

Changes to particulate versus mineral-associated soil carbon after 50 years of litter manipulation in forest and prairie experimental ecosystems

Kate Lajtha · Kimberly L. Townsend ·
Marc G. Kramer · Christopher Swanston ·
Richard D. Bowden · Knute Nadelhoffer

Received: 28 September 2013 / Accepted: 13 February 2014 / Published online: 2 March 2014
© Springer International Publishing Switzerland 2014

Abstract Models of ecosystem carbon (C) balance generally assume a strong relationship between 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 mixed deciduous forest and in 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 %) compared to increases in 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 forested 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 % decline in mineral-associated carbon in the intermediate (1.85–2.4 g cm⁻³) density pool; isotopic data suggest that it was preferentially younger C that was lost. 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

Responsible Editor: Sharon A. Billings

K. Lajtha (✉)
Department of Crop and Soil Sciences, Oregon State
University, Corvallis, OR 97331, USA
e-mail: lajthak@science.oregonstate.edu;
kate.lajtha@oregonstate.edu

K. L. Townsend
Department of Crop and Soil Sciences, Oregon State
University, Corvallis, OR 97331, USA

M. G. Kramer
Soil and Water Science Department, University of
Florida, Gainesville, FL 32611, USA

C. Swanston
Northern Research Station, USDA Forest Service,
Houghton, MI 49931, USA

R. D. Bowden
Department of Environmental Science, Allegheny
College, Meadville, PA 16335, USA

K. Nadelhoffer
Department of Ecology and Evolutionary Biology,
University of Michigan, Ann Arbor, MI 48109, USA

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 labile C did not change. In prairie soils, $\delta^{13}\text{C}$ values suggest a loss of recent C₄-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 vulnerable to significant C loss 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.

Keywords Carbon sequestration · Carbon stabilization · Density fractionation · Detrital manipulation treatments · DIRT · Forest · Prairie · Radiocarbon dating · Soil organic matter · SOM

Introduction

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), constitutes approximately two-thirds of the terrestrial C pool, and is estimated to be about 2,300 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 CO₂ so the balance is nearly equal (Schlesinger and Andrews 2000; Trumbore 2006). SOM can follow a number of trajectories as it is processed within soil: it can be stabilized, leached to surface waters as dissolved organic matter (DOM), transformed (e.g. into microbial biomass, modified or cleaved by enzymes, partially degraded), or mineralized by microbes and returned to the atmosphere as CO₂. 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; Schmidt et al. 2011). 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 biochemical composition of litter inputs, but the resulting effects on SOM stability and turnover cannot now be predicted accurately.

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. 2006; 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. Changes in net primary productivity (NPP) and thus litterfall are predicted in many ecosystems with climate change (Melillo et al. 1993; King et al. 1997; Raich et al. 2006), but it is not clear if there will be parallel changes in SOM stores. 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. However, many factors could cause non-linearities in the relationship between litter inputs and C sequestration in soil. 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 biochemical composition and quantity of C inputs. 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’ (Kuzakov et al. 2000), and thus enhanced microbial respiration in response to additional plant litter inputs—or root activity—could increase destabilization of stored SOM, paradoxically decreasing C sequestration in soil.

Here we assess soil carbon loss or gain due to a series of long-term (50 year) litter removal and addition treatments in the Detrital Input and Removal Treatment (DIRT) plots established by Dr. Francis

Hole in the University of Wisconsin Arboretum in 1956. The experimental plots that we used are located in two oak dominated mixed deciduous forests and two restored prairie ecosystems, and involve manipulations of either detrital leaf or prairie grass litter. The forested plot manipulations included aboveground litter doubling and removal; the prairie manipulations included aboveground litter removal, aboveground-only litter additions (i.e. no belowground inputs), and no above or belowground inputs. Our objectives were to (1) quantify bulk soil C changes after 50 years of litter manipulation and (2) determine changes in mineral and particulate C pools and the biochemical characteristics of these pools after 50 years of treatment. 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 on older SOM pools, would have ended. We also hypothesized that effects of C additions would be seen only in free, light fraction pools (sensu Sollins et al. 2009) and that mineral and aggregate-C would be relatively resistant to litter additions, or saturated, even over decadal time spans. In prairie soils, we hypothesized that root inputs and aboveground litter inputs would contribute equally to soil C. Although other studies have suggested that roots contribute more to stabilized SOM than does aboveground litter (Rasse et al. 2005), we based our hypothesis on the idea that root activity, such as the exudation of relatively labile organic acids, could cause substantial priming of older SOM (Sulzmann et al. 2005) and thus could cause a decrease in total SOM content which would not be detectable with analyses of SOM biochemical composition or biomarker analysis. We used $\delta^{13}\text{C}$ and ^{14}C isotopic analysis coupled with incubation experiments and sequential density fractionation to investigate the extent to which litter inputs and removals are coupled with SOM stocks and concentrations, and use turnover of different pools to help understand the observed changes.

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 two prairie (Curtis Prairie 1 and 3, 43.038N, –89.431E) sites within the University of Wisconsin Arboretum. Mean annual precipitation for the UW Arboretum is 928 mm, with a mean annual minimum temperature of 0.7 °C and mean annual maximum temperature of 14.2 °C (Kucharik et al. 2006).

Wingra Woods is a 52 acre deciduous forest dominated by mixed oaks (*Quercus alba* and *Q. velutina*) and underplanted with sugar maple (*Acer saccharum*), basswood (*Tilia americana*) and beech (*Fagus grandifolia*). Other typical northern species were planted to make the area resemble the sugar maple forests of northern Wisconsin. Noe Woods is a 41 acre forest also dominated by mixed oaks, typical of a forest that developed on former savanna sites. Most of the larger oaks were about 150 years old in 2006. 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). The Curtis Prairie sites are restored prairies dominated by big bluestem, *Andropogon gerardi*, and Indiangrass, *Sorghastrum nutans*, 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 forest and prairie manipulations were established in 1956, coincidentally just prior to the steep rise in “bomb carbon” ^{14}C signal in the atmosphere, and are described in greater detail in Nielsen and Hole (1963). Mean soil pH in the control plots was 4.9, but was greater in litter addition plots (5.9). Mean soil pH was 5.3 in the prairie surface soils.

Treatments consisted both litter additions and removals (Table 1). Forested plots were subject to three treatments that were applied annually: Control, No aboveground Litter (and thus C inputs to soil are only from roots), and Double (aboveground) Litter. Roots were intact in all plots. In prairies, the 4 treatments were Control, No aboveground Litter (and thus C inputs to soil are only from roots), No Roots (and thus C inputs to soil are only from aboveground litter), and No Inputs (both above- and below-ground inputs excluded).

Sample collection and handling

Soils were previously collected from plots in 1984 and 1997 and archived at the University of Michigan.

Table 1 Treatment and methods of the DIRT plots installed at the Wisconsin Arboretum in 1956

	Treatment	Method
Forest	Control (CTL)	“Undisturbed” ^a plots with normal above and below ground litter inputs allowed.
	Double Litter (DL)	“Litter doubled” ^a by adding aboveground litter inputs removed annually from No Litter plots.
	No Litter (NL)	“Litter continually removed” ^a (annually) by raking aboveground inputs and proportionately redistributing to Double Litter plots.
Prairie	Control (CTL)	“Undisturbed” ^a plots with normal above and below ground litter inputs
	No Input (NI)	Vegetation aboveground is “clipped frequently” ^a and removed, thus minimizing both above and belowground production. Encroachment is precluded with metal barriers 15 cm above ground and 30 cm belowground.
	No Litter (NL)	Vegetation is “harvested annually” ^a from plots in autumn, leaving roots intact
	No Root (NR)	Vegetation aboveground is “clipped frequently” and removed, thus minimizing both above and belowground production on site. Aboveground litter (mulch) is transferred annually from No Litter treatments, in autumn. Encroachment is precluded with metal barriers 15 cm above ground and 30 cm belowground. Originally designated as “clipped and mulched” ^a

^a Official plot designations given in Nielsen and Hole (1963)

These soils were analyzed for total C and N only. In July of 2006, soils were collected from each of the 4 sites (2 forest, 2 prairie) to mark the 50th anniversary of the experiment. Within each site, we randomly established four 3 × 3 m subplots that were at least 0.5 m from a plot edge. Within each of the four subplots, the O horizon was removed and two replicate mineral soil samples were collected to a depth of 10 cm with a hammer soil corer with a 6.25 cm diameter. These two soil cores were combined within each subplot to yield four soil samples per experimental plot. Samples were sieved to 2 mm and homogenized. Field moist soil was used for laboratory incubations, and the remainder was air dried.

Incubation experiments

The cumulative respiration rate in a 28 day laboratory incubation was used to estimate the size of a labile or bioavailable carbon pool (McLauchlan and Hobbie 2004). Field-moist, 30 g soil samples from the 2006 collection were measured into 50 ml Erlenmeyer flasks. Samples were stored in an incubator at 25 °C with open containers of water to maintain humidity. After a two day equilibration period, respiration rates were measured daily for a week, then every 4 days. Flasks were stoppered and initial headspace samples were taken using a 1 mL-calibrated syringe. Flasks were then left stoppered, allowing CO₂ to accumulate for at least an hour before a final gas sample was taken. Gas samples were analyzed on a Hewlett Packard 5700A gas chromatograph fitted with a 2 m Poropak R 80/100 column and thermal conductivity detector. Small aliquots were dried at 60 °C to correct for initial water content.

Sequential density fractionation

Soil subsamples were composited from within each field site and sequentially fractionated by density using sodium polytungstate following Sollins et al. (2006). Target fractions were <1.65, 1.65–1.85, 1.85–2.0, 2.0–2.2, 2.2–2.4, 2.4–2.65, and >2.65 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.

δ¹³C isotopic analysis

Carbon and N concentrations and δ¹³C 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 mm) were ground finely with a zirconium mortar and pestle, and loaded into tin boats. ¹³C 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 δ¹³C. 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 bulk soils and density fractions 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 900°C for 4 h to convert the organic C to CO₂. The CO₂ was subsequently isolated, added to a reaction chamber, and reduced with excess H and a conditioned iron catalyst at 550°C for up to 6 h. 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, Livermore, CA, USA. 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 (¹⁴C:¹²C 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 ¹⁴C data involves so-called “bomb ¹⁴C.” Bomb ¹⁴C was generated in the 1950s and 1960s from above-ground thermonuclear testing, which roughly doubled the amount of ¹⁴C in the atmosphere (Hua and Barbetti 2004; Reimer et al. 2004). This elevated atmospheric ¹⁴C was subsequently incorporated into carbon reservoirs such as vegetation and soils. Samples that contain substantial bomb ¹⁴C will have $\Delta^{14}\text{C}$ values above 0 ‰, whereas samples with values near or below 0 ‰ are dominated by pre-bomb ¹⁴C and have either incorporated little or no carbon from the atmosphere since 1950 (e.g., Torn et al. 2009) or else have preferentially lost bomb carbon. The atmospheric $\Delta^{14}\text{C}$ value in 1956 was 38 ‰, which was after ¹⁴C had begun to increase but well below the average

Table 2 SOC concentration and content and bulk density, in forested DIRT plots at the Wisconsin Arboretum, sampled in 2006 after 50 years of detrital manipulation

	Control	Double Litter	No Litter
SOC concentration (mg C g ⁻¹ soil)			
Noe			
Mean (SE)	34.3 (1.4) ^a	47.4 (4.3) ^b	15.6 (1.8) ^c
% difference from control		+37	−55
Wingra			
Mean (SE)	28.4 (2.8) ^a	38.9 (1.3) ^b	12.9 (1.5) ^c
% difference from control		+37	−54
Bulk Density (g cm ⁻³)			
Noe			
Mean (SE)	1.05 (0.02) ^a	0.90 (0.02) ^b	1.23 (0.05) ^c
Wingra			
Mean (SE)	1.04 (0.03) ^a	1.01 (0.03) ^a	1.37 (0.02) ^b
SOC content (g C m ⁻²)			
Noe			
Mean (SE)	3,634 (152) ^a	4,694 (137) ^a	1,914 (219) ^b
% difference		+29	−47
Wingra			
Mean (SE)	2,958 (295) ^a	3,925 (133) ^a	1,768 (203) ^b
% difference		+33	−40

Values are means of four 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

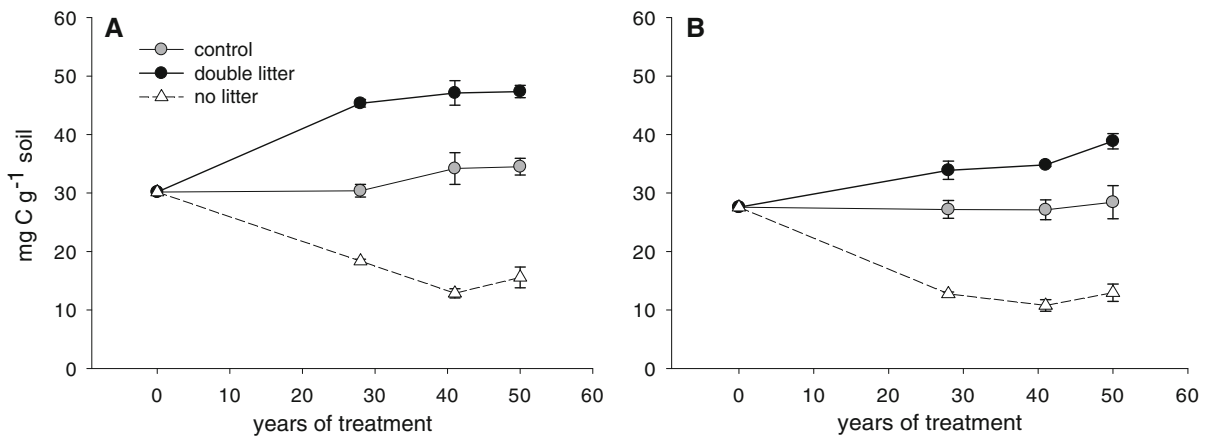


Fig. 1 SOC concentrations for Noe Woods (a) and Wingra Woods (b) in the Wisconsin DIRT plots in 1984, 1997 and 2006. Values are means $\pm$ 1 standard error, $n = 4$. Significant

differences in values between treatments within a site in 2006 are shown in Table 2. When SE bars are not shown it is because the SE was smaller than the symbol

Northern Hemisphere peak of 933 ‰ in 1963 (Hua and Barbetti 2004). Because all experimental plots began with low ^{14}C values in soil, bomb ^{14}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 soil organic carbon (SOC) dynamics.

Statistical analysis

For total C and N from 1984, 1997 and 2006 and respiration data from 2006, means per treatment are comprised of 4 replicate samples per experimental plot. For these datasets one-way ANOVA, using SigmaPlot version 12 (Systat Software, Inc., San Jose, CA, USA), 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 $p = 0.05$.

Due to budgetary constraints we combined subsamples into one homogenous sample per experimental plot for density fractionation. Thus $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$ on fractions consist of single values, so it was not possible to run statistical tests. Total C values were pooled within ecosystems by treatment (e.g., data for Noe and Wingra Woods were averaged for each experimental treatment), however, with $n = 2$ we were not able to run statistical tests. The numbers in bold in Table 4, then, represent where both sites comprising the mean followed the same trend of either increase or decrease in SOC relative to the Control.

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 (Fig. 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 (data in see Table 6 in Appendix). 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 versus No Root plots. No Input plots lost 69–71 % of total soil C content after 50 years. Soil N followed patterns of soil C, although

Table 3 SOC concentration and content in prairie DIRT plots at the Wisconsin Arboretum, sampled in 1997 and 2006 after 41 and 50 years of detrital manipulation

	Control	No Input	No Roots	No Litter
1997 Curtis Prairie 1				
SOC concentration (mg C g ⁻¹ soil)				
Mean (SE)	21.2 (0.9) ^a	8.7 (0.3) ^b	15.9 (1.9) ^c	22.0(1.0) ^a
% difference		-59	-57	+4
Bulk Density (g cm ⁻³)				
Mean (SE)	1.11 (0.01) ^a	1.20 (0.02) ^{ab}	1.31 (0.01) ^b	0.96 (0.02) ^c
% difference				
SOC content (g C m ⁻²)				
Mean (SE)	2,349 (65) ^a	1,049 (28) ^b	2,067 (123) ^a	2,114 (50) ^a
% difference		-55	-25	-10
2006 Curtis Prairie 1				
SOC concentration (mg C g ⁻¹ soil)				
Mean (SE)	27.4 (1.9) ^a	7.9 (0.5) ^b	16.1 (1.7) ^c	21.0 (0.5) ^c
% difference		-71	-41	-24
SOC content (g C m ⁻²)				
Mean (SE)	3,030 (204) ^a	949 (65) ^b	2,098 (225) ^c	2,014 (52) ^c
% difference		-69	-31	-34
2006 Curtis Prairie 3				
SOC concentration (mg C g ⁻¹ soil)				
Mean (SE)	24.6 (0.8) ^a	10.8 (0.5) ^b	18.0 (1.6) ^c	24.9 (0.6) ^a
% difference		-56	-28	+1
SOC content (g C m ⁻²)				
Mean (SE)	2,720 (90) ^a	1,304 (62) ^b	2,295 (210) ^a	2,389 (57) ^a
% difference		-52	-16	-12

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

values were more variable (data in see Table 7 in Appendix).

Incubation experiments

Cumulative respiration values over the 28 day incubation in the forest sites ranged from 28.8 $\mu\text{g C g}^{-1}$ soil to 132.1 $\mu\text{g C g}^{-1}$ soil, or 1.9 mg C g⁻¹ C in soil to 3.1 mg C g⁻¹ C in soil (Fig. 2). On a per g soil basis, Double Litter respiration values were higher than Control, and No Litter values were lower than Control. However, when respiration was normalized on a per g soil C basis, these differences largely disappeared. Cumulative respiration values in the prairie soils ranged from 22.1 $\mu\text{g C g}^{-1}$ soil to 96.7 $\mu\text{g C g}^{-1}$ soil, or 2.8 mg C g⁻¹ C in soil to 3.4 mg C g⁻¹ C in soil. On a per gram soil basis in the prairie soils, 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.

Sequential density fractionation

Mean soil mass recoveries after sequential density fractionation across ecosystems and treatments were high (96–100 %) and total C recovery averaged 84 % (± 1.7 SE; data in see Table 8 in Appendix). In all treatments the majority (69–78 %) of soil material was in the 2.4–2.65 g cm³ range. However, the proportion of C was distributed across the 3 fractions that comprise the 1.65–2.65 g cm⁻³ range more uniformly, with no one of these density fractions containing more than 29 % of the total C. Data were analyzed both as

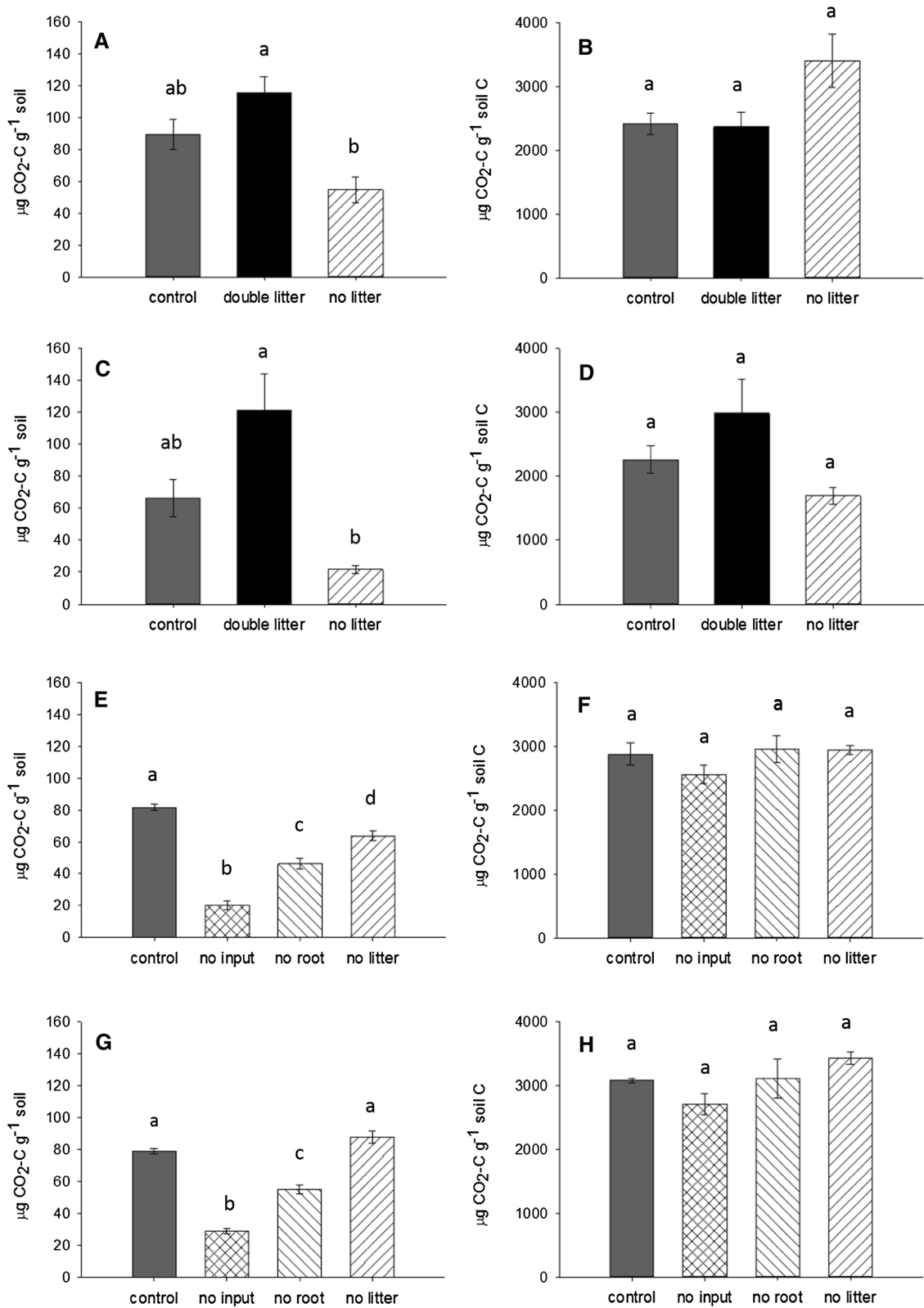


Fig. 2 Cumulative respiration after a 28 day incubation of bulk soil by treatment from Noe Woods (a, b), Wingra Woods (c, d), Curtis Prairie 1 (e, f), and Curtis Prairie 3 (g, h). Values are means $\pm$ 1 standard error, $n = 4$, letters indicate significant differences between means (p value ≤ 0.05). 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 $\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil C}$

separate fractions, and also grouped into “functional” categories following Hatton et al. (2012): light ($<1.85\text{ g cm}^{-3}$ density fractions), aggregate (>1.85 to $<2.4\text{ g cm}^{-3}$ fractions), and mineral ($>2.4\text{ g cm}^{-3}$) fractions.

In the forest sites, C in the two lightest density fractions (from <1.65 to 1.85 g cm^{-3}) were significantly higher in Double Litter plots compared to Controls and were lower in the No Litter plots (Fig. 3; Table 4). In both forested plots, the $1.85\text{--}2.0\text{ g cm}^{-3}$ fraction was significantly greater in Double Litter plots than Controls (Fig. 3), and although the next two density fractions ($2.0\text{--}2.2$, $2.2\text{--}2.4\text{ g cm}^{-3}$) 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 4). We infer that C additions to particles in this aggregate pool caused slightly heavier particles ($>2.0\text{--}2.4\text{ g cm}^{-3}$) to become lighter and thus be captured in the $1.85\text{--}2.0\text{ g cm}^{-3}$ pool.

In the prairie sites, C in the four lightest density fractions (from <1.65 to 2.2 g cm^{-3}) were highest in control plots and were lowest in the No Input plots (Fig. 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 forested plots with litter removed had less negative, or heavier, $\delta^{13}\text{C}$ values than control or Double Litter plots across density fractions, particularly in the heaviest fractions (Fig. 4). Forested soils had a positive trend in $\delta^{13}\text{C}$ with particle density although with a decline at the heaviest

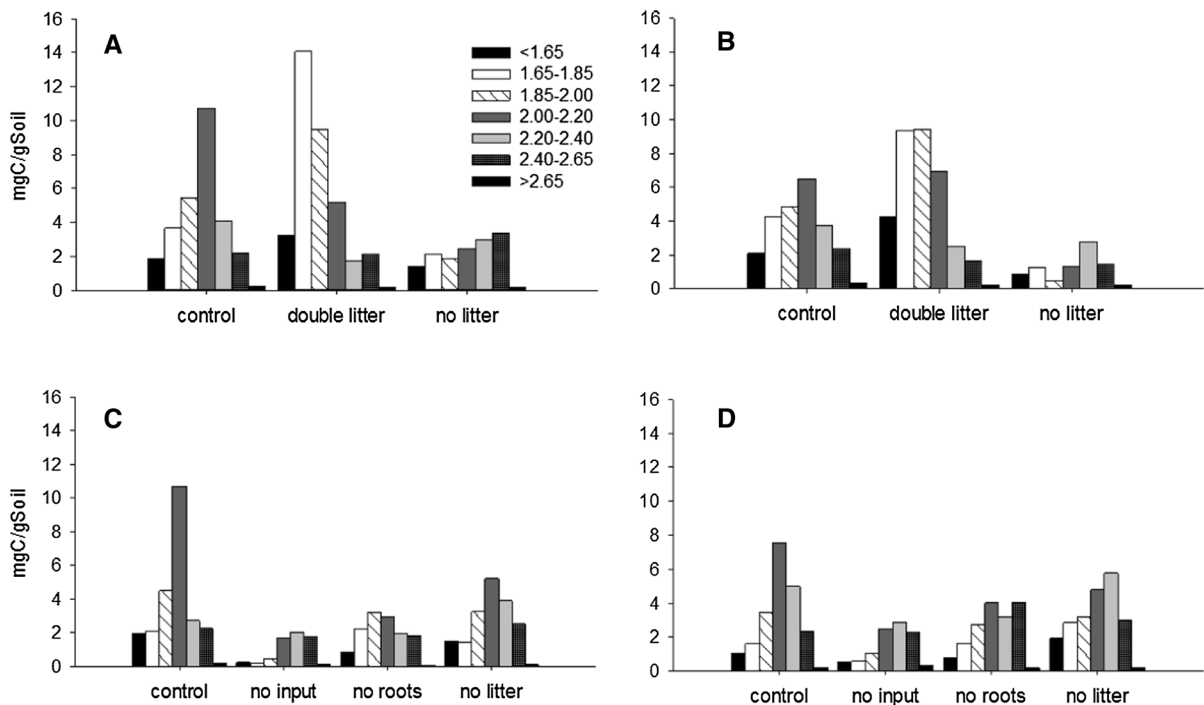


Fig. 3 SOC concentration in density fractions of Noe Woods (a), Wingra Woods (b), Curtis Prairie 1 (c), and Curtis Prairie 3 (d) plots in the Wisconsin DIRT plots after 50 years. Values are individual data points

Table 4 SOC concentration changes (%) in the Wisconsin DIRT plots relative to control plots by density fraction

Site/treatment	Light (OM) fraction	Intermediate (aggregate) fraction	Heavy fraction
Prairie removal (NI)	-74 (11)	-68 (6)	-11(9)
Prairie removal (NR)	-16 (6)	-46 (6)	20 (30)
Prairie removal (NL)	26 (38)	-23 (6)	15 (5)
Forest removal (NL)	-51 (11)	-67 (2)	4 (30)
Forest addition (DL)	164 (35)	3 (15)	-18 (9)

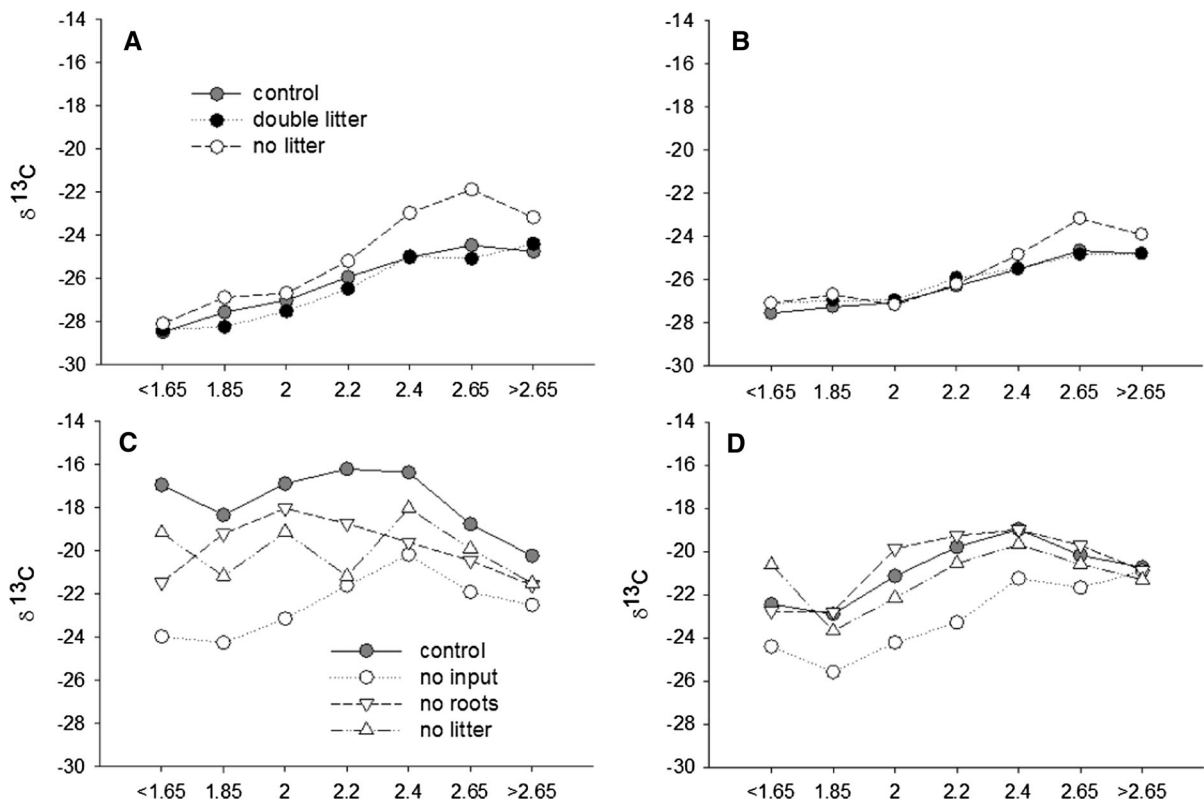
Individual fractions were pooled into three categories: Light fraction ($<1.85 \text{ g cm}^{-3}$), Intermediate fraction ($1.85\text{--}2.4 \text{ g cm}^{-3}$), and Heavy fraction ($>2.4 \text{ g cm}^{-3}$). Values are mean (SE), $n = 2$. Values in bold indicate treatments where both samples (one per site) followed the same pattern compared to control values

fraction. The prairie sites had an elliptical pattern in $\delta^{13}\text{C}$ with particle density, with maximum $\delta^{13}\text{C}$ values in intermediate density fractions and lower $\delta^{13}\text{C}$ values

in the lightest and heaviest fractions, and were more enriched in ^{13}C than the forested sites.

Radiocarbon in bulk soils and soil fractions

In general, C concentration in bulk soils was strongly related to ^{14}C abundance, indicating a positive relationship between increasing C stores and higher proportions of more recent C (Fig. 5). In the one forest (Noe Woods) where $\Delta^{14}\text{C}$ was measured, bulk soils and density fractions from the No Litter plots had $\Delta^{14}\text{C}$ values between 35–90 ‰ lower than those of the control (Table 5). In the prairie sites, control soils had also clearly accumulated bomb ^{14}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, consistent with the interpretation that 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 ‰.

**Fig. 4** $\delta^{13}\text{C}$ values 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 Wisconsin DIRT plots. Values are individual data points

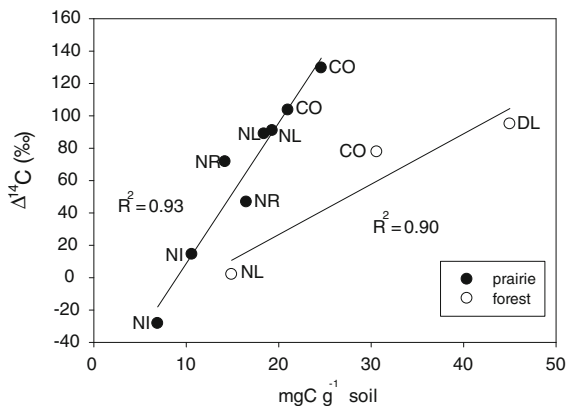


Fig. 5 Relationship between $\Delta^{14}\text{C}$ and SOC concentration of bulk soil for the two Curtis Prairie sites and Noe Woods. Each point is one composite sample. NI no input, NR no root, NL no litter, CO control

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, consistent with an interpretation that less carbon in that fraction was incorporated since 1950. Another interpretation could be that there was a greater efflux of “new” C from this pool, but this seems highly unlikely. The next heaviest fraction ($>2.2\text{--}2.4 \text{ g cm}^{-3}$) 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}C . The patterns of ^{14}C abundance in density fractions from the Noe Forest control and No Litter treatments were similar, whereas ^{14}C abundance with increasing density followed a different pattern in the Double Litter fractions. The ^{14}C of some density fractions ($1.65\text{--}1.85$ and $1.85\text{--}2.0 \text{ g cm}^{-3}$) appeared higher than the corresponding fractions from the Control, whereas the heaviest three fractions appeared somewhat lower. Most litter removal fractions at $>1.65\text{--}1.85 \text{ g cm}^{-3}$ had markedly depleted signals in the prairie No Inputs plots, likely indicative of charcoal incorporation from historic burning. Although most plots trended to have depleted signals in the $>1.65\text{--}1.85 \text{ g cm}^{-3}$ fraction, this effect was partly masked by addition of bomb ^{14}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 (Fig. 5) and mineral fractions (Fig. 6) in treatment plots for ^{14}C values relative to the control values. Bulk carbon loss in litter removal plots

trended strongly with ^{14}C changes ($r^2 = 0.95, p < 0.01$). The magnitude of carbon loss in the $1.8\text{--}2.4 \text{ g cm}^{-3}$ mineral fraction corresponded to increasingly negative ^{14}C values relative to the control ($r^2 = 0.98, p < 0.01$) 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 ($6 \pm 36 \%$ standard deviation). There was an increase of carbon in this fraction due to some of the detrital manipulations, as well as a decrease. A strong relationship between the amount of C decline and the change in ^{14}C was found in this fraction (Fig. 6) ($r^2 = 0.44, p < 0.01$).

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 no significant change in C content after 50 years of litter addition. Thus our results are consistent with our initial hypothesis suggesting that either 50 years is too short a time frame to see changes in mineral soil C, or else that surface ($0\text{--}10 \text{ cm}$) mineral C pools are saturated, and thus may never increase in response to increased litter inputs.

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; Crow et al. 2009a; Lajtha et al. 2013; Bowden et al. 2014; Fekete et al. 2014), and thus either no increase or slight decreases in both total and labile C. Priming is 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. Further analyses of other DIRT sites can indicate when accumulation of litter inputs becomes greater than the priming of older SOM in forested ecosystems.

The apparent increase in ^{14}C abundance in the light ($<1.85 \text{ g cm}^{-3}$) and first intermediate ($1.85\text{--}2.0 \text{ g cm}^{-3}$) mineral density fractions from the Noe Woods Double Litter corresponded with large increases in the C concentration: 164 % in the $1.65\text{--}1.85 \text{ g cm}^{-3}$ fraction and 175 % in the $1.85\text{--}2.00 \text{ g cm}^{-3}$ fraction, which we interpret to mean that these fractions received higher proportions of more recent bomb ^{14}C relative to the

Table 5 $\Delta^{14}\text{C}$ in ‰ (SE) of bulk soil and density fractions for the DIRT plots in Curtis Prairie and Noe Woods

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

The reported values represent individual analytical samples

^a Sample not run

Control. These two 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}C in those fractions. It is possible that even as increased C inputs from litter were accumulating in the light (<1.85 g cm^{−3}) fractions, these C inputs were resulting in priming effects in the denser fractions (Kuziyakov 2002), reducing the net accumulation of C and more recent bomb ^{14}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^{−3}) 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 g cm^{−3}) 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 not significantly affected by increased litter inputs.

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 g m^{−2} year^{−1} averaged across both forests, Double Litter plots accumulated about 5 %

of total litter added to the plots over 50 years, primarily in the lightest fractions that are subject to rapid turnover if environmental conditions were to change.

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 the intermediate ($1.8\text{--}2.4\text{ g cm}^{-3}$), aggregate fraction were equal to declines in the light fraction. In contrast, there was no consistent trend in the heaviest ($>2.4\text{ g cm}^{-3}$) pool of mineral-stabilized C. 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. Diochon 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 review) attributed this C decline to mineral-associated C loss.

Loss in C content in the $1.8\text{--}2.4\text{ g cm}^{-3}$ fraction of litter removal plots compared to controls were strongly correlated to declines in $\Delta^{14}\text{C}$ (Fig. 6). An increase in $\Delta^{14}\text{C}$ would have indicated incorporation of atmospheric bomb C, which was about 38 ‰ $\Delta^{14}\text{C}$ in 1956 at plot establishment and rose rapidly to $>900\text{ ‰}$ by 1964. Conversely, a decline in $\Delta^{14}\text{C}$ indicates no incorporation, or else preferential loss, of this high- $\Delta^{14}\text{C}$ bomb C. In the $>2.4\text{ g cm}^{-3}$ fraction, small declines in C content resulted in greater ^{14}C isotope changes in the residual material than the $1.8\text{--}2.4\text{ g cm}^{-3}$ fraction. The results suggest that treatments resulted in a proportionally greater loss of pre-bomb carbon, or a lack of incorporation of bomb ^{14}C , into the $>2.4\text{ g cm}^{-3}$ density fraction per unit of C loss. This is consistent with the idea

that the densest fractions engage in less carbon exchange, with proportionately more of the organic matter in a particle having direct, stable mineral interaction (Sollins et al. 2009). Likewise, the lighter mineral fractions, with greater overall carbon loading and lower proportional direct mineral interaction, could be expected to engage in much more dynamic carbon exchange with correspondingly greater bomb ^{14}C accumulation. Although these processes are clearest when comparing the Controls and treatments, they are also clearly visible in the control data alone (Table 5). Differences in ^{13}C values between the $1.8\text{--}2.4\text{ g cm}^{-3}$ fraction and the $>2.4\text{ g cm}^{-3}$ fraction further support this interpretation (Fig. 7) since the denser mineral fraction has a ^{13}C isotope signature more consistent with forest (pre-conversion to prairie). An apparent inconsistency with the idea of a stable dense fraction is the gain in carbon content and mass in the $>2.4\text{ g cm}^{-3}$ we observed relative to the control (Fig. 6). One possible explanation is that as the lighter mineral fraction ($1.85\text{--}2.4\text{ g cm}^{-3}$) lost carbon from its organo-mineral particles, these particles became denser ($>2.4\text{ g cm}^{-3}$), explaining both the loss from the $1.85\text{--}2.4\text{ g cm}^{-3}$ fraction as well as the gain in carbon content and mass in the $>2.4\text{ g cm}^{-3}$ fraction.

Patterns of $\delta^{13}\text{C}$ across density fractions in both forest and prairie soils followed those observed in other soils compiled in a larger, global dataset (Throop et al. 2013; Fig. 7). As has been observed in other forested

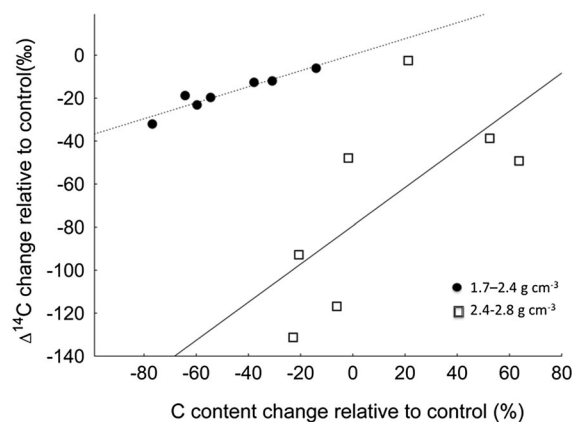


Fig. 6 Relationship between C content change relative to control (%) and $\Delta^{14}\text{C}$ (‰) change relative to control (‰) in two density fraction pools for all litter removal treatments in Noe Forest and the two Curtis Prairie sites

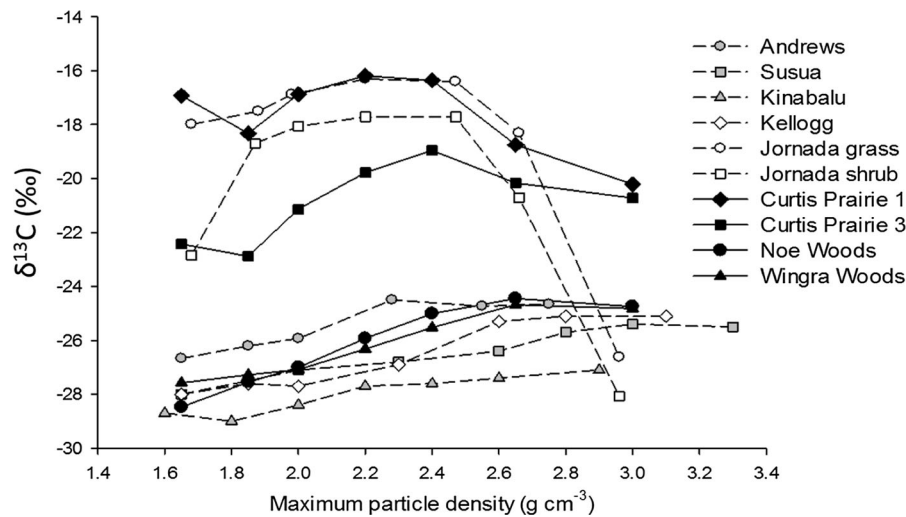


Fig. 7 Patterns of $\delta^{13}\text{C}$ in soil sequential density fractions from the control plots in the Wisconsin DIRT sites plotted in comparison with data from soils studied by Throop et al. (2013)

and Sollins et al. (2009). Wisconsin DIRT sites are plotted with black symbols, comparison data with gray

soils from deserts to the tropics, these soils showed a positive linear trend in $\delta^{13}\text{C}$ with increasing particle density, which has been interpreted to be indicative of increased microbial processing and age. This interpretation is consistent with our ^{14}C results demonstrating less bomb carbon accumulation, or greater age, with increasing density. The commonly observed slight decrease in the heaviest fraction, also seen on both forested soils here, has been interpreted to indicate sorption of less processed or else primary plant matter (Sollins et al. 2009). The heavier $\delta^{13}\text{C}$ signatures in the forested No Input plots compared to control plots, especially in the highest density fractions, are consistent with a signature of older, more processed SOM, as also seen in the ^{14}C data. The less negative $\delta^{13}\text{C}$ signatures in the prairie soils compared to the forested soils are indicative of a mixed C3–C4 grassland. The elliptical pattern in $\delta^{13}\text{C}$ with increasing density is consistent with a greater C3 influence in the older, heavier density fractions, possibly reflecting older forest-derived C before prairie restoration. Similarly, the more negative $\delta^{13}\text{C}$ values in prairie plots with litter removed compared to control plots across all density fractions also likely reflects an older, more C3-derived vegetation influence, also consistent with our ^{14}C results.

Several recent studies have suggested that root and rhizosphere-derived C is equally or more important to stable soil C than is aboveground litter (Kramer

et al. 2010; Mendez-Millan et al. 2010; Clemmensen et al. 2013), with much of this work coming from the analysis of root versus shoot biomarkers rather than decadal analyses of SOM accumulation. Other studies have suggested that the importance of shoot versus root inputs might be highly ecosystem dependent, and dependent on the types of inputs, such as woody debris versus labile leaf litter (Crow et al. 2009b). We hypothesized that priming, or the selective degradation of older SOM by fresh inputs of organic substrates, could cause a discrepancy between biomarker analyses of SOM chemistry and total C accumulation in soils: in other words, inputs could both appear as new SOM while also causing a decline in older SOM. Rhizosphere activity and root exudation have been shown to accelerate the loss of older SOC (Kuzyakov 2002; Ekschmitt et al. 2008; Drake et al. 2013) as have fresh leaf inputs (Crow et al. 2009b). In 1997, 41 years after the start of the experiment, only plots without roots showed significant declines in soil C, agreeing with reports highlighting the importance of root C. However, after 50 years, declines in soil C in plots with root inputs but without aboveground litter were similar to declines in plots without roots but with aboveground inputs, and thus we did not find that the presence of roots was more critical than the presence of aboveground litter for C stabilization.

There was no clear evidence that soil C lability 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 composition appeared to change in litter removal plots; soils with litter excluded had lower $\Delta^{14}\text{C}$ values indicative of lower net ^{14}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 vulnerable to significant C loss 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 SOM dynamics.

Acknowledgments This study was supported in part by NSF Grants 0817064 and 1257032. We thank Kevin McSweeney and Brad Herrick for assistance in the field and granting us permission to sample in the plots, Fox Peterson for statistical and editorial help, and Jim LeMoine for laboratory help.

Appendix

See Tables 6, 7, 8

Table 6 Soil Organic Nitrogen (SON) concentration and content and bulk density, in forested DIRT plots at the Wisconsin Arboretum, sampled in 2006 after 50 years of detrital manipulation

	Control	Double Litter	No Litter
SON concentration (mg N g ⁻¹ soil)			
Neo			
Mean (SE)	2.91 (0.09) ^a	2.83 (0.31) ^a	1.50 (0.26) ^b
% difference		+2.79	−48.57
Wingra			
Mean (SE)	2.06 (0.16) ^a	3.12 (0.16) ^b	1.10 (0.08) ^c
% difference		+51.67	−46.68
SON content (g N m ⁻²)			
Neo			
mean (SE)	306.54 (9.52) ^a	255.02 (27.98) ^a	184.06 (31.70) ^b
% difference		−16.81	−39.96
Wingra			
Mean (SE)	213.91 (16.95) ^a	314.05 (16.02) ^b	149.69 (11.47) ^a
% difference		+46.82	−30.02

Values are means of four 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

Table 7 SON concentration and content in prairie DIRT plots at the Wisconsin Arboretum, sampled in 1997 and 2006 after 41 and 50 years of detrital manipulation

	Control	No Input	No Roots	No Litter
1997 Curtis Prairie 1				
SON concentration (mg N g ⁻¹ soil)				
Mean (SE)	1.70 (0.02) ^a	0.89 (0.01) ^b	1.33 (0.05) ^c	1.68 (0.04) ^a
% difference		-47.75	-21.62	-0.98
SON content (g N m ⁻²)				
Mean (SE)	188.20 (3.38) ^a	106.99 (2.37) ^b	173.91 (6.12) ^a	161.96 (4.29) ^a
% difference		-43.15	-11.54	-13.95
2006 Curtis Prairie 1				
SON concentration (mg N g ⁻¹ soil)				
Mean (SE)	2.04 (0.08) ^a	0.96 (0.02) ^b	1.36 (0.11) ^c	1.87 (0.04) ^a
% difference		-53.18	-33.45	-8.44
SON content (g N m ⁻²)				
Mean (SE)	225.84 (9.09) ^a	115.18 (2.70) ^b	177.45 (14.94) ^c	179.81 (3.79) ^c
% difference		-49.00	-21.43	-20.38
2006 Curtis Prairie 3				
SON concentration (mg N g ⁻¹ soil)				
Mean (SE)	1.95 (0.07) ^a	1.10 (0.05) ^b	1.50 (0.12) ^c	1.95 (0.07) ^a
% difference		-43.72	-23.07	-0.48
SON content (g N m ⁻²)				
Mean (SE)	216.11 (7.50) ^a	132.49 (5.88) ^b	196.28 (15.14) ^a	187.02 (7.08) ^a
% difference		-38.69	-9.18	-13.46

Values are means of four 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

Table 8 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 Noe Woods control	C mg g ⁻¹ fraction	% total C	Mass % bulk soil Wingra Woods control	C mg g ⁻¹ fraction	% total C
<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
Total recovery (%)	98.3		76.9	98.4		82.9
Noe Woods double litter			Wingra Woods double litter			
<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

Table 8 continued

	Noe Woods double litter			Wingra Woods double litter		
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
Total recovery (%)	96.9		71.4	96.3		93.0
	Noe Woods no litter			Wingra Woods no litter		
<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
Total recovery (%)	99.3		88.2	99.8		72.6
	Curtis Prairie 1 control			Curtis Prairie 3 control		
<1.65	0.8	261.0	6.9	0.4	265.8	4.0
1.65–1.85	0.9	247.4	7.4	0.6	251.7	6.4
1.85–2	2.8	162.1	15.7	1.9	186.4	13.6
2–2.2	12.9	82.9	37.2	8.8	85.5	29.3
2.2–2.4	5.5	49.7	9.5	8.5	58.6	19.5
2.4–2.65	70.3	3.2	7.9	67.1	3.5	9.3
>2.65	5.2	3.5	0.6	6.1	3.7	0.9
Total recovery (%)	98.3		85.3	93.4		82.9
	Curtis Prairie 1 no input			Curtis Prairie 3 no input		
<1.65	0.1	273.4	3.2	0.2	253.2	4.8
1.65–1.85	0.1	265.2	2.2	0.2	282.5	5.5
1.85–2	0.3	165.0	5.7	0.5	197.1	10.0
2–2.2	3.4	50.2	21.5	3.1	79.0	23.0
2.2–2.4	8.0	25.2	25.8	6.7	43.5	26.9
2.4–2.65	77.0	2.3	22.4	73.8	3.1	21.4
>2.65	6.3	1.8	1.5	11.5	3.1	3.3
Total recovery (%)	95.0		82.2	96.0		94.9
	Curtis Prairie 1 no roots			Curtis Prairie 3 no roots		
<1.65	0.3	282.5	5.3	0.3	253.8	4.3
1.65–1.85	0.9	251.8	14.0	0.7	229.1	9.1
1.85–2	2.0	159.3	20.1	1.7	165.7	15.1
2–2.2	4.2	70.6	18.4	5.4	74.2	22.0
2.2–2.4	6.6	29.7	12.3	6.3	50.1	17.4
2.4–2.65	78.1	2.3	11.2	77.6	5.2	22.0
>2.65	3.1	2.4	0.5	5.8	3.7	1.2
Total recovery (%)	95.3		81.8	97.9		91.1

Table 8 continued

	Curtis Prairie 1 no litter		Curtis Prairie 3 no litter			
<1.65	0.6	253.5	7.0	0.8	243.9	7.5
1.65–1.85	0.7	211.7	6.6	1.3	222.5	11.2
1.85–2	2.1	154.6	15.0	2.0	156.1	12.4
2–2.2	6.7	77.8	24.1	5.2	92.2	18.7
2.2–2.4	7.8	50.1	18.1	8.4	68.5	22.5
2.4–2.65	74.1	3.4	11.7	74.9	4.0	11.6
>2.65	3.8	3.0	0.5	5.7	3.6	0.8
Total recovery (%)	95.8		83.0	98.3		84.7

References

- Battin TJ, Luyssaert S, Kaplan LA, Aufdenkampe AK, Richter A, Tranvik LJ (2009) The boundless carbon cycle. *Nat Geosci* 2:598–600
- Binkley D, Aber J, Pastor J, Nadelhoffer K (1986) Nitrogen availability in some Wisconsin Forests—comparisons of resin bags and on-site incubations. *Biol Fertil Soils* 2:77–82
- Bowden RD, Deem L, Nadelhoffer K, Plante A, Lajtha K (2014) Above and belowground litter Influences on soil organic matter and carbon in a temperate deciduous forest. *Soil Sci Soc Am J* (in press)
- Chung H, Ngo KJ, Plante A, Six J (2010) Evidence for carbon saturation in a highly structured and organic-matter-rich soil. *Soil Sci Soc Am J* 74:130–138
- Clemmensen KE, Bahr A, Ovaskainen O, Dahlberg A, Ekblad A, Wallander H, Stenlid J, Finlay RD, Wardle DA, Lindahl BD (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* 339:1615–1618
- Compton JE, Boone RD (2002) Soil nitrogen transformations and the role of light fraction organic matter in forest soils. *Soil Biol Biochem* 34:933–943
- Crow SE, Lajtha K, Bowden RD, Yano Y, Brant JB, Caldwell BA, Sulzman EW (2009a) Increased coniferous needle inputs accelerate decomposition of soil carbon in an old-growth forest. *For Ecol Manag* 258:2224–2232
- Crow SE, Lajtha K, Filley TR, Swanston CW, Bowden RD, Caldwell BA (2009b) Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. *Glob Change Biol* 15:2003–2019
- Diochon AC, Kellman L (2009) Physical fractionation of soil organic matter: destabilization of deep soil carbon following harvesting of a temperate coniferous forest. *J Geophys Res* 114:G01016
- Drake JE, Darby BA, Giasson M-A, Kramer MA, Phillips RP, Finzi AC (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, Kandeler E, Poll C, Brune A, Buscot F, Friedrich M, Gleixner G, Hartmann A, Kästner M, Marhan S, Miltner A, Scheu S, Wolters V (2008) Soil-carbon preservation through habitat constraints and biological limitations on decomposer activity. *J Plant Nutr Soil Sci* 171:27–35
- Fang C, Smith P, Moncrieff JB, Smith JU (2005) Similar response of labile and resistant soil organic matter pools to changes in temperature. *Nature* 433:57–59
- Fekete I, Kotrocó Z, Varga C, Nagy PT, Tóth JA, Bowden RD, Várbró G, Lajtha K (2014) Alterations in forest detritus input influence soil carbon concentration and soil respiration in a Central-European deciduous forest. *Soil Biol Biochem* (in press)
- Field CB, Raupach MR (eds) (2004) The global carbon cycle: integrating humans, climate, and the natural world. Island Press, Washington, DC, p 529
- Foley JA, Ramankutty N (2004) A primer on the terrestrial carbon cycle: what we don't know but should. In: Field CB, Raupach MR (eds) The global carbon cycle: integrating humans, climate and the natural world. Island Press, Washington, DC, p 529
- Giardina CP, Ryan MG (2000) Soil warming and organic carbon content. *Nature* 408:789–790
- Gottschalk P, Smith JU, Wattenbach M, Bellarby J, Stehfest E, Arnell N, Osborn TJ, Jones C, Smith P (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, Kleber M, Zeller B, Moni C, Plante AF, Townsend K, Gelhaye L, Lajtha K, Derrien D (2012) Transfer of litter-derived N to soil mineral-organic associations: evidence from decadal ^{15}N tracer experiments. *Org Geochem* 42:1489–1501
- Hoosbeek MR, Scarascia-Mugnozza GE (2009) Increased litter build up and soil organic matter stabilization in a poplar plantation after 6 years of atmospheric CO_2 enrichment (FACE): final results of POP-EuroFACE compared to other forest FACE experiments. *Ecosystems* 12:220–239
- Hua Q, Barbetti M (2004) Review of tropospheric bomb ^{14}C data for carbon cycle modeling and age calibration purposes. *Radiocarbon* 46:1273–1298
- Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol Appl* 10:423–436
- John B, Yamashita T, Ludwig B, Flessa H (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, Guggenberger G (2003) Mineral surfaces and soil organic matter. *Eur J Soil Sci* 54:219–236

- King AW, Post WM, Wullschlegel SD (1997) The potential response of terrestrial carbon storage to changes in climate and atmospheric CO₂. *Clim Change* 35:199–227
- Kramer C, Trumbore S, Fröberg M, Dozal LMC, Zhang D, Xu X, Santos GM, Hanson PJ (2010) Recent (<4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. *Soil Biol Biochem* 42:1028–1037
- Kramer MG, Sanderman J, Chadwick OA, Chorover J, Vitousek PM (2012) Long-term carbon storage through retention of dissolved aromatic acids by reactive particles in soil. *Glob Change Biol* 18:2594–2605
- Kucharik CJ, Fayram NJ, Cahill KN (2006) A paired study of prairie carbon stocks, fluxes, and phenology: comparing the world's oldest prairie restoration with an adjacent remnant. *Glob Change Biol* 12:122–139
- Kuzyakov Y (2002) Review: factors affecting rhizosphere priming effects. *J Plant Nutr Soil Sci* 165:382–396
- Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. *Soil Biol Biochem* 32:1485–1498
- Lajtha K, Peterson F, Nadelhoffer K, Bowden R (2013) Twenty years of litter and root manipulations: insights into multi-decadal soil dynamics. *Soil Sci Soc Am J* 67:1418–1427
- Leifeld J, Fuhrer J (2005) The temperature response of CO₂ production from bulk soils and soil fractions is related to soil organic matter quality. *Biogeochemistry* 75:433–453
- Liski J, Perruchoud D, Karjalainen T (2002) Increasing carbon stocks in the forest soils of western Europe. *For Ecol Manag* 169:159–175
- Llorente M, Glaser B, Turrión MB (2010) Storage of organic carbon and Black carbon in density fractions of calcareous soils under different land uses. *Geoderma* 159:31–38
- Mayzelle MM, Krusor ML, Bowden RD, Lajtha K, Six JW (2014) The effects of decreasing carbon saturation deficit on temperate forest soil carbon cycling. *Soil Sci Soc Am J* (in press)
- McFarlane KJ, Schoenholtz SH, Powers RF, Perakis SS (2010) Soil Organic Matter Stability in Intensively Managed Ponderosa Pine Stands in California. *Soil Sci Soc Am J* 74:979–992
- McLauchlan KK, Hobbie SE (2004) Comparison of labile soil organic matter fractionation techniques. *Soil Sci Soc Am J* 68:1616–1625
- Melillo JM, McGuire AD, Kicklighter DW, Moore BI, Voron-smarty CJ, Schloss AL (1993) Global climate change and terrestrial net primary production. *Nature* 363:234–240
- Mendez-Millan M, Dignac M-F, Rumpel C, Rasse DP, Derenne S (2010) Molecular dynamics of shoot versus root biomarkers in an agricultural soil estimated by natural abundance ¹³C labelling. *Soil Biol Biochem* 42:169–177
- Nielsen GA, Hole FD (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 RL, Parshotam AA, Salt GJ (2002) Carbon turnover in two soils with contrasting mineralogy under long-term maize and pasture. *Aust J Soil Res* 40:127–136
- Paul KI, Polglase PJ, Richards GP (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). *For Ecol Manag* 177:485–501
- Plante AF, Conant RT, Stewart CE, Paustian K, Six J (2006) Impact of soil texture on the distribution of soil organic matter in physical and chemical fractions. *Soil Sci Soc Am J* 70:287–296
- Post WM, Peng T-H, Emanuel WR, King AW, Dale VH, DeAngelis DL (1990) The global carbon cycle. *Am Sci* 78:310–326
- Raich JW, Schlesinger WH (1992) The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* 44:81–99
- Raich JW, Russell AE, Kitayama K, Parton WJ, Vitousek PM (2006) Temperature influences carbon accumulation in moist tropical forests. *Ecology* 87:76–87
- Rasse DP, Rumpel C, Dignac M-F (2005) Is soil carbon mostly root carbon? mechanisms for a specific stabilisation. *Plant Soil* 269:341–356
- Reimer PJ, Brown TA, Reimer RW (2004) Reporting and calibration of post-bomb ¹⁴C data. *Radiocarbon* 46:1299–1304
- Richter DD, Markewitz D, Trumbore SE, Wells CG (1999) Rapid accumulation and turnover of soil carbon in a re-establishing forest. *Nature* 400:56–58
- Schlesinger WH, Andrews JA (2000) Controls on soil respiration: implications for climate change. *Biogeochemistry* 48:7–20
- Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kogel-Knabner I, Lehmann J, Manning DAC, Nannipieri P, Rasse DP, Weiner S, Trumbore SE (2011) Persistence of soil organic matter as an ecosystem property. *Nature* 478:49–56
- Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant Soil* 241:155–176
- Sollins P, Swanston C, Kleber M, Filley T, Kramer M, Crow S, Caldwell B, Lajtha K, Bowden R (2006) Organic C and N stabilization in a forest soil: evidence from sequential density fractionation. *Soil Biol Biochem* 38:3313–3324
- Sollins P, Kramer MG, Swanston C, Lajtha K, Filley T, Aufdenkampe AK, Wagai R, Bowden RD (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 CE, Paustian K, Conant RT, Plante AF, Six J (2009) Soil carbon saturation: implications for measurable carbon pool dynamics in long-term incubations. *Soil Biol Biochem* 41:357–366
- Stuiver M, Polach HA (1977) Reporting of C-14 data. *Radiocarbon* 19:355–363
- Sulzman EW, Brant JB, Bowden RD, Lajtha K (2005) Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO₂ efflux in an old growth coniferous forest. *Biogeochemistry* 73:231–256
- Throop HL, Lajtha K, Kramer M (2013) Density fractionation and ¹³C reveal changes in soil carbon following woody encroachment in a desert ecosystem. *Biogeochemistry* 112:409–422
- Torn MS, Trumbore SE, Chadwick OA, Vitousek PM, Hendricks DM (1997) Mineral control of soil organic carbon storage and turnover. *Nature* 389:170–173

- Torn MS, Swanston CW, Castanha C, Trumbore S (2009) Storage and turnover of organic matter in soil. In: Huang PM, Senesi N (eds) Biophysico-chemical processes involving natural nonliving organic matter in environmental systems. Wiley, Hoboken, pp 219–272
- Townsend AR, Vitousek PM, Desmarais DJ, Tharpe A (1997) Soil carbon pool structure and temperature sensitivity inferred using CO₂ and ¹³CO₂ incubation fluxes from five Hawaiian soils. *Biogeochemistry* 38:1–17
- Trumbore S (2006) Carbon respired by terrestrial ecosystems—recent progress and challenges. *Glob Change Biol* 12: 141–153
- Wynn JG, Bird MI, Vellen L, Grand-Clement E, Carter J, Berry SL (2006) Continental-scale measurement of the soil organic carbon pool with climatic, edaphic, and biotic controls. *Global Biogeochem Cycles* 20:GB1007