in Europe by various researchers, including the Harvard Forest, 1990 the Bousson Experimental Forest, 1991, the H.J. Andrews Experimental Forest, 1997, the Sikfokut Forest, Eger, Hungary, in 2001, and the Michigan Biological Station in 2004. These DIRT sites added plot trenching to allow for the analysis of the role of above- vs. below-ground detrital inputs on SOM stabilization. The stated goal of the DIRT project is to *assess how rates and sources of plant litter in organic puts control the accumulation and dynamics of organic matter and nutrients in forest soils over decadal time scales* (Naddelhoffer, 2004).

The Francis Hole experiment establishes a SOM story that extends over half a century. We use the data gathered from the Francis Hole DIRT plots to examine the trajectory of SOM change and to establish a methodology for examining SOM changes at other DIRT sites.

The experimental plots are located in two oak dominated mixed deciduous forests (recovering from occasional cutting) and restored prairie (restored pasture and

cropped land), and involve manipulations of either detrital leaf litter or prairie grasses. In the forested sites, treatments include aboveground litter additions and removal; roots were intact in all treatments. In the prairie systems, aboveground inputs were not increased, but treatments include root only inputs, aboveground only inputs, and no above or root inputs. Treatments were started in 1956 in the Wingra Woods, Noe Woods, and the Curtis Prairie.

Following the assumptions of many models that soil C stores should be coupled to litter inputs, we hypothesized that increased detrital inputs in forested ecosystems would result in significant increases in total soil C and that priming effects, generally seen as an immediate effect of new carbon substrate additions, would no longer be detected. If true, this suggests that the forest soils in the University of Wisconsin Arboretum may not generally be C saturated, and after 50 years we predicted that we would see increases in both short and intermediate cycling C pools. In the prairie soils, we hypothesized that root inputs contribute more to C sequestration than do aboveground inputs (Rasse et al. 2005), and thus elimination of root inputs would have a stronger effect on C destabilization than would the elimination of aboveground litter.

## Chapter 2: Materials and Methods

## Site description

Litter manipulation plots are located in two forested (Wingra Woods, Noe Woods; 43.046N, -89.426E and 43.038N, -89.441E, respectively) and three grassland (Curtis Prairie, 43.038N, -89.431E) sites within the University of Wisconsin Arboretum. Mean annual precipitation for the UW Arboretum is 928mm, with a mean annual minimum temperature of 0.7 °C and mean annual maximum temperature of 14.2 °C (Kucharik et al. 2006).

Noe Woods is a 41 acre forest dominated by white (*Quercus alba*) and black oaks (*Q. veluntina*). Surveyor's records indicate that Noe Woods was an oak savanna at the time of European settlement around 1840. It developed into a white and black oak dominated forest after settlement. A period of limited cutting and stump sprouting occurred from 1900 to 1910. High levels of black oak mortality were recorded from 1956 to 1983 due to oak wilt disease. Regeneration was dominated by black cherry, box elder, and American elm (McCune et al. 1985). Oak mortality and subsequent regeneration by non-oak species accounts for the mixed hardwood forest we find there today. The soils in the forest stands are well drained silt-loam Alfisols derived from glacial deposits overlaid by a loess cap, and have mull-type forest floors (Binkley et al. 1986). Soil types are fine-silty/fine-loamy, mixed, superactive, mesic Typic Hapludalfs and fine-loamy over sandy or sandy-skeletal mixed, active, mesic Mollic Hapludalfs. These soil series are very deep and very well drained .

Wingra Woods is a 20 hectare deciduous forest dominated by oaks and underplanted with sugar maple, basswood and beech. It is gradually changing to a shady forest with sugar maple as the dominant species. Northern species such as

hemlock and yellow birch were planted to make the area resemble the sugar maple forests of northern Wisconsin. The soils are fine-silty, mixed, superactive, mesic Typic Hapludalfs with deep profiles and good drainage.

The Curtis Prairie sites are restored prairies, created in 1940 (Prairie 1) and 1956 (Prairie 3) on land that had previously been cultivated and used for horse pasture. The parent material is glacial loess, and soils are fine-silty, mixed, superactive mesic Typic Endoaquolls. All DIRT manipulations were established in 1956, coincidentally just prior to the steep rise in “bomb carbon”  $^{14}\text{C}$  signal in the atmosphere, and are described in Nielsen and Hole (1963; Table 1). More information about the maintenance protocol can be found in the appendix.

#### Sample collection and handling

Experimental plots are 4 by 4 meters, except for Double litter plots in both forested sites which are 1 by 3 meters and one of the burn plots in Curtis Prairie 3 which is 4 by 8 meters.

Soils were collected from plots in 1984 and 1997 and archived at the University of Michigan. In July of 2006, soils were collected from Noe Woods, Wingra Woods and two of the Curtis Prairie sites to mark the 50<sup>th</sup> anniversary of the experiment. Where present, the O horizon was removed and the top 10 cm of mineral soil were collected with a hammer soil corer with a 6.25 cm diameter. Four replicates were collected from each plot, one from each corner. Samples were taken half a meter from the corner of the plot to minimize edge effects. Two soil cores were combined for each replicate. Samples were flown back to Oregon State University

for processing and analysis. Samples were sieved to 2 mm and homogenized. Field moist soil was stored in a refrigerator prior to analysis. The remainder was air dried.

#### Laboratory incubation for labile carbon

Field moist, 30 g soil samples were measured into 50 ml Erlenmeyer flasks. Samples were stored in an incubator at 25°C with open containers of water to maintain humidity. Samples were weighed twice a week and water was added if needed to maintain the correct moisture level. Respiration rates were measured on days 2, 4, 6, 8, 10, 24 and 28. Flasks were stoppered and initial gas samples were taken using a 1 mL-calibrated syringe. Flasks were then left stoppered, allowing CO<sub>2</sub> to accumulate for at least an hour before a final sample was taken. Gas samples were analyzed on a Hewlett Packard 5700A gas chromatograph fitted with a 2m Poropak R 80/100 column and thermal conductivity detector. The cumulative respiration rate over a 28 day period was used to calculate a labile or bioavailable carbon pool (McLaughlan and Hobbie 2004).

#### Acid hydrolysis

Recalcitrant soil C was determined following the protocol of Paul et al. (2006) and Plante et al. (2006b) with slight modification. Light fraction (<1.65) particulate matter was removed from soils using sodium polytungstate prior to hydrolysis in 6N HCl for 18 hours at 100°C. Residues were then rinsed with at least 500 ml of distilled, dionized water on a GF-F glass filter. Supernatant was discarded. The

remaining residue was oven dried and ground using a Spex Certimill 8000 and analyzed for total carbon and nitrogen using a Costech CHN elemental analyzer.

#### Sequential density fractionation

Soil replicates from each plot were composited and sequentially fractionated by density using sodium polytungstate following Sollins et al. (2006). Target fractions were  $<1.65 \text{ g cm}^{-3}$ ,  $1.65 - 1.85 \text{ g cm}^{-3}$ ,  $1.85 - 2.0 \text{ g cm}^{-3}$ ,  $2.0 - 2.2 \text{ g cm}^{-3}$ ,  $2.2 - 2.4 \text{ g cm}^{-3}$ ,  $2.4 - 2.65 \text{ g cm}^{-3}$ , and  $>2.65 \text{ g cm}^{-3}$ . Each recovered fraction was dried and ground in a Spex Certimill 8000 and analyzed for total carbon and nitrogen using a Costech CHN elemental analyzer.

#### $\delta^{13}\text{C}$ Isotopic analysis

Carbon, N and  $\delta^{13}\text{C}$  for bulk soil and density fractions were measured with a coupled continuous-flow elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) system with a Carlo-Erba model 1108 EA interfaced to a Thermo-Finnigan Delta Plus XP IRMS. Dry samples ( $<2 \text{ mm}$ ) were ground finely with a zirconium mortar and pestle, and loaded into tin boats.  $^{13}\text{C}$  data are reported relative to the Pee Dee Belemnite (PDB) standard. Precision of in-house standards, which had been calibrated using international standards, was typically better than 0.2 per mil for  $\delta^{13}\text{C}$ . One standard was run for every 10 unknowns, and 2 blanks and conditioning and calibration standards were included at the beginning and end of each run. Samples were run in duplicate and were always within the range of the standards. Analysis of internal standards indicated an analytical error of  $<5 \%$  for N and  $<2 \%$  for C.

Samples were analysed at the light stable isotope facility of the University of California, Santa Cruz.

#### Radiocarbon sample preparation and analysis

Aliquots of samples sufficient to provide 1 mg C were weighed in quartz glass tubes along with CuO and Ag. The tubes were evacuated and sealed, and then the samples combusted in a muffle furnace at 900C for 4 hours to convert the organic C to CO<sub>2</sub>. The CO<sub>2</sub> was subsequently isolated, added to a reaction chamber, and reduced with excess H and a conditioned iron catalyst at 550C for up to 6 hours. The resulting graphite was measured on the Van de Graaff FN accelerator mass spectrometer to an average precision of 4‰ at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory. Radiocarbon data are expressed according to Stuiver and Polach (1977) as  $\Delta^{14}\text{C}$ , the deviation in parts per thousand from the absolute international standard activity (<sup>14</sup>C:<sup>12</sup>C ratio of oxalic acid corrected for decay since 1950). The  $\Delta^{14}\text{C}$  values were adjusted for mass-dependent fractionation based on measured  $\delta^{13}\text{C}$  values for each fraction.

An interpretive technique used in conjunction with the <sup>14</sup>C data involves so-called “bomb <sup>14</sup>C.” Bomb <sup>14</sup>C was generated in the 1950s and 1960s from above-ground thermonuclear testing, which roughly doubled the amount of <sup>14</sup>C in the atmosphere (Hua and Barbetti 2004, Reimer et al. 2004). This elevated atmospheric <sup>14</sup>C was subsequently incorporated into carbon reservoirs such as vegetation and soils. Samples that contain substantial bomb <sup>14</sup>C will have  $\Delta^{14}\text{C}$  values above 0 ‰, whereas samples with values near or below 0 ‰ are dominated by pre-bomb <sup>14</sup>C and have

incorporated little or no carbon from the atmosphere since 1950 (e.g., Torn et al. 2009). The atmospheric  $\Delta^{14}\text{C}$  value in 1956 was 38 ‰, which was after  $^{14}\text{C}$  had begun to increase but well below the average Northern Hemisphere peak of 933 ‰ in 1963 (Hua and Barbetti 2004). Beginning with such low  $^{14}\text{C}$  values in treated plots, bomb  $^{14}\text{C}$  would be expected to accumulate proportionally to additions of recent (>1950) C, with deviations in different treatments and soil fractions providing some insight into SOC dynamics.

### Statistical analysis

For total C and N from 1984, 1997 and 2006 (figure 1, table 2, table 3, table 4) and respiration data (figure 2), means are comprised of 4 replicates. For these datasets one-way ANOVA, using SigmaPlot version 12 (Systat Software, Inc., San Jose, CA), was utilized to compare means. A Tukey HSD *post hoc* test was used for comparison of means if a significant p-value was found. Significance for the contrasts was set at  $p = 0.05$  and significant p-values were reported.

Due to budgetary constraints we were only able to fractionate on subsample from each sample by density, instead combining replicates into one homogenous sample. Data sets for density fractions therefore consist of single values, including C concentration (figure 3),  $\delta^{13}\text{C}$  patterns by density (figures 4 and 5), and  $\Delta^{14}\text{C}$  patterns (figure 6 and 7, table 6), so it was not possible to run statistical tests.

Table 6 consists of pooled density fraction data averaged across sites. With an  $n=2$  we were not able to run statistical tests. The numbers in bold represent where

both sites comprising the mean followed the same trend of either increase or decrease in SOC relative to the control.

## Chapter 3: Results

### Bulk C response to detrital manipulation

There were significant differences in soil C among detrital treatments in the forested plots, both in the most recent sampling (Table 2) and over time (Figure 1). After 50 years, surface soil C concentration increased by 37% in Double Litter plots compared to Controls in both forests. Soil N followed patterns of soil C, although values were more variable. Because bulk density decreased slightly in Double Litter plots, the increase in C content increased slightly less compared to Controls (29-33%). Bulk C concentration decreased in all sites where litter was excluded. In the forested No Litter plots, bulk C concentration decreased by ~55% after 50 years (Table 2). Because bulk density increased significantly in No Litter plots, the decrease in C content was 40-47%.

In prairie exclusion plots, C losses also increased over time (1997 – 2006). In 1997 in Curtis Prairie 1, soil C concentration in the top 10 cm was significantly lower than Control in No Input and No Roots plots; No Litter plots did not differ from Control (Table 3). By 2006 No Litter plots showed slight, but significant decreases in soil C compared to Controls for Prairie 1 but not Prairie 3; there were no differences between C content loss in No Litter vs. No Root plots. No Input plots lost 69-71% of total soil C content after 50 years.

### Short-term incubation for labile C

Cumulative respiration values over the 28 day incubation in the forest sites range from 28.8  $\mu\text{g C} \cdot \text{g}^{-1}$  soil to 132.1  $\mu\text{g C} \cdot \text{g}^{-1}$  soil, or 1.9  $\text{mg C} \cdot \text{g}^{-1}$  C in soil to 3.1  $\text{mg C} \cdot \text{g}^{-1}$  C in soil (Figure 2). On a per g soil basis, Double Litter values were higher

than Control, and No Litter values were lower than Control. However, on a per g soil C basis, a measure of the lability of C in the soil sample, these differences largely disappeared. Cumulative respiration values in the prairie soils were generally slightly lower on a per g soil basis than in the two forested sites, ranging from  $22.1 \mu\text{g C} \cdot \text{g}^{-1}$  soil to  $96.7 \mu\text{g C} \cdot \text{g}^{-1}$  soil, but slightly higher on a per g soil C basis, ranging from  $2.8 \text{mg C} \cdot \text{g}^{-1}$  C in soil to  $3.4 \text{mg C} \cdot \text{g}^{-1}$  C in soil. On a per gram soil basis, Control and No Litter soils had the highest respiration rates, followed by No Root, and then No Input. As in the forested sites, much of this variation disappeared when calculated on a per g soil C basis.

#### Acid hydrolysis estimate of recalcitrant C

Percent recalcitrant C ranged from 46.0 to 54.3% in the Curtis Prairie plots and from 51.1 to 70.5% in the forested plots. There was no pattern in recalcitrant C corresponding to site or treatment.

#### Sequential density fractionation

Mean soil mass recoveries across ecosystems and treatments were high (96 – 100%) and total C recovery averaged 83% ( $\pm 1.7$  s.e.; Table 4). In all treatments and controls the majority (69-78%) of soil material was in the  $2.4\text{-}2.65 \text{ g cm}^3$  range. However, the proportion of carbon was distributed across the  $1.65\text{-}2.65$  range more uniformly (Table 4), with no one density fraction containing more than 29% of the total C. Data were analyzed both as separate fractions, and also grouped into

“functional” categories following Hatton et al. (2012): light (<1.85 density fractions), aggregate (>1.85 – <2.4 fractions), and mineral (>2.4) fractions.

In the forest sites, C in the two lightest density fractions (from <1.65 to 1.85 g cm<sup>-3</sup>) were significantly higher in Double Litter plots compared to Controls and were lower in the No Litter plots (Figure 3, Table 5). In both forested plots, the 1.85 – 2.0 g cm<sup>-3</sup> fraction was significantly greater in Double Litter plots than Controls (Figure 3), and although the next two density fractions were slightly lower in Double Litter than in Control, averaged over all aggregate fractions, Double Litter intermediate fractions were not significantly different from Control (Table 5). We infer that C additions to this aggregate pool caused the transfer of slightly heavier fractions (>2.0 – 2.4) to move into the 1.85 – 2.0 g cm<sup>-3</sup> pool.

In the prairie sites, C in the four lightest density fractions (from <1.65 to 2.2 g cm<sup>-3</sup>) were highest in Control plots and were lowest in the No Input plots (Figure 3). No Root and No Litter plots were intermediate. Although litter removals generally resulted in C loss from both light and aggregate fraction pools, No Litter plots in Curtis Prairie 3 actually increased C in some pools. The heaviest fraction did not show consistent trends with litter exclusion. In No Input plots, losses were similar between light and intermediate density pools.

#### Patterns of $\delta^{13}\text{C}$ across soils and density fractions

In both Noe and Wingra Woods, soils from plots with litter removed had less negative, or heavier,  $\delta^{13}\text{C}$  values than Control or Double Litter plots across all density fractions, although differences were slight, and this pattern was more pronounced for

Noe than for Wingra Woods (Figure 4). Patterns of  $\delta^{13}\text{C}$  followed those in other forested soils compiled in a larger dataset (Throop et al. 2013);  $\delta^{13}\text{C}$  increased with increasing density until the  $> 2.65 \text{ g cm}^{-3}$  fraction, and then leveled or slightly declined (Figure 5). In contrast to results seen in the forested sites, soils from prairie plots with litter removed generally had more negative, or lighter  $\delta^{13}\text{C}$  values than Control plots across all density fractions, although this pattern was more pronounced in Curtis Prairie 3 than in Prairie 1, where only No Input  $\delta^{13}\text{C}$  values were consistently lighter than Control values (Figure 4). Patterns of  $\delta^{13}\text{C}$  followed those of a desert grassland shown in Throop et al. (2013), although with a less severe drop in  $\delta^{13}\text{C}$  in the heaviest fraction (Figure 5), and showed  $\delta^{13}\text{C}$  signatures consistent with those expected for a mixed C3-C4 grassland.

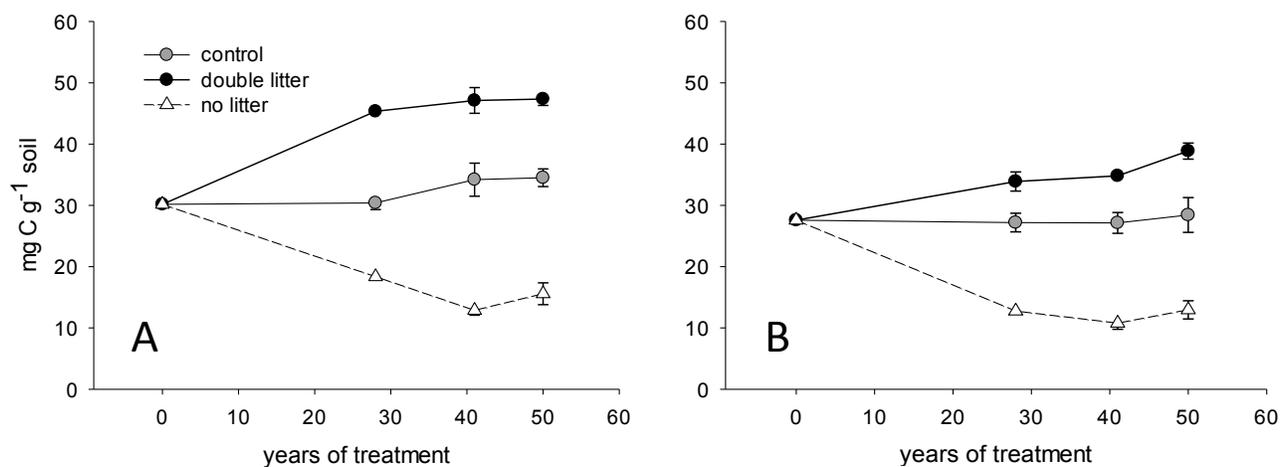
#### Radiocarbon in bulk soils and soil fractions

In general, greater C concentration in bulk soils was strongly related to greater  $^{14}\text{C}$  abundance, indicating greater C stores and higher proportions of more recent C (Figure 6). In the one forest (Noe Woods) where  $\Delta^{14}\text{C}$  was measured, soils and fractions from the No Litter plots had  $\Delta^{14}\text{C}$  values between 35-90 ‰ lower than those of the Control (Table 6). In the prairie sites, Control soils had also clearly accumulated bomb  $^{14}\text{C}$  (more recent), and  $\Delta^{14}\text{C}$  trended higher in Control plots than in any of the removal plots, with No Input plots having the lowest  $\Delta^{14}\text{C}$  values, indicating less carbon has been incorporated since 1950. The  $\Delta^{14}\text{C}$  of bulk soil and density fractions from the No Input plots in the prairies ranged near or below 0 ‰.

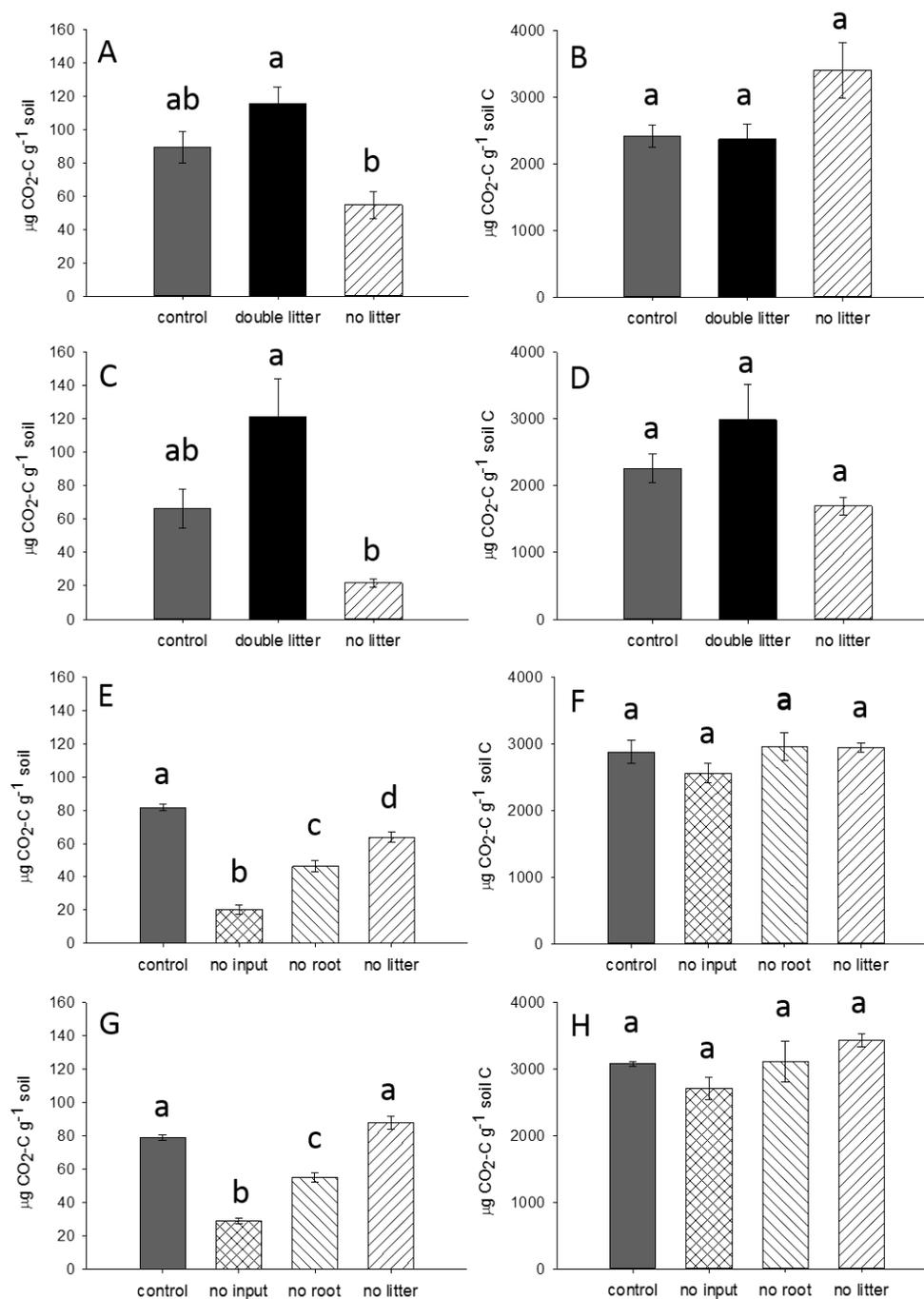
In all soils across all treatments,  $\Delta^{14}\text{C}$  of the heaviest fraction ( $>2.65 \text{ g cm}^{-3}$ ) was always negative and always more negative than any other fraction in the soil profile, indicating less carbon in that fraction was incorporated since 1950. The next heaviest fraction ( $>2.2 - 2.4 \text{ g cm}^{-3}$ ) also had low  $\Delta^{14}\text{C}$  values, and the lighter fractions tended to have the highest  $\Delta^{14}\text{C}$  values, indicating light fraction incorporated more bomb  $^{14}\text{C}$ . The patterns of  $^{14}\text{C}$  abundance in density fractions from the Noe Forest Control and No Litter treatments were similar, whereas  $^{14}\text{C}$  abundance with increasing density followed a different pattern in the Double Litter fractions. The  $^{14}\text{C}$  of some density fractions (1.65-1.85 and 1.85-2) appeared higher than the corresponding fractions from the Control, whereas the heaviest three fractions appeared somewhat lower. Most litter removal fractions at  $>1.65 - 1.85 \text{ g cm}^{-3}$  had markedly depleted signals in the prairie No Inputs plots, likely indicative of charcoal incorporation from burning. Although most plots trended to have depleted signals in the  $>1.65 - 1.85 \text{ g cm}^{-3}$  fraction, this effect was partly masked by addition of bomb  $^{14}\text{C}$  in the other plots. Otherwise, across treatments within each ecosystem,  $\Delta^{14}\text{C}$  patterns followed that of bulk soils.

There were significant relationships between C content change in bulk soil (Figure 6) and mineral fractions (Figure 7) in treatment plots for  $^{14}\text{C}$  values relative to the Control values. Bulk carbon loss in litter removal plots trended strongly with  $^{14}\text{C}$  changes ( $r^2=0.95, P<0.01, n=6$ ). The magnitude of carbon loss in the 1.8-2.4 mineral fraction corresponded to increasingly negative  $^{14}\text{C}$  values relative to the control ( $r^2=0.98, P<0.01, n=6$ ) up to -40 ‰. Carbon in the  $> 2.4 \text{ g cm}^{-3}$  density fraction had both a positive and negative response to detrital removal manipulations (6% +36%

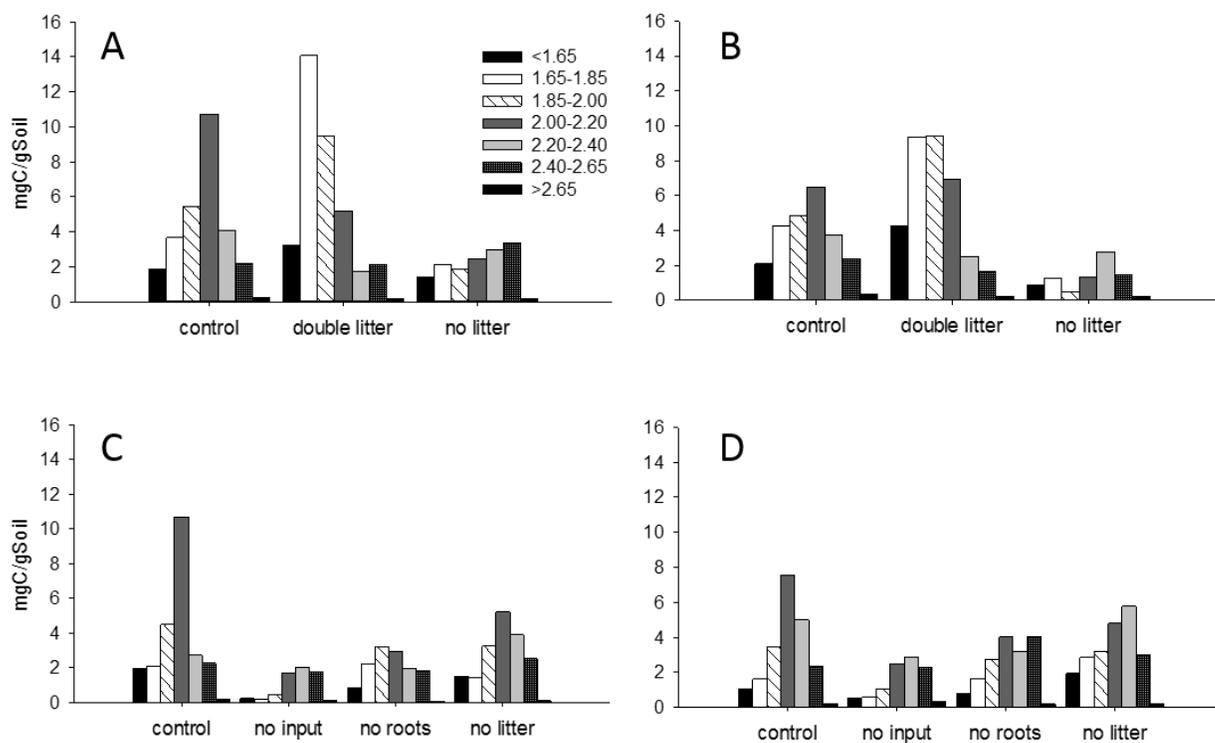
standard deviation). There was an increase of carbon in this fraction due to some of the detrital manipulations, as well as a decrease (Figure 7). A strong relationship between the amount of C decline and the change in  $^{14}\text{C}$  was found in this fraction (Figure 7) ( $r^2 = 0.44$ ,  $P < 0.01$ ,  $n = 6$ ).



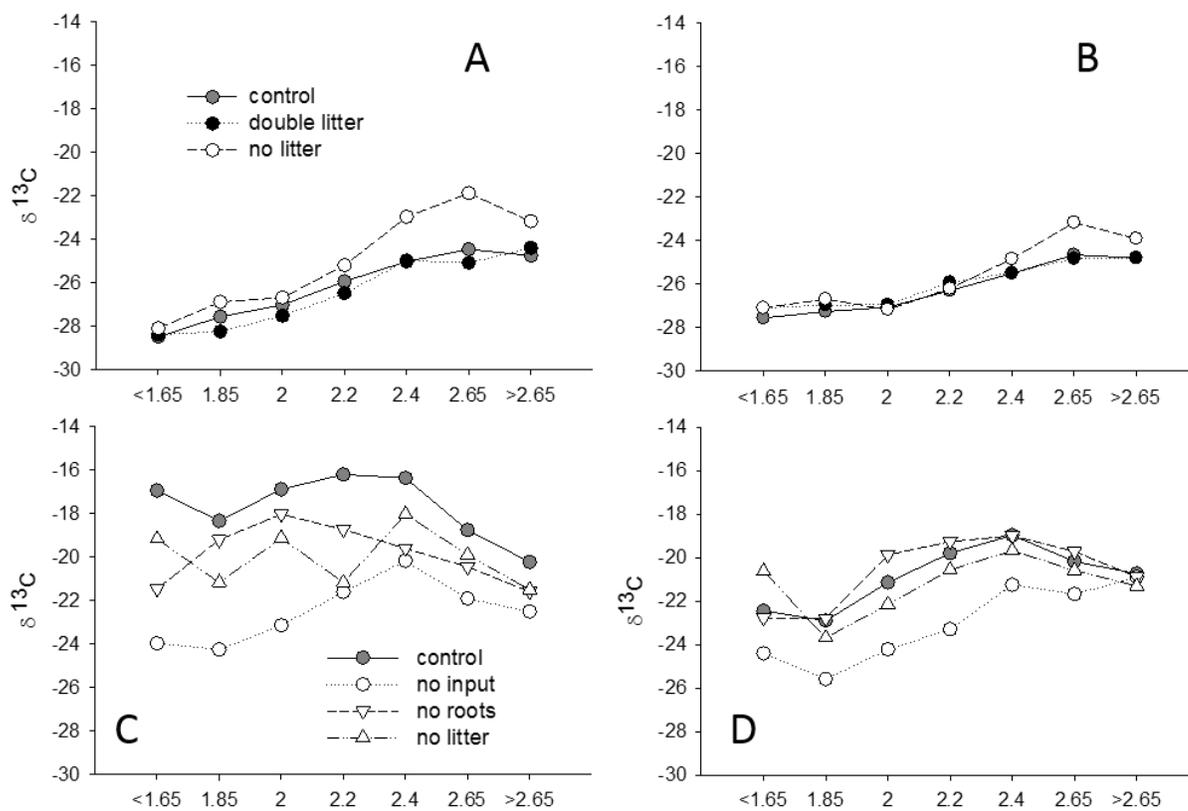
**Figure 1** Soil Organic Carbon (SOC) concentrations for the Noe Woods (A) and Wingra Woods (B) sites of the Francis Hole Detrital Input and Removal Treatments in 1984, 1997 and 2006. Values are means  $\pm$  1 standard error,  $n=4$ . Significant differences in values between treatments within a site in 2006 are shown in Table 2.



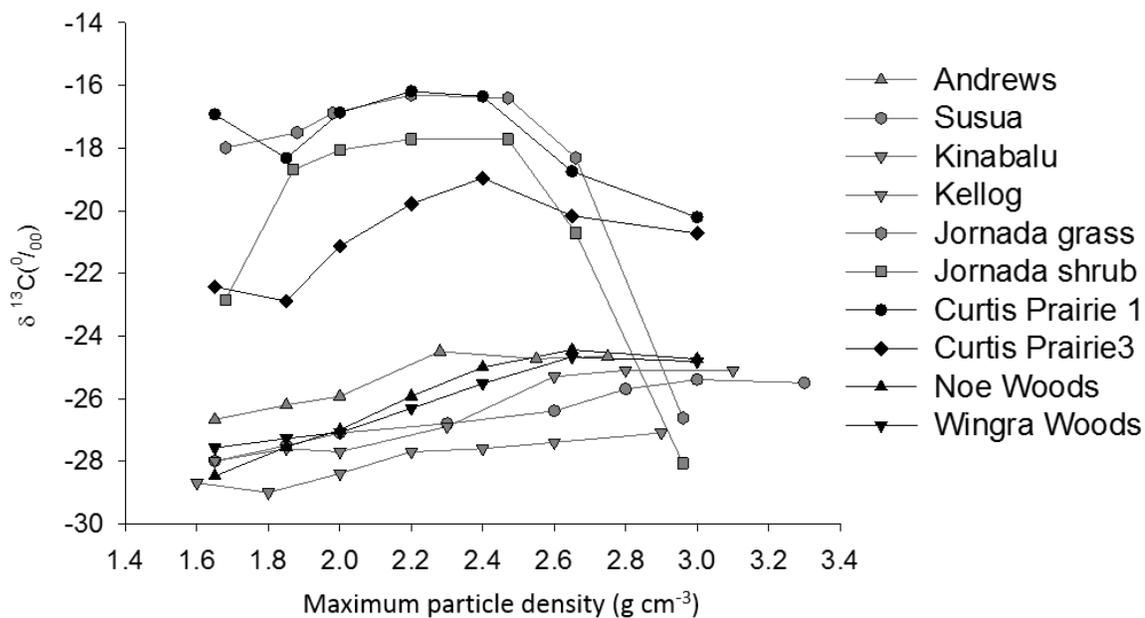
**Figure 2** Cumulative respiration after a 28 day incubation of bulk soil by treatment from Noe Woods (A, B), Wingra Woods (C, D), Curtis Prairie 1 (E, F), and Curtis Prairie 3 (G,H). Values are means  $\pm$  1 standard error,  $n=4$ , letters indicate significant differences between means ( $P$ -value  $\leq 0.05$ ). Note that the left column is on a different scale than the right column. Values in left column are expressed in  $\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil}$ , values in right column are expressed in  $\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil C}$ .



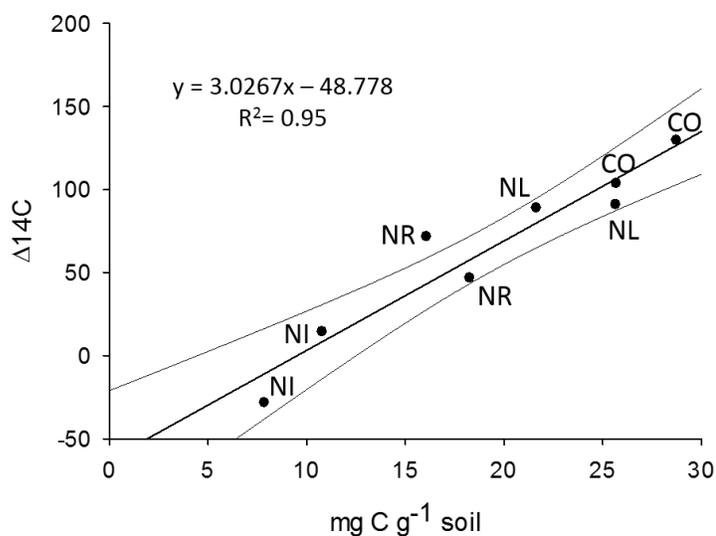
**Figure 3** SOC concentration in density fractions of Noe Woods (A), Wingra Woods (B), Curtis Prairie 1 (C), and Curtis Prairie 3 (D) plots in the Francis Hole Dirt Experiment by treatment. Values are individual data points.



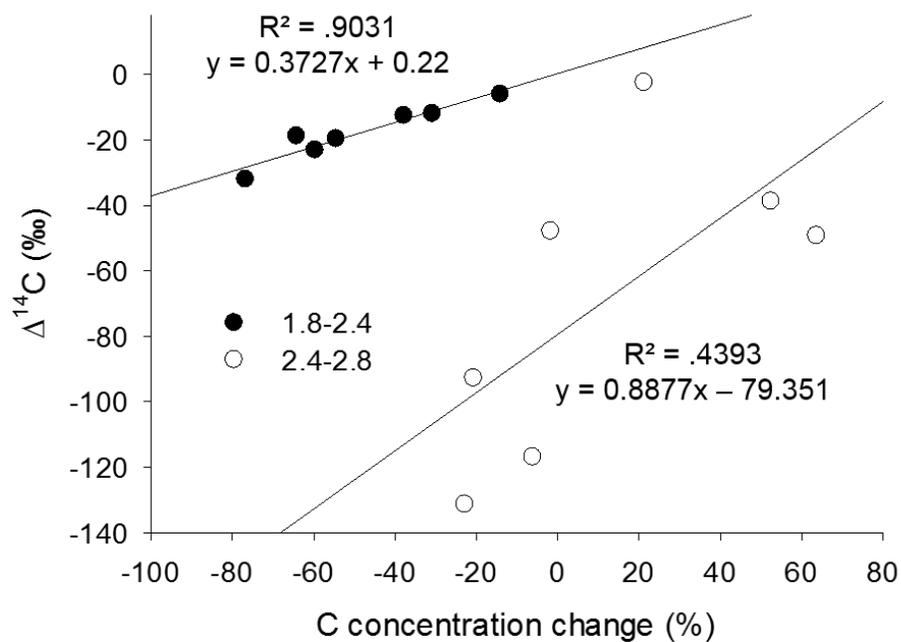
**Figure 4** Patterns of  $\delta^{13}\text{C}$  by density fraction of soils sampled after 50 years of detrital manipulation from Noe Woods (A), Wingra Woods (B), Curtis Prairie 1 (C) and Curtis Prairie 3 (D), sites in the Francis Hole DIRT Experiment. Values are individual data points.



**Figure 5** Patterns of  $\delta^{13}\text{C}$  in soil sequential density fractions from the control plots in the Francis Hole DIRT sites plotted in comparison with data from soils studied by Throop et al. (2013) and Sollins et al. (2009). Francis Hole DIRT sites are plotted with black symbols, comparison data with gray.



**Figure 6** Relationship between  $\Delta^{14}\text{C}$  and soil organic carbon concentration of bulk soil for the two Curtis Prairie sites of the Francis Hole DIRT plots. NI, No Input, NR, No Root, NL, No Litter, CO, Control



**Figure 7** Relationship between mineral fraction C content change (%) and  $\Delta^{14}\text{C}$  (‰) across all detrital removal treatments (Forest and Prairie combined)

**Table 1** Treatment and methods of the Francis Hole Experimental Detrital Input and Removal Treatments (DIRT) plots installed at the Wisconsin Arboretum in 1956.

	Treatment	Method
Forest	Control (CTL)	“Undisturbed” ‡ plots with normal above and below ground litter inputs allowed.
	Double Litter (DL)	“Litter doubled” ‡ by adding aboveground litter inputs removed annually from No Litter plots.
	No Litter (NL)	“Litter continually removed” ‡ (annually) by raking aboveground inputs and proportionately redistributing to Double Litter plots.
	Bare (B) †	Aboveground inputs are removed annually by raking; although originally intended to be a root exclusion plot, large tree roots are present.
	OA-less (OA) †	Stripped of 15.2 cm of organic soil and refilled with mineral soil at initiation in 1956.
Prairie	Control (CTL)	“Undisturbed” ‡ plots with normal above and below ground litter inputs
	No Input (NI)	Vegetation both above and belowground is “clipped frequently” ‡ to a minimum.
	No Root (NR)	Vegetation aboveground is clipped, killing vegetation and eliminating roots. Aboveground litter (mulch) is transferred annually from “harvested annually” ‡ (No Litter) treatment, in autumn. Originally designated as “clipped and mulched” ‡
	No Litter (NL)	Vegetation is “harvested annually” ‡ from plot in autumn, leaving roots intact.
	Burned (B) †	Aboveground vegetation is “burned” ‡ biennially in the spring.

“†” indicates treatments were not included in our analyses because plot boundaries or treatment maintenance regimes were inconsistent or not recorded over the period of this study. “‡” indicates official plot designations given in Nielsen and Hole (1963).

**Table 2** Soil Organic Carbon (SOC) and Soil Organic Nitrogen (SON) concentration and content and bulk density, in forest Francis Hole Experimental DIRT plots at the Wisconsin Arboretum, sampled in 2006 after 50 years of detrital manipulation. Values are means of 4 replicate subsamples (1 standard error). Significance was determined by one-way ANOVA followed by a Tukey HSD post hoc test. Means followed by different letters in superscript were significantly different from each other at  $P \leq 0.05$ . % difference refers to the percent difference between the mean for each treatment relative to the mean for control.

	Control	Double Litter	No Litter
<b>SOC concentration (mg C g<sup>-1</sup> soil)</b>			
Noe			
mean (SE)	34.53 (1.44) <sup>a</sup>	47.36 (4.26) <sup>b</sup>	15.57 (1.78) <sup>c</sup>
% difference		+37.18	-54.89
Wingra			
mean (SE)	28.43 (2.84) <sup>a</sup>	38.86 (1.32) <sup>b</sup>	12.94 (1.48) <sup>c</sup>
% difference		+36.68	-54.47
<b>Bulk Density (g/cm<sup>3</sup>)</b>			
Noe			
mean (SE)	1.05 (0.02) <sup>a</sup>	0.90 (0.02) <sup>b</sup>	1.23 (0.05) <sup>c</sup>
Wingra			
mean (SE)	1.04 (0.03) <sup>a</sup>	1.01 (0.03) <sup>a</sup>	1.37 (0.02) <sup>b</sup>
<b>SOC content (g C m<sup>-2</sup>)</b>			
Noe			
mean (SE)	3634.35 (151.59) <sup>a</sup>	4693.94 (137.29) <sup>a</sup>	1914.03 (219.22) <sup>b</sup>
% difference		+29.15	-47.34

Wingra

mean (SE)	2958.28 (295.21) <sup>a</sup>	3925.19 (132.98) <sup>a</sup>	1767.75 (202.72) <sup>b</sup>
% difference		+32.68	-40.24

**SON concentration (mg N g<sup>-1</sup> soil)**

Noe

mean (SE)	2.91 (0.09) <sup>a</sup>	2.83 (0.31) <sup>a</sup>	1.50 (0.26) <sup>b</sup>
% difference		+2.79	-48.57

Wingra

mean (SE)	2.06 (0.16) <sup>a</sup>	3.12 (0.16) <sup>b</sup>	1.10 (0.08) <sup>c</sup>
% difference		+51.67	-46.68

**SON content (g N m<sup>-2</sup>)**

Noe

mean (SE)	306.54 (9.52) <sup>a</sup>	255.02 (27.98) <sup>a</sup>	184.06 (31.70) <sup>b</sup>
% difference		-16.81	-39.96

Wingra

mean (SE)	213.91 (16.95) <sup>a</sup>	314.05 (16.02) <sup>b</sup>	149.69 (11.47) <sup>a</sup>
% difference		+46.82	-30.02

**Table 3** Soil Organic Carbon (SOC) and Soil Organic Nitrogen (SON) concentration and content in prairie Francis Hole Experimental DIRT plots at the Wisconsin Arboretum, sampled in 1997 and 2006 after 41 and 50 years of detrital manipulation. Values are means of 4 replicate subsamples (1 standard error). Significance was determined by one-way ANOVA followed by a Tukey HSD post hoc test. Means followed by different letters in superscript were significantly different from each other at  $P \leq 0.05$ . % difference refers to the percent difference between the mean for each treatment relative to the mean for control.

1997 Curtis Prairie				
	Control	No Input	No Roots	No Litter
<b>SOC concentration (mg C g<sup>-1</sup> soil)</b>				
mean (SE)	21.23 (0.91) <sup>a</sup>	8.72 (0.34) <sup>b</sup>	15.86 (1.93) <sup>c</sup>	22.00(0.95) <sup>a</sup>
% difference		-58.92	-25.30	+3.64
<b>Bulk Density (g/cm<sup>3</sup>)</b>				
mean (SE)	1.11 (0.01) <sup>a</sup>	1.20 (0.02) <sup>ab</sup>	1.31 (0.01) <sup>b</sup>	0.96 (0.02) <sup>c</sup>
<b>SOC content (g C m<sup>-2</sup>)</b>				
mean (SE)	2348.92 (65.20) <sup>a</sup>	1049.40 (28.43) <sup>b</sup>	2067.16 (123.24) <sup>a</sup>	2114.12 (50.39) <sup>a</sup>
% difference		-55.32	-25.01	-10.00
<b>SON concentration (mg N g<sup>-1</sup> soil)</b>				
mean (SE)	1.70 (0.02) <sup>a</sup>	0.89 (0.01) <sup>b</sup>	1.33 (0.05) <sup>c</sup>	1.68 (0.04) <sup>a</sup>
% difference		-47.75	-21.62	-0.98
<b>SON content (g N m<sup>-2</sup>)</b>				
mean (SE)	188.20 (3.38) <sup>a</sup>	106.99 (2.37) <sup>b</sup>	173.91 (6.12) <sup>a</sup>	161.96 (4.29) <sup>a</sup>
% difference		-43.15	-11.54	-13.95

## 2006 Curtis Prairie 1

	Control	No Input	No Roots	No litter
<b>SOC concentration (mg C g<sup>-1</sup> soil)</b>				
mean (SE)	27.41 (1.85) <sup>a</sup>	7.88 (0.54) <sup>b</sup>	16.07 (1.73) <sup>c</sup>	20.95 (0.54) <sup>c</sup>
% difference		-71.24	-41.36	-23.55
<b>SOC content (g C m<sup>-2</sup>)</b>				
mean (SE)	3029.87 (204.30) <sup>a</sup>	949.19 (64.86) <sup>b</sup>	2097.69 (225.22) <sup>c</sup>	2014.23 (52.01) <sup>c</sup>
% difference		-68.67	-30.77	-33.52
<b>SON concentration (mg N g<sup>-1</sup> soil)</b>				
mean (SE)	2.04 (0.08) <sup>a</sup>	0.96 (0.02) <sup>b</sup>	1.36 (0.11) <sup>c</sup>	1.87 (0.04) <sup>a</sup>
% difference		-53.18	-33.45	-8.44
<b>SON content (g N m<sup>-2</sup>)</b>				
mean (SE)	225.84 (9.09) <sup>a</sup>	115.18 (2.70) <sup>b</sup>	177.45 (14.94) <sup>c</sup>	179.81 (3.79) <sup>c</sup>
% difference		-49.00	-21.43	-20.38

## 2006 Curtis Prairie 3

	Control	No Input	No Roots	No litter
<b>SOC concentration (mg C g<sup>-1</sup> soil)</b>				
mean (SE)	24.61 (0.81) <sup>a</sup>	10.83 (0.51) <sup>b</sup>	17.59 (1.61) <sup>c</sup>	24.86 (0.59) <sup>a</sup>
% difference		-56.00	-28.54	+1.01
<b>SOC content (g C m<sup>-2</sup>)</b>				
mean (SE)	2720.32 (89.54) <sup>a</sup>	1303.66 (61.55) <sup>b</sup>	2295.24 (209.98) <sup>a</sup>	2389.37 (56.81) <sup>a</sup>
% difference		-52.08	-15.63	-12.17
<b>SON concentration (mg N g<sup>-1</sup> soil)</b>				
mean (SE)	1.95 (0.07) <sup>a</sup>	1.10 (0.05) <sup>b</sup>	1.50 (0.12) <sup>c</sup>	1.95 (0.07) <sup>a</sup>
% difference		-43.72	-23.07	-0.48

**SON content (g N m<sup>-2</sup>)**

mean (SE)	216.11 (7.50) <sup>a</sup>	132.49 (5.88) <sup>b</sup>	196.28 (15.14) <sup>a</sup>	187.02 (7.08) <sup>a</sup>
% difference		-38.69	-9.18	-13.46

**Table 4** Mass recovery, carbon (C) concentration and C recovery by density fraction of soils from Noe Woods, Wingra Woods and the Curtis Prairie sites of the Francis Hole DIRT plots sampled in 2006.

Density Fractions	Mass % bulk soil	C mg g <sup>-1</sup> fraction	% total C	Mass % bulk soil	C mg g <sup>-1</sup> fraction	% total C
<b>Noe Woods Control</b>			<b>Wingra Woods Control</b>			
<1.65	0.6	333.7	5.1	0.6	335.8	7.2
1.65-1.85	1.6	235.8	10.0	1.8	229.3	14.6
1.85-2	3.4	158.8	14.9	3.0	159.0	16.6
2-2.2	12.2	87.7	29.3	6.9	81.2	22.3
2.2-2.4	6.3	64.9	11.1	5.4	74.2	12.9
2.4-2.65	69.1	3.2	6.0	72.0	2.4	8.2
>2.65	5.2	4.2	0.6	8.7	4.0	1.1
<b>Total recovery (%)</b>	98.3		76.9	98.4		82.9
<b>Noe Woods Double Litter</b>			<b>Wingra Woods Double Litter</b>			
<1.65	1.0	309.8	6.4	1.3	339.1	11.6
1.65-1.85	6.6	212.2	27.9	4.1	236.4	25.4
1.85-2	6.2	152.3	18.8	5.9	162.0	25.5
2-2.2	5.9	88.0	10.3	8.5	93.9	18.7
2.2-2.4	3.0	56.2	3.4	3.4	69.2	6.8
2.4-2.65	68.6	3.1	4.2	67.0	3.3	4.4
>2.65	5.4	2.8	0.3	6.2	3.7	0.7
<b>Total recovery (%)</b>	96.9		71.4	96.3		93.0
<b>Noe Woods No Litter</b>			<b>Wingra Woods No Litter</b>			
<1.65	0.4	332.8	8.8	0.3	332.5	7.5
1.65-1.85	0.8	264.3	13.0	0.5	255.2	11.1
1.85-2	1.0	184.6	11.4	0.3	132.5	4.0
2-2.2	2.9	85.4	15.0	2.5	53.6	11.4
2.2-2.4	5.3	56.4	18.3	5.4	51.4	24.1
2.4-2.65	83.2	4.0	20.5	84.2	1.7	12.5
>2.65	5.7	3.4	1.2	6.7	3.5	2.1
<b>Total recovery (%)</b>	99.3		88.2	99.8		72.6

	<b>Curtis Prairie 1 Control</b>			<b>Curtis Prairie 3 Control</b>		
<b>&lt;1.65</b>	0.8	261.0	6.9	0.4	265.8	4.0
<b>1.65-1.85</b>	0.9	247.4	7.4	0.6	251.7	6.4
<b>1.85-2</b>	2.8	162.1	15.7	1.9	186.4	13.6
<b>2-2.2</b>	12.9	82.9	37.2	8.8	85.5	29.3
<b>2.2-2.4</b>	5.5	49.7	9.5	8.5	58.6	19.5
<b>2.4-2.65</b>	70.3	3.2	7.9	67.1	3.5	9.3
<b>&gt;2.65</b>	5.2	3.5	0.6	6.1	3.7	0.9
<b>Total recovery (%)</b>	98.3		85.3	93.4		82.9
	<b>Curtis Prairie 1 No Input</b>			<b>Curtis Prairie 3 No Input</b>		
<b>&lt;1.65</b>	0.1	273.4	3.2	0.2	253.2	4.8
<b>1.65-1.85</b>	0.1	265.2	2.2	0.2	282.5	5.5
<b>1.85-2</b>	0.3	165.0	5.7	0.5	197.1	10.0
<b>2-2.2</b>	3.4	50.2	21.5	3.1	79.0	23.0
<b>2.2-2.4</b>	8.0	25.2	25.8	6.7	43.5	26.9
<b>2.4-2.65</b>	77.0	2.3	22.4	73.8	3.1	21.4
<b>&gt;2.65</b>	6.3	1.8	1.5	11.5	3.1	3.3
<b>Total recovery (%)</b>	95.0		82.2	96.0		94.9
	<b>Curtis Prairie 1 No Roots</b>			<b>Curtis Prairie 3 No Roots</b>		
<b>&lt;1.65</b>	0.3	282.5	5.3	0.3	253.8	4.3
<b>1.65-1.85</b>	0.9	251.8	14.0	0.7	229.1	9.1
<b>1.85-2</b>	2.0	159.3	20.1	1.7	165.7	15.1
<b>2-2.2</b>	4.2	70.6	18.4	5.4	74.2	22.0
<b>2.2-2.4</b>	6.6	29.7	12.3	6.3	50.1	17.4
<b>2.4-2.65</b>	78.1	2.3	11.2	77.6	5.2	22.0
<b>&gt;2.65</b>	3.1	2.4	0.5	5.8	3.7	1.2
<b>Total recovery (%)</b>	95.3		81.8	97.9		91.1
	<b>Curtis Prairie 1 No Litter</b>			<b>Curtis Prairie 3 No Litter</b>		
<b>&lt;1.65</b>	0.6	253.5	7.0	0.8	243.9	7.5
<b>1.65-1.85</b>	0.7	211.7	6.6	1.3	222.5	11.2
<b>1.85-2</b>	2.1	154.6	15.0	2.0	156.1	12.4
<b>2-2.2</b>	6.7	77.8	24.1	5.2	92.2	18.7
<b>2.2-2.4</b>	7.8	50.1	18.1	8.4	68.5	22.5
<b>2.4-2.65</b>	74.1	3.4	11.7	74.9	4.0	11.6

<b>&gt;2.65</b>	3.8	3.0	0.5	5.7	3.6	0.8
<b>Total recovery (%)</b>	95.8		83.0	98.3		84.7

**Table 5** Mean percent SOC concentration changes (SE) in Francis Hole DIRT plots sampled in 2006 relative to control plots by density fraction. Individual fractions were pooled into 3 categories: Light fraction ( $<1.85 \text{ g/cm}^3$ ), Intermediate fraction ( $1.85 \text{ g/cm}^3$  to  $2.4 \text{ g/cm}^3$ ), and Heavy fraction ( $>2.4 \text{ g/cm}^3$ ). Values are mean (SE),  $n=2$ . Bold values indicate that SE is smaller than the mean.

Site/Treatment	Light (OM) Fraction	Intermediate (Aggregate)Fraction	Heavy Fraction
Prairie Removal (NI)	<b>-74% (11)</b>	<b>-68% (6)</b>	<b>-11% (9)</b>
Prairie Removal (NR)	<b>-16% (6)</b>	<b>-46% (6)</b>	20% (30)
Prairie Removal (NL)	26% (38)	<b>-23% (6)</b>	<b>15% (5)</b>
Forest Removal (NL)	<b>-51% (11)</b>	<b>-67% (2)</b>	4% (30)
Forest Addition (DL)	<b>164% (35)</b>	3% (15)	<b>-18% (9)</b>

**Table 6**  $\Delta^{14}\text{C}$  per thousand (error) of bulk soil and density fractions for the Francis Hole DIRT plots for Curtis Prairie and Noe Woods. The reported values represent individual analytical samples.

		<b>Control</b>	<b>No Input</b>	<b>No Roots</b>	<b>No Litter</b>
<b>Curtis Prairie 1</b>	<b>Bulk</b>	129.7 (4.6)	-28.2(4.2)	71.8 (4.4)	88.9 (5.2)
	<b>&lt;1.65</b>	90.9 (4.5)	2.1 (4.1)	42.6 (4.4)	68.0 (4.7)
	<b>1.65-1.85</b>	98.2 (5.2)	-104.6 (3.7)	74.2 (4.8)	75.9 (4.4)
	<b>1.85-2</b>	134.1 (3.5)	-42.6 (3.9)	116.0 (4.4)	107.0 (4.4)
	<b>2-2.2</b>	151.4 (4.5)	-2.3 (3.8)	111.3 (6.5)	118.8 (4.7)
	<b>2.2-2.4</b>	139.6 (5.8)	-11.9 (5.9)	63.5 (4.4)	111.3 (4.5)
	<b>2.4-2.65</b>	36.8 (4.3)	-94.5 (5.2)	-56.0 (3.4)	-11.1 (4.0)
	<b>&gt;2.65</b>	-25.1 (3.7)	-166.7 (2.9)	-123.3 (4.4)	†
		<b>Control</b>	<b>No Inputs</b>	<b>No Roots</b>	<b>No Litter</b>
<b>Curtis Prairie 3</b>	<b>Bulk</b>	103.7 (4.6)	14.5 (4.2)	46.8 (4.3)	91.0 (4.5)
	<b>&lt;1.65</b>	88.4 (4.4)	14.8 (5.5)	31.8 (4.3)	78.0 (4.6)
	<b>1.65-1.85</b>	69.8 (4.4)	-50.2 (2.8)	18.4 (4.2)	57.9 (4.5)
	<b>1.85-2</b>	98.5 (3.7)	-17.4 (3.5)	74.8 (4.6)	86.3 (4.6)
	<b>2-2.2</b>	125.0 (4.7)	3.7 (3.6)	84.9 (4.6)	118.8 (4.4)
	<b>2.2-2.4</b>	115.1 (4.6)	-7.6 (3.1)	66.1 (4.3)	102.4 (4.3)
	<b>2.4-2.65</b>	52.2 (4.8)	-64.6 (3.9)	3.0 (4.2)	49.7 (4.1)
	<b>&gt;2.65</b>	-27.5 (4.1)	-113.7 (3.6)	-66.7 (3.9)	-23.2 (4.0)
		<b>Control</b>	<b>Double Litter</b>	<b>No Litter</b>	
<b>Noe Woods</b>	<b>Bulk</b>	77.8 (4.5)	95.1 (4.8)	2.0 (4.1)	
	<b>&lt;1.65</b>	91.7 (4.2)	75.4 (4.6)	55.8 (4.1)	
	<b>1.65-1.85</b>	51.3 (3.9)	101.3 (4.5)	-1.4 (4.1)	
	<b>1.85-2</b>	88.9 (4.0)	126.3 (4.7)	36.9 (4.3)	
	<b>2-2.2</b>	98.3 (4.3)	103.2 (4.6)	33.6 (3.7)	
	<b>2.2-2.4</b>	87.2 (4.2)	41.6 (4.3)	7.7 (3.9)	
	<b>2.4-2.65</b>	36.0 (4.1)	9.8 (4.1)	-2.7 (3.9)	
	<b>&gt;2.65</b>	-17.1 (3.5)	-31.9 (4.0)	-75.0 (3.8)	

## Chapter 4: Discussion

The increase in bulk soil C in surface horizons in the forested sites after addition was substantial, but the increase was due principally to an increase in the light fraction (free particulate) carbon; intermediate density fractions showed little change in C content after 50 years of litter addition. To date, data from other DIRT sites, all of which are younger, have not shown such increases in either bulk or light fraction C, but instead have shown signs of priming (Sulzman et al. 2005, Lajtha et al. 2013, Bowden et al. 2013, Fekete et al. 2013). Priming is thought to be a relatively short-lived phenomenon (Hoosbeek and Scarascia-Mugnozza 2009), and thus it is possible that we missed the early phase of priming, although our earliest measurements were after 28 years as compared to 20 years for Harvard Forest and Bousson. The apparent increase in  $^{14}\text{C}$  abundance in the light (1.65-1.85) and first two intermediate (1.85-2.0, 2.0-2.2) mineral density fractions from the Noe Woods Double Litter corresponded with large increases in the C concentration indicating these fractions received higher proportions of more recent bomb  $^{14}\text{C}$  relative to the Control. These three density fractions appear to be primary reservoirs for the overall increase in total SOC in the Double Litter plots. Conversely, slight declines in the amount of C held in the heaviest three mineral fractions corresponded with somewhat lower net accumulation of  $^{14}\text{C}$  in those fractions. It is possible that even as increased C inputs from litter were accumulating in the light (<1.85) fractions, these C inputs were resulting in priming effects in the denser fractions (Kuzyakov 2002), reducing the net accumulation of C and more recent bomb  $^{14}\text{C}$ . Because the increase in soil C content in the litter addition treatments was in the free particulate fraction, and not in denser (1.85-2.4, or >2.4 g cm<sup>-3</sup>) soil fractions associated with aggregates or stabilized by association

with minerals, we conclude that there is little evidence for increased interaction (or potential increased stabilization) with minerals or in aggregates, with the exception for the lightest (1.85-2.0) mineral density fraction. Thus at least at the 50 year time scale, the pool of soil C associated with longer turnover times appears to be at or near equilibrium with climate and mineralogy, and is not substantially affected by increased C inputs.

Significant increases in total soil C content were also found in a litter addition experiment in a moist tropical forest (Leff et al. 2012) almost immediately, suggesting that the balance between C stabilization from increased inputs and C destabilization from priming is complex, and site-specific. Similarly, Liao et al. (2006) saw large C increases in free light particulate, macroaggregate, and microaggregate fractions following large productivity increases with woody plant invasion of dry grasslands. However, our results from this long-term litter manipulation experiment have significant implications for models of management effects on soil C sequestration. Most models assume a direct link between litterfall and soil C sequestration, although C accumulation is only a small fraction of litterfall; Paul et al. (2003) predicted that after 40 years of afforestation, less than 3% of cumulative NPP would accumulate in soil. Assuming a mean litterfall of  $182 \text{ g m}^{-2} \text{ yr}^{-1}$  averaged across both forests, Double Litter plots accumulated about 5% of total litter added to the plots over 50 years.

In both forested and prairie sites, litter removal treatments resulted in substantial C concentration declines, with C losses in the forested sites with litter exclusion (40 and 47%) greater than C gains with litter doubling (29 and 33%). The

pattern of C loss varied by density fraction relative to bulk soil. Although we predicted that we would see the greatest changes in the litter exclusion treatments in the light fraction pools as was seen for the litter addition treatments in the forest, declines in light fraction were equal to declines in the intermediate ( $1.8 - 2.4 \text{ g cm}^{-3}$ ), aggregate fraction; the heaviest ( $>2.4 \text{ g cm}^{-3}$ ) pool of mineral-stabilized C had both a positive and negative response to the detrital treatments. Other studies have shown that light fraction organic matter is highly sensitive to management and changes in ecosystem productivity (McFarlane et al. 2010, McLauchlan and Hobbie 2004, Compton and Boone 2002) but these studies have generally observed C increases, rather than C declines due to lowered litter input. Diachon and Kellman (2009) observed decreases in mineral-associated, heavy fraction C that paralleled light fraction C losses after timber harvest, and Llorente et al. (2010) observed mineral C losses in reforestation of former cultivated land, similar to results of John et al. (2005) who observed lower mineral C storage under forest than under grassland. Similarly, Richter et al. (1999) showed bulk C decline in soil at depth due to reforestation of an abandoned field, and Mobley et al. (In Prep) attributed this C decline to mineral-associated C loss. The loss of C observed in the intermediate mineral fraction may best be explained by reduced abundance of preferred substrates (e.g. light fraction particulate C) available to microbes during decomposition.

The magnitude of carbon loss in the  $1.8\text{-}2.4 \text{ g cm}^{-3}$  fraction corresponded to increasingly negative  $^{14}\text{C}$  values in the remaining mineral C, which suggests that younger (bomb) mineral-associated SOC was preferentially lost due to the litter exclusion treatments, or that no significant gain of bomb  $^{14}\text{C}$  occurred. The carbon

response in the  $>2.4 \text{ g cm}^{-3}$  density fraction to detrital removal manipulations was both positive and negative, and also corresponded to steeper  $^{14}\text{C}$  declines as carbon content declined relative to the control (Fig. 7). These results suggest that an increase in carbon content in that fraction may have occurred due to the loss of carbon in the lighter ( $1.85\text{-}2.4 \text{ g cm}^{-3}$ ) mineral fraction, thereby increasing the density of the organo-mineral particle and causing that particle to “shift” into the  $>2.4 \text{ g cm}^{-3}$  pool. Declines in carbon content observed in the  $1.8\text{-}2.4$  and  $> 2.4 \text{ g cm}^{-3}$  density fractions likely occurred due to preferential microbial consumption of more recent (bomb) SOC.

In the  $>2.4 \text{ g cm}^{-3}$  fraction, small declines in C content resulted in greater  $^{14}\text{C}$  isotope changes in the residual material than the  $1.8\text{-}2.4 \text{ g cm}^{-3}$  fraction. The results suggest that a proportionally greater loss of pre-bomb carbon, or a lack of incorporation of bomb  $^{14}\text{C}$ , into the  $>2.4 \text{ g cm}^{-3}$  density fraction per unit of C loss. The difference in patterns of C loss and change in  $^{14}\text{C}$  observed between the  $1.85\text{-}2.4$  and  $>2.4 \text{ g cm}^{-3}$  mineral fraction suggest more active carbon exchange (loss and gain) in the lighter fraction. One reason for this difference in C exchange may be the lower carbon loading per unit of specific surface area of the mineral surface (Sollins et al. 2009) in the denser mineral fraction, which may have enhanced the stabilization of carbon accumulating in this fraction more uniformly via mineral interaction. Another factor may be that less aggregation of younger bomb C material is occurring in the denser mineral fraction since the C content is so low in that mineral fraction. A third factor may be the lack of incorporation of bomb  $^{14}\text{C}$  (more recent carbon) due to the OM removal treatments.

Recent studies have suggested that root and rhizosphere-derived C is more important to stable soil C than is aboveground litter, and thus we expected to see greater effects on total soil C with the elimination of root inputs compared to the elimination of litter inputs in the prairie plots where root inputs were minimized. Indeed, in 1997, 41 years after the start of the experiment, only plots without roots showed significant declines in soil C. However, after 50 years, declines in soil C in plots with roots but without aboveground litter were similar to declines in plots without roots, in terms of SOC content and thus we did not find that the presence of roots was more critical than the presence of aboveground litter for C stabilization. This is not evidence that root C and shoot C have equal contributions to C stabilization in soils, however; by eliminating roots, we also eliminated rhizosphere activity and root exudation, which have been shown to accelerate the loss of SOC (Kuzyakov 2002; Eckschmitt et al. 2008; Drake et al. 2013). Thus our No Root plots eliminated both root C inputs as well as potential root-induced priming.

There was no clear evidence that soil C quality changed in litter addition plots in the forested sites, even as total amounts changed.  $\delta^{13}\text{C}$  values and incubation estimates of labile C were similar between Control and Double Litter soils, indicating there were no strong changes in the character of organic C in those soils. In contrast, C characteristics appeared to change in litter removal plots; soils with litter excluded had lower  $\Delta^{14}\text{C}$  values indicative of lower net  $^{14}\text{C}$  addition,  $\delta^{13}\text{C}$  values indicative of loss of fresh plant-derived C, and decreases in all light fraction C pools, although incubation estimates of C quality did not change.

Other studies have shown a loss in mineral C associated with land use change such as reforestation (Richter et al. 1999, Diochon and Kellman 2009, Llorente et al. 2010). Our study suggests that lighter (1.85-2.4) mineral fractions are more susceptible to C loss due to OM manipulations than are heavier fractions, which have been shown to contain carbon with the longest mean residence times. While Diochon and Kellman (2009) point out the need to differentiate mineral from light fraction C changes, here we show that further refinement and insight into organo-mineral C fractions is needed. Taken together, our results suggest surface mineral soils may be more vulnerable to loss than to gain, in association with disturbance, land use change, or perhaps even climate change over century - decadal timescales, and also highlight the need for longer-term experimental manipulations to study soil organic matter dynamics.

## Chapter 5: Conclusion

Given the importance of SOC in the global C cycle, understanding its sensitivity to management, disturbance, and temperature/moisture regime change is critical. Climate changes can be expected to change both quantity and quality of litter inputs, but the resulting effects on SOM stability and turnover cannot now be predicted accurately. The Francis Hole Experimental Plots give us an opportunity to explore SOC trajectories at the decadal timescale.

Following the assumptions of many models that soil C stores should be coupled to litter inputs, we hypothesized that increased detrital inputs in forested ecosystems would result in significant increases in total soil C and that priming effects, generally seen as an immediate effect of new carbon substrate additions, would no longer be detected. If true, this suggests that the forest soils in the University of Wisconsin Arboretum may not generally be C saturated, and after 50 years we predicted that we would see increases in both short and intermediate cycling C pools. We did see a significant increase in total SOC concentration in the Double Litter plots in both forested sites but this difference wasn't enough to provide a significant difference in total SOC content when bulk density is factored in. We did find an increase in SOC content in the 3 lightest fractions (<1.65, 1.65-1.85, 1.85-2.0) of the Double Litter plots for both forested sites but the increases in the 1.85-2.0 fractions weren't enough to create an overall increase in the intermediate cycling C pool (1.85-2.4). The lack of increase in SOC concentration in the intermediate fraction could be due to priming effects increasing microbial respiration from those fractions. In current respiration dataset from the soils sampled in 2006, there were no significant differences in respiration between Control and Double Litter plots,

indicating that if priming was a contributing factor to SOC dynamics, it is no longer a significant factor. This means that we can't assume the priming observed at other DIRT sites will continue indefinitely.

In the prairie soils, we hypothesized that root inputs contribute more to C sequestration than do aboveground inputs (Rasse et al. 2005), and thus elimination of root inputs would have a stronger effect on C destabilization than would the elimination of aboveground litter. Although we do see a significant decrease in SOC concentration in No Root relative to No Litter and Control in 1997 and the difference is close to significant in 2006, the difference between No Root and No Litter disappear when we take bulk density into account and look at SOC content. The density fraction data shows that neither No Root nor No Litter are as depleted in SOC in the two lightest fractions (<1.65 and 1.65 to 1.85) as the same fractions in No Input.  $\delta^{13}\text{C}$  patterns, which are indicative of loss of fresh plant-derived C are similar for No Root and No Litter and are distinct from No Input for both Prairie sites.  $\Delta^{14}\text{C}$  patterns are also similar for No Root and No Litter and intermediate between Control and No Input. Overall, there is little to suggest that elimination of roots has more of an effect on SOC stabilization than aboveground litter in this restored prairie site after 50 years of treatment.

#### Limitations of this study

While I feel fortunate to have had the opportunity to work on the Francis Hole Experimental plots, figuring out how to best present the data and determining how far to take conclusions based on this data set has not been without challenge. The feature that contributes most to the strength of this study, its longevity, also leads to the

largest challenges. We had no control over the original experimental design, as it was put in place in 1956. We had to work with a lack of replication, there was only one plot of each treatment per site, which made statistical analysis more difficult and restricted the scope of our conclusions. Additionally, the study would have been more balanced if we had been able to explore the effect of root removal in the forested sites.

#### Future research in DIRT

The network of DIRT sites that have been established starting in 1990 have improved upon the study design and will enable researchers to explore SOM dynamics in a number of different ecosystems. These DIRT sites added plot trenching to allow for the analysis of the role of above- vs. below-ground detrital inputs on SOM stabilization. They create the opportunity to explore SOM stabilization mechanisms so as to better predict changes in C cycling due to climate change.

## Bibliography

- Battin, T. J., S. Luysaert, L. A. Kaplan, A. K. Aufdenkampe, A. Richter, and L. J. Tranvik. 2009. The boundless carbon cycle. *Nature Geoscience* **2**:598 - 600.
- Binkley, D., J. Aber, J. Pastor, and K. Nadelhoffer. 1986. Nitrogen Availability in Some Wisconsin Forests - Comparisons of Resin Bags and on-Site Incubations. *Biology and Fertility of Soils* **2**:77-82.
- Bruun, S., I. K. Thomsen, B. T. Christensen, and L. S. Jensen. 2008. In search of stable soil organic carbon fractions: a comparison of methods applied to soils labelled with <sup>14</sup>C for 40 days or 40 years *European Journal of Soil Science* **59** 247-256.
- Chung, H., K. J. Ngo, A. Plante, and J. Six. 2010. Evidence for Carbon Saturation in a Highly Structured and Organic-Matter-Rich Soil. *Soil Sci Soc Am J* **74** 130-138.
- Compton, J. E. and R. D. Boone. 2002. Soil nitrogen transformations and the role of light fraction organic matter in forest soils. *Soil Biology and Biochemistry* **34**:933-943.
- Diochon, A. C. and L. Kellman. 2009. Physical fractionation of soil organic matter: Destabilization of deep soil carbon following harvesting of a temperate coniferous forest. *J. Geophys. Res. - Biogeosciences* **114**:G01016
- Drake, J. E., B. A. Darby, M.-A. Giasson, M. A. Kramer, R. P. Phillips, and A. C. Finzi. 2013. Stoichiometry constrains microbial response to root exudation- insights from a model and a field experiment in a temperate forest. *Biogeosciences* **10**:821-838.

Ekschmitt, K., E. Kandeler, C. Poll, A. Brune, F. Buscot, M. Friedrich, G. Gleixner, A. Hartmann, M. Kästner, S. Marhan, A. Miltner, S. Scheu, and V. Wolters.

2008. Soil-carbon preservation through habitat constraints and biological limitations on decomposer activity. *Journal of Plant Nutrition and Soil Science* 171:27-35.

Fang, C., P. Smith, J. B. Moncrieff, and J. U. Smith. 2005. Similar response of labile and resistant soil organic matter pools to changes in temperature. *Nature* 433:57-59.

Fekete, I., Z. Kotroczó, C. Varga, P. T. Nagy, J. A. Tóth, R. D. Bowden, G. Várbíró, and K. Lajtha. 2013. Alterations in forest detritus input influence soil carbon concentration and soil respiration in a Central-European deciduous forest. *Soil Biology and Biochemistry* **in press**.

Field, C. B. and M. R. Raupach, eds. 2004. *The global carbon cycle: integrating humans, climate, and the natural world*. Island Press, Washington, D.C., 529 pp.

Foley, J.A. and N. Ramankutty. 2004. A primer on the terrestrial carbon cycle: What we don't know but should. In *The Global Carbon Cycle: Integrating Humans, Climate and the Natural World*. C.B. Field and M.R. Raupach, Editors. Island Press, Washington, D.C., 529 pp.

Fu, S., and W. Cheng. 2002. Rhizosphere priming effects on the decomposition of soil organic matter in C4 and C3 grassland soils. *Plant and Soil* 238:289-294.

Giardina, C. P. and M. G. Ryan. 2000. Soil warming and organic carbon content. *Nature* 408:789-790.

- Gomez-Casanovas, N., R. Matamala, D.R. Cook, and M.A. Gonzalez-Meler. 2012. Net ecosystem exchange modifies the relationship between the autotrophic and heterotrophic components of soil respiration with abiotic factors in prairie grasslands. *Global Change Biology* 18:2532-2545.
- Gottschalk, P., J. U. Smith, M. Wattenbach, J. Bellarby, E. Stehfest, N. Arnell, T. J. Osborn, C. Jones, and P. Smith. 2012. How will organic carbon stocks in mineral soils evolve under future climate? Global projections using RothC for a range of climate change scenarios. *Biogeosciences* 9:3151-3171.
- Hatton, P.-J., M. Kleber, B. Zeller, C. Moni, A. F. Plante, K. Townsend, L. Gelhaye, K. Lajtha, and D. Derrien. 2012. Transfer of litter-derived N to soil mineral-organic associations: evidence from decadal <sup>15</sup>N tracer experiments. *Organic Geochemistry* 42:1489–1501.
- Heimann, M. and M. Reichstein. 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* 451:289-292.
- Hoosbeek, M. R. and G. E. Scarascia-Mugnozza. 2009. Increased Litter Build Up and Soil Organic Matter Stabilization in a Poplar Plantation After 6 Years of Atmospheric CO<sub>2</sub> Enrichment (FACE): Final Results of POP-EuroFACE Compared to Other Forest FACE Experiments. *Ecosystems* 12:220-239.
- Hua, Q., and M. Barbetti. 2004. Review of Tropospheric Bomb <sup>14</sup>C Data for Carbon Cycle Modeling and age Calibration Purposes. *Radiocarbon* 46:1273-1298.
- Jandl, R., M. Lindner, L. Vesterdal, B. Bauwens, R. Baritz, F. Hagedorn, D. W. Johnson, K. Minkinen, and K. A. Byrne. 2007. How strongly can forest management influence soil carbon sequestration? *Geoderma* 137:253-268.

- Jobbágy, E. G. and R. B. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* **10**:423-436.
- John, B., T. Yamashita, B. Ludwig, and H. Flessa. 2005. Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. *Geoderma* **128**:63–79.
- Kaiser, K. and G. Guggenberger. 2003. Mineral surfaces and soil organic matter. *European Journal of Soil Science* **54**:219-236.
- King, A. W., W. M. Post, and S. D. Wullschlegel. 1997. The potential response of terrestrial carbon storage to changes in climate and atmospheric CO<sub>2</sub>. *Climatic Change* **35**:199-227.
- Kramer, M. G., J. Sanderman, O. A. Chadwick, J. Chorover, and P. M. Vitousek. 2012. Long-term carbon storage through retention of dissolved aromatic acids by reactive particles in soil. *Global Change Biology* **18**:2594-2605.
- Kucharik, C. J., N. J. Fayram, and K. N. Cahill. 2006. A paired study of prairie carbon stocks, fluxes, and phenology: comparing the world's oldest prairie restoration with an adjacent remnant. *Global Change Biology* **12**:122-139.
- Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science* **165**:382-396.
- Kuzyakov, Y., J. K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem* **32**:1485-1498.
- Lajtha, K., F. Peterson, K. Nadelhoffer, and R. Bowden. 2013. Twenty Years of Litter and Root Manipulations: Insights into Multi-Decadal SOM Dynamics *Soil*

Science Society of America Journal **in review**.

- Lal, R. 2010. Managing Soils and Ecosystems for Mitigating Anthropogenic Carbon Emissions and Advancing Global Food Security. *BioScience* **60**:708-721.
- Leifeld, J. and J. Fuhrer. 2005. The temperature response of CO<sub>2</sub> production from bulk soils and soil fractions is related to soil organic matter quality. *Biogeochemistry* **75**:433-453.
- Liao, J. D., T. W. Boutton, and J. D. Jastrow. 2006. Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. *Soil Biology and Biochemistry* **38**:3184-3196.
- McCune, B. and G. Cottam. 1985. The successional status of a southern Wisconsin oak woods. *Ecology* **66**(4):1270-1278.
- Liski, J., D. Perruchoud, and T. Karjalainen. 2002. Increasing carbon stocks in the forest soils of western Europe. *Forest Ecology and Management* **169**:159-175.
- Llorente, M., B. Glaser, and M. B. Turrión. 2010. Storage of organic carbon and Black carbon in density fractions of calcareous soils under different land uses. *Geoderma* **159**:31-38.
- Mayzelle, M. M., M. L. Krusor, R. D. Bowden, K. Lajtha, and J. W. Six. in review. The Effects of Decreasing Carbon Saturation Deficit on Temperate Forest Soil Carbon Cycling. *Soil Science Society of America Journal*.
- McFarlane, K. J., S. H. Schoenholtz, R. F. Powers, and S. S. Perakis. 2010. Soil Organic Matter Stability in Intensively Managed Ponderosa Pine Stands in California. *Soil Sci Soc Am J* **74**:979-992.
- McLauchlan, K. K. and S. E. Hobbie. 2004. Comparison of labile soil organic matter

- fractionation techniques. *Soil Science Society of America Journal* **68**:1616-1625.
- Melillo, J. M., A. D. McGuire, D. W. Kicklighter, B. I. Moore, C. J. Vorosmarty, and A. L. Schloss. 1993. Global climate change and terrestrial net primary production. *Nature* **363**:234-240.
- Nadelhoffer, K. J., R. D. Boone, R. D. Bowden, J. D. Canary, J. Kaye, P. Micks, A. Ricca, J. A. Aitkenhead, K. Lajtha, and W. H. McDowell. 2004. The DIRT experiment: litter and root influences on forest soil organic matter stocks and function. Pages 300-315 in D. Foster and J. Aber, editors. *Forests in Time: The Environmental Consequences of 1000 Years of Change in New England*. Yale University Press.
- Nielsen, G. A. and F. D. Hole. 1963. A study of the natural processes of incorporation of organic matter into soil in the University of Wisconsin Arboretum. *Wis. Acad. Rev.* **52**:213-227.
- Parfitt, R. L., A. A. Parshotam, and G. J. Salt. 2002. Carbon turnover in two soils with contrasting mineralogy under long-term maize and pasture. *Aust. J. Soil Res.* **40**:127-136.
- Paul, E. A., S. J. Morris, R. T. Conant, and A. F. Plante. 2006. Does the Acid Hydrolysis-Incubation Method Measure Meaningful Soil Organic Carbon Pools? *Soil Sci. Soc. Am. J.* **70**:1023-1035.
- Paul, K. I., P. J. Polglase, and G. P. Richards. 2003. Predicted change in soil carbon following afforestation or reforestation, and analysis of controlling factors by linking a C accounting model (CAMFor) to models of forest growth (3PG),

- litter decomposition (GENDEC) and soil C turnover (RothC). *Forest Ecology and Management* **177**:485-501.
- Plante, A. F., R. T. Conant, C. E. Stewart, K. Paustian, and J. Six. 2006a. Impact of Soil Texture on the Distribution of Soil Organic Matter in Physical and Chemical Fractions. *Soil Science Society of America Journal* **70**:287-296
- Plante, A. F., R. T. Conant, E. A. Paul, K. Paustian, and J. Six. 2006b. Acid hydrolysis of easily dispersed and microaggregate-derived silt- and clay-sized fractions to isolate resistant soil organic matter. *European Journal of Soil Science* **57**:456-467.
- Post, W. M., T.-H. Peng, W. R. Emanuel, A. W. King, V. H. Dale, and D. L. DeAngelis. 1990. The global carbon cycle. *American Scientist* **78**:310-326.
- Raich, J. W. and W. H. Schlesinger. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* **44**:81-99.
- Raich, J. W., A. E. Russell, K. Kitayama, W. J. Parton, and P. M. Vitousek. 2006. Temperature influences carbon accumulation in moist tropical forests. *Ecology* **87**:76-87.
- Reimer, P.J., T.A. Brown, and R.W. Reimer. 2004. Reporting and Calibration of Post-Bomb <sup>14</sup>C Data. *Radiocarbon* **46**:1299-1304.
- Richter, D. D., D. Markewitz, S. E. Trumbore, and C. G. Wells. 1999. Rapid accumulation and turnover of soil carbon in a re-establishing forest. *Nature* **400**:56-58.
- Schlesinger, W. H. and J. A. Andrews. 2000. Controls on Soil Respiration: Implications for Climate Change *Biogeochemistry* **48**:7-20.
- Six, J., R. T. Conant, E. A. Paul, and K. Paustian. 2002. Stabilization mechanisms of

soil organic matter: Implications for C-saturation of soils. *Plant and Soil* **241**:155 - 176.

- Sollins, P., C. Swanston, M. Kleber, T. Filley, M. Kramer, S. Crow, B. Caldwell, K. Lajtha, and R. Bowden. 2006. Organic C and N stabilization in a forest soil: evidence from sequential density fractionation. *Soil Biology and Biochemistry* **38**:3313-3324.
- Sollins, P. C. Swanston, M. Kramer. 2007. Stabilization and destabilization of soil organic matter- a new focus. *Biogeochemistry* **85**:1-7.
- Sollins, P., M. G. Kramer, C. Swanston, K. Lajtha, T. Filley, A. K. Aufdenkampe, R. Wagai, and R. D. Bowden. 2009. Sequential density fractionation across soils of contrasting mineralogy: evidence for both microbial- and mineral-controlled soil organic matter stabilization *Biogeochemistry* **96**:209-231.
- Stewart, C. E., K. Paustian, R. T. Conant, A. F. Plante, and J. Six. 2009. Soil carbon saturation: Implications for measurable carbon pool dynamics in long-term incubations. *Soil Biology & Biochemistry* **41** 357-366.
- Stuiver, M., and H.A. Polach. 1977. Reporting of C-14 Data. *Radiocarbon* 19:355-363.
- Stuiver, M., P.J. Reimer, and T.F. Braziunas. 1998. High-precision radiocarbon age calibration for terrestrial and marine samples. *Radiocarbon* 40:1127-1151.

## Appendix

### Francis Hole Plot Site and Treatment Information from an undated typed document on file at the University of Wisconsin Arboretum

Curtis Prairie is a 60 acre restored prairie with three sets of experimental plots within it. Site 1 is located at the eastern end, Site 2 is just west of Site 1 and Site 3 is located at the western end of the prairie.

There are 5 plots, one each of four different treatments and one control. Plots B-E form a block while plot A is slightly removed from the other plots.

All the plots in Site 1 are 12' by 12'. In Site 2, plot A is 12' by 24' and the rest of the plots are 12' by 12'.

The management regimes are identical for the two sites and are as follows according to an undated report entitled "Maintenance of long-term soil study plots":

- Burned- This plot is burned whenever the prairie is burned.
- No Input 'bare'- Kept bare and free of all vegetation by light hoeing (or pulling), avoiding any mixture of organic matter into the soil. Rake off plant material. Hoeing every two weeks, April 1 to November 15. Not burned
- No Roots 'mulched'- All living plants removed by same light hoeing as B, but left in mulch. Harvested plant material from D spread over this plot evenly every fall (late October, before frost). Not burned
- No Litter 'harvested'- Entire plot mowed in late October, harvest raked up and transferred evenly to plot C. Not burned
- Control- No treatment except that not more than one seeded in-tree should be permitted at a time. The older of any two should be removed. Not burned

Site 4 is Noe woods, a 10 hectare mixed deciduous forest. There are 4 plots with 4 management regimens. A, B and D are 12' by 12'. C is 3' by 9'. The Control plot is referred to as "any quadrat elsewhere nearby". The treatments are as follows:

- OAless 'A-1'- soil layer removed in 1956. No further treatment.
- Bare- Hoe shallowly, like B plots in the prairie, to keep free of vegetation. Rake off plant material. Avoid mixing organic matter into soil.
- Double litter- Each fall (first half of November) add fresh litter collected from three 9 ft<sup>2</sup> quadrants elsewhere evenly over this plot (leaves, twigs, acorns; avoid old decaying litter lying below). 3 screens one yard square can be laid out to collect the fresh litter if desired. (not entirely clear here, three quadrants added in...)
- No litter 'leafless'- Should be raked free of leaves every two weeks from April 1 to November 15

Site 5 is Wingra woods, a 20 hectare mixed deciduous forest. The management regimen is the same as for Noe woods, with the side note that growing

vegetation, including moss, should be allowed to remain in D, the leafless plot. There is an additional note that the Double litter plot must be reestablished the summer of 1974.

Included was an annual work program for 2 men.

April 1 to November 1: every two weeks

- Hoe and weed B plots in prairie (1,2,3)
- Hoe and weed C (mulched) plots in 1, 2, 3 (leaving mulch as intact as possible).
- Rake C (leafless) plots in Site 4 (Noe) and 5 (Wingra)
- Hoe and weed B in Noe and Wingra

Early November (or late October)

- Harvest D plots in Sites 1, 2,3
- Rake up harvest and transfer harvest from plots D to C plots in 1,2,3
- Gather new litter from 3 sq yards of woods and add evenly to C plots in Noe and Wingra