CONVERSION FROM AGRICULTURE TO GRASSLAND BUILDS SOIL ORGANIC MATTER ON DECADAL TIMESCALES

Kendra K. McLauchlan,[1,3] Sarah E. Hobbie,[1] and Wilfred M. Post[2]

[1]*University of Minnesota, Department of Ecology, Evolution, and Behavior, Saint Paul, Minnesota 55108 USA*
[2]*Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-6335 USA*

Abstract. Soil organic matter (SOM) often increases when agricultural fields are converted to perennial vegetation, yet decadal scale rates and the mechanisms that underlie SOM accumulation are not clear. We measured SOM accumulation and changes in soil properties on a replicated chronosequence of former agricultural fields in the midwestern United States that spanned 40 years after perennial-grassland establishment. Over this time period, soil organic carbon (SOC) in the top 10 cm of soil accumulated at a constant rate of 62.0 g·m^{-2}·yr^{-1}, regardless of whether the vegetation type was dominated by C_3 or C_4 grasses. At this rate, SOC contents will be equivalent to unplowed native prairie sites within 55–75 years after cultivation ceased. Both labile (short turnover time) and recalcitrant (long turnover time) carbon pools increased linearly for 40 years, with recalcitrant pools increasing more rapidly than expected. This result was consistent across several different methods of measuring labile SOC. A model that investigates the mechanisms of SOM formation suggests that rapid formation of stable carbon resulted from biochemically resistant microbial products and plant material. Former agricultural soils of the Great Plains may function as carbon sinks for less than a century, although much of the carbon stored is stable.

Key words: agriculture; carbon sequestration; chronosequence; cultivated-field conversion to grassland; restored grasslands and soil organic C; soil organic matter.

INTRODUCTION

Over the past two centuries, agriculture has depleted the planet's largest terrestrial carbon (C) pool, soil organic carbon (SOC), by 55 Pg (Amundson 2001). The loss of C from soils to the atmosphere appears to be reversible as converting tilled land to perennial grassland transfers C from the atmosphere to soil. Although studies show short-term soil accumulation rates of 30 to 60 g C·m^{-2}·yr^{-1} on former agricultural fields (Post and Kwon 2000), the causes of the variation in this rate, the multidecadal scale rates of accumulation, and the residence times of the newly formed SOC are poorly known.

Quantifying the rate of increase in soil organic matter (SOM) is important for understanding global C cycles, the long-term fertility of agricultural systems and restored grasslands, and how long it takes grassland systems to recover from agriculture. There have been several different estimates of this rate, which vary widely (Gebhart et al. 1994, Burke et al. 1995). Different types of studies have been used to investigate the dynamics of SOC after cessation of agriculture in grassland systems in North America: repeated sampling of old fields (Knops and Tilman 2000), paired sites (Baer et al.

2000), and unreplicated chronosequences (Jastrow 1987). Many of these studies focus on changes in surface soils during the first decade after agricultural abandonment, and estimated rates of SOC accumulation significantly vary. Therefore, despite decades of study, the multidecadal accumulation rate and residence time of SOC that forms after conversion to perennial grassland are unknown—it is not known how long initial increases in SOC content persist, how labile and recalcitrant SOC fractions accumulate, and how vegetation composition affects the accumulation of different SOC fractions.

What causes the remaining variation in the rate of SOC accumulation on grasslands (summarized by Post and Kwon [2000])? One possibility is that SOC accumulation is influenced by soil-forming factors other than time, such as climate, vegetation, topography, or parent material, that vary from site to site (Jenny 1941). We were particularly interested in the role of grassland vegetation in affecting rate of SOC accumulation on former agricultural fields. Unfortunately, it has been difficult to isolate the role of the vegetation factor in previous studies because they observed abandoned agricultural fields where vegetation colonization and succession dynamics are unavoidable (Odum 1960, Burke et al. 1995). Model results have suggested that certain invasive grasses have the potential to alter SOC content differently than native plants (Ogle et al. 2004), and a study that compared converted and abandoned grass-

lands with different vegetation composition found differences in ecosystem properties, including SOC, between grassland types (Christian and Wilson 1999).

Most studies of SOC accumulation have focused on measuring total SOC, whereas SOC is a heterogeneous substance consisting of compounds with different turnover times (Parton et al. 1987). In particular, SOC comprises at least two conceptual pools: a small labile pool with short turnover time, and a larger recalcitrant pool with long turnover time (Oades 1988, Krull et al. 2003). The relative amounts of labile and recalcitrant compounds determine the timeframe and longevity of soil C sequestration (Paul et al. 2001a, 2003). Some studies have examined the short-term (under one decade) increases in labile C pools after cessation of agriculture (Robles and Burke 1998). However, the quantities of different types of SOC formed in former agricultural fields over multiple decades are virtually unknown.

Former agricultural lands of the northern Great Plains were depleted in SOM by decades of cultivation (Tiessen et al. 1982, Mann 1986), and the establishment of grasslands on these soils through federal conservation programs provides an opportunity to study the rate and type of SOM accumulation. To address the uncertainties about SOC increases with cessation of agriculture and the role of vegetation type in affecting the dynamics of soil recovery, we established a chronosequence of sites in western Minnesota (USA) that had been taken out of agricultural production and converted to perennial grassland using three different seed mixes (a C_3 grass, *Bromus inermis*; a *Bromus*–legume mix; and native C_4 grasses) at different times over the past 40 years. The chronosequence was replicated with respect to field age, or number of years since tillage ceased. Parent material, topography, and climate are similar for all sites. We measured plant and soil characteristics at these sites including total SOC and nitrogen (N) contents, labile and recalcitrant C pool sizes using several techniques, soil aggregate size, soil potential net N mineralization rates, and plant productivity and tissue chemistry. Similar seed mixes had been used for establishing grassland vegetation over the entire 40-year period and current vegetation corresponds to seeding mix so that vegetation and time since conversion are not confounded.

Materials and Methods

Site description

We sampled soils and vegetation at 33 sites in western Minnesota (USA), in an area ~50 km in radius. Soil parent material of all sites is calcareous glacial till from the Laurentide Ice Sheet. The vegetation before European settlement of the area in the mid-late 19th century was tallgrass prairie but now it is almost exclusively an agricultural landscape dominated by corn (*Zea mays*) and soybeans (*Glycine max*). Mean annual precipitation is 650 mm/yr; mean annual temperature is 6°C (Staff 1997).

We chose former agricultural fields that had been plow-tilled for at least 20 years to meet the assumptions of depleted soil organic matter (SOM) pools at the time of conversion to perennial grassland. We chose 20 years as the minimum length of tillage because the greatest depletion of SOM occurs within the first few decades of conversion from native grasslands to tilled agricultural fields, and subsequently SOM levels stabilize at a new level in agricultural fields (Mann 1986, Davis et al. 2003). All former agricultural fields were used for row-crop production but details of land management differed among sites. Although sites in this study likely differed in initial SOM content at the time of conversion from agriculture to perennial grassland, these differences do not systematically vary over the duration of the chronosequence. We sampled at a single topographic position, the shoulder slope, to minimize variation in SOM due to landscape position.

We sampled three plots on each of 31 former agricultural fields and two native-prairie sites. Each site served as a replicate, with results from each plot averaged within a site. Sites were either privately owned but enrolled in the Conservation Reserve Program (CRP) or owned by the U.S. Fish and Wildlife Service. Each site was deliberately converted from agriculture to grassland because of federal programs whose purposes range from restoring waterfowl habitat to reducing agricultural production. Thus, we assumed that there was no systematic bias from 1960 to 2000 in the type of land chosen for grassland establishment or the vegetation used for conversion. At the time of conversion, sites were planted with one of three different seed mixes developed for soil conservation in the Prairie Pothole region. The seed mixes used for grassland establishment were: (1) monospecific stands of *Bromus inermis*, a perennial, nonnative C_3 grass, (2) C_3 perennial grasses (often *Bromus inermis*) planted with legumes (often *Medicago sativa*), and (3) perennial, native C_4 grasses, often *Andropogon gerardii*, *Sorghastrum nutans*, and *Panicum virgatum*. Comparison of seed-mix composition and current vegetation composition showed that all of the species that were planted were present at each site, and no plant functional groups that had not been part of the seed mix were present. Although individual sites had not changed dramatically in vegetation composition over the duration of the chronosequence, we chose to sample sites that represented a variety of grassland types to test the hypothesis that vegetation composition would affect SOM accumulation. The vegetation of the unplowed prairie sites contained a mixture of C_3 grasses, C_4 grasses, forbs, and legumes native to western Minnesota. None of the sites are grazed; some have been burned occasionally since their conversion to grassland. Infrequent burning does not significantly affect SOC levels (Fynn et al. 2003).

Soil measurements

At each site, five 1.9-cm-diameter soil cores were taken to 10 cm depth in each of three 2 × 2 m plots and composited by plot. Some of the fresh soil was sieved to 2 mm to remove rocks and plant material. Some analyses were performed on this fresh soil, and the remainder was air-dried and ground to a powder. Some of the fresh soil was passed through a 4-mm sieve and immediately air-dried for aggregate size analysis. Total C and N of ground soil samples were determined on an elemental analyzer (Model ECS 4010, COS-TECH Analytical, Valencia, California, USA), after phosphoric acid treatment to remove carbonates. Microbial biomass of fresh soils was determined by chloroform fumigation and direct extraction (Anderson and Joergensen 1997).

Light- and heavy-fraction material were isolated from air-dried soil passed through a 2-mm sieve by flotation with a heavy liquid (aqueous sodium iodide) adjusted to a density of 1.7 g/cm³ (Jastrow 1996). Samples were sonicated to disrupt soil structure with an energy of 225 J/mL applied across 12 min. The light fractions correspond to poorly decomposed, relatively labile SOM, and the heavy fractions to mineral-associated, more recalcitrant SOM (Gregorich et al. 1989). Heavy-fraction material was proportional to light-fraction material, and so we report only the light fraction. Soil samples (1 g) were refluxed for 16 h in digestion tubes with 10 mL of 6 mol/L hydrochloric acid solution (Paul et al. 2001*b*). The residue was isolated, washed with 100 mL deionized water, dried in an 80°C oven, weighed, and analyzed for total C and N by combustion. The chemically resistant residue, or unhydrolyzable fraction, represents soil C pools that are extremely resistant to microbial degradation. The choice of method used to isolate labile and recalcitrant C pools may in part determine the pool size, so we chose several different methods (McLauchlan and Hobbie 2004).

Soil structure was measured by mechanically sieving to separate aggregates of different sizes into five water-stable aggregate size classes: >2000 μm, 2000 to >1000 μm, 1000 to >500 μm, 500 to >250 μm, and ≥250 μm. An index of aggregate size, geometric mean diameter, GMD, was calculated using the formula

$$GMD = \exp\left(\sum \frac{m_i \log x_i}{m_s}\right)$$

where m_i is the mass of aggregates in a size class with average diameter x_i and m_s is the mass of the sample (Kemper and Chepil 1965). Soil particle size distribution followed the hydrometer method (Gee and Bauder 1986). Soil calcium was extracted with 1 mol/L ammonium acetate and analyzed with inductively coupled plasma atomic-emission spectrometry at the Research Analytical Laboratory at the University of Minnesota (Saint Paul, Minnesota, USA).

We calculated potential net N-mineralization rates as the difference in inorganic-N pools (NO_3^--N + NH_4^+-N) between the beginning and end of an aerobic 28-d incubation at 22°C (Gebhart et al. 1994). Inorganic N was measured by extraction with 1 mol/L potassium chloride solution followed by analysis on an Alpkem autoanalyzer fitted with a cadmium coil (FS 3000, OI Analytical, College Station, Texas, USA).

Vegetation measurements

Aboveground net primary productivity (ANPP) was estimated by clipping peak standing aboveground biomass in a 0.075-m² area in each plot, separating live material, drying, and weighing. Belowground net primary productivity (BNPP) was estimated by measuring root growth into a standard 5-cm-diameter, 20-cm-deep cylinder of root-free soil during the 2001 growing season (Cuevas and Medina 1988). Root biomass was separated from soil, dried, and weighed. The variable net primary productivity (NPP) used for analysis was the sum of ANPP and BNPP. Although these values vary from year to year in response to weather variations (Knapp and Smith 2001), it is likely that our measurement of NPP was representative of relative differences between vegetation types over the duration of the chronosequence. Additionally, NPP did not vary significantly with field age, indicating that NPP likely did not vary within each field over the duration of the chronosequence.

Total aboveground plant C, N, and δ¹³C were analyzed at the University of California Davis Stable Isotope Laboratory (Davis, California, USA) using a Europa Hydra 20/20 mass spectrometer (Europa Scientific, Cambridge, UK). Composition of C fractions in aboveground plant material was determined using the forage fiber method (Van Soest 1963), where plant material was sequentially digested into fractions that correspond with soluble cell contents, cellulose, hemicellulose, and lignin on a forage fiber analyzer (ANKOM 200; ANKOM Technology, Macedon, New York, USA). Aboveground plant chemistry generally corresponds to belowground plant chemistry for many grassland species (Craine et al. 2002).

Because not all plant functional groups were represented at all sites, we used a summary variable, aboveground plant δ¹³C, that was measured at all sites to test the role of vegetation in determining soil properties. Vegetation δ¹³C reflects both the relative abundance of C_3 and C_4 vegetation at a site and variation in independent vegetation traits. Vegetation characteristics are evenly distributed across the chronosequence: field age did not significantly predict aboveground plant δ¹³C ($r^2 = 0.02$, $P > 0.4$) or aboveground plant percentage N ($r^2 = 0.01$, $P > 0.4$).

Statistical analyses

All statistical analyses were performed with JMP 5.0 (SAS Institute 2002). A multiple stepwise regression

TABLE 1. Parameter values for four different simulations using a simple model of soil organic carbon (SOC) dynamics to investigate mechanisms of SOC accumulation.

		Simulations			
Parameter	Rate†	$b = 0,$ $I_3 > 0$	$b = 0,$ $I_3 = 0$	$b > 0,$ $I_3 = 0$	$b > 0,$ $I_3 > 0$
a	transfer of C from labile to microbial biomass pool	0.565	0.627	0.695	0.200
b	abiotic condensation reactions	0	0	0.315	0.481
g	microbial humification	0.825	1.640	1.022	1.577
d	transfer of C from microbial biomass to labile pool	0.214	0.237	0.321	0.211
e	transfer of C from recalcitrant to microbial biomass pool	0.040	0.055	0.049	0.079
h	microbial respiration	1.646	0.808	1.301	0.934
I_1	plant inputs to labile pool	137.0	152.9	231.1	165.4
I_3	plant inputs to recalcitrant pool	103.34	0	0	4.38

Notes: Parameter values were determined using a simplex method to minimize differences between measured and simulated SOC pools. Many optimizations were performed, starting with different initial values for the parameters. See *Results*: *Model* for more information.

† The units of all rates are (yr^{-1}) except for I_1 and I_3 which are (g C·m^{-2}·yr^{-1}).

using backward elimination with $P = 0.05$ as the cutoff for significance was used to test the effects of several potential predictor variables on SOC content. To assess the effect of vegetation on soil organic-carbon (SOC) accumulation rate, we used a least-squares regression model with SOC as the response variable, and vegetation variables, field age, and their interaction term as predictor variables. To highlight additional bivariate relationships of interest, we used simple linear regression to calculate the predictive power of field age for light fraction C, unhydrolyzable C, GMD, net N mineralization, microbial biomass C, and incubation labile C. We also used simple linear regression to explore the relationship of the summary vegetation variable, aboveground plant δ^{13}C, to soil properties and other vegetation traits. It is important to note that two different variables may each significantly predict a particular response variable but are not necessarily correlated with one another.

Model

There are currently several mathematical models of SOM dynamics, many of which have been useful in agricultural systems (Smith et al. 1997). We found that a commonly used model, ROTHC (Jenkinson 1990), adequately simulated changes in total SOC after conversion from agriculture to grassland, the situation found in this study. However, while ROTHC explicitly models microbial byproducts and recalcitrant plant materials such as lignin, the two postulated mechanisms for formation of recalcitrant SOC, it could not replicate the rapid increase in recalcitrant SOC pools observed in this study. Therefore, we developed a simple, general mathematical model to examine the mechanisms of rapid formation of recalcitrant C.

The model contained three SOC pools: microbial biomass C, labile C, and a recalcitrant C pool that includes unhydrolyzable C and remaining nonlabile C. In the model, C enters the labile pool from portions of plant inputs and microbial biomass such as proteins and carbohydrates; microbes use C from both the labile

and recalcitrant pools; and C enters the recalcitrant pool from inputs of resistant plant and microbial products such as chitin, other cell-wall constituents, and complex polysaccharides, as well as from abiotic condensation reactions of labile C.

The model consisted of three ordinary differential equations:

$$\frac{dx_1}{dt} = -(a + b)x_1 + dx_2 + I_1$$

$$\frac{dx_2}{dt} = ax_1 - (g + d + h)x_2 + ex_3$$

$$\frac{dx_3}{dt} = bx_1 + gx_2 - ex_3 + I_3$$

where x_1 is the labile C pool, x_2 is the microbial biomass C pool, and x_3 is the recalcitrant C pool (see Table 1 for other parameters). All simulations were initialized with the same pool sizes. Parameters, including inputs, were obtained by minimizing the function

$$\sum_{j=1}^{2} \sum_{i=1}^{3} w_i [x_i(t_j) - \bar{x}_i(t_j)]^2$$

where $w_1 = 10$, $w_2 = 20$, $w_3 = 1$, $t_1 = 40$ years $t_2 = 1000$ years (or native), $x_i(t_j)$ is the simulated pool size at time t_j, and $\bar{x}_i(t_j)$ is the measured pool size at time t_j. The weights, w_i, were chosen to equalize the importance of all the pools in the optimization. Model simulations and parameter optimizations using measurements were performed using PopTools (Hood 2004) within Microsoft Excel.

To distinguish the relative importance of different mechanisms of recalcitrant SOM formation (recalcitrant plant inputs, microbial humification, and abiotic condensation reactions) we performed model optimizations with different parameter values, systematically changing the variables that determine the accumulation rate of the recalcitrant C pool. Specifically, we used five scenarios where abiotic condensation reactions, microbial humification, and recalcitrant plant inputs to soil were each set to zero or unconstrained.

RESULTS

Accumulation of SOM

Over the entire 40-year duration of the chronosequence, total soil organic carbon (SOC) increased at a rate of 62.0 $g \cdot m^{-2} \cdot yr^{-1}$ in the top 10 cm of soil (Fig. 1A). Field age, or time since cultivation, was the primary determinant of SOC content. A multiple stepwise regression showed that variation in total SOC among fields was significantly predicted by field age, and was not predicted by other factors such as topography (percentage slope), aboveground plant chemistry (percentage N, percentage lignin), NPP, soil texture (percentage clay), or soil calcium content. Percentage clay ranged from 10.8% to 30% (a factor of three) and NPP ranged from 140 to 589 g/m^2 (a factor of four) among sites. SOC in soils from 10 to 20 cm depth showed a similar pattern to the soils at 0–10 cm depth, accumulating at the rate of 46.7 $g \cdot m^{-2} \cdot yr^{-1}$ ($r^2 = 0.30$, $P < 0.002$).

A linear equation was the best fit for the relationship between field age and total SOC, and the slope of this regression equation was used to calculate the rate of SOC accumulation (Fig. 1A). Thus, the rate of SOC accumulation remained constant over the first 40 years of restoration and did not decrease as C levels increased. If this rate remains constant after the 40-year time period that we observed, SOC content of surface soils in the former agricultural fields we studied will be equivalent to that of unplowed native prairie in 55–75 years after grassland establishment.

Both labile (as measured by light-fraction C) and recalcitrant (as measured by unhydrolyzable C) pools of soil C began to increase immediately after establishment of perennial grasslands (Fig. 1B and C). This pattern was also demonstrated with an additional technique for quantifying the labile C pool (soil respiration from a laboratory incubation), indicating that these results are consistent across different techniques used to measure labile C (K. K. McLauchlan, *unpublished data*). Microbial biomass C, a portion of the empirically-measured labile C pool, also continued to increase over 40 years (Fig. 1C). Both labile and recalcitrant C pools increased linearly over the 40-year time span of the chronosequence and recalcitrant C increased faster than labile C. Much of the rate of increase in SOC over 40 years can be attributed to an increase in unhydrolyzable C, which increased at a rate of 43.0 $g \cdot m^{-2} \cdot yr^{-1}$ while light fraction C increased at the much smaller rate of 3.5 $g \cdot m^{-2} \cdot yr^{-1}$. However, labile C pools as measured independently by light-fraction C or soil respiration are not large enough to empirically match the quantity of SOC unaccounted for by unhydrolyzable C.

Soil N increased concomitantly with SOC but at a slower rate of 4.7 $g \cdot m^{-2} \cdot yr^{-1}$. N supply available for plant and microbial uptake, measured as potential net N mineralization in a laboratory incubation, declined steadily over time since cessation of agriculture (Fig.
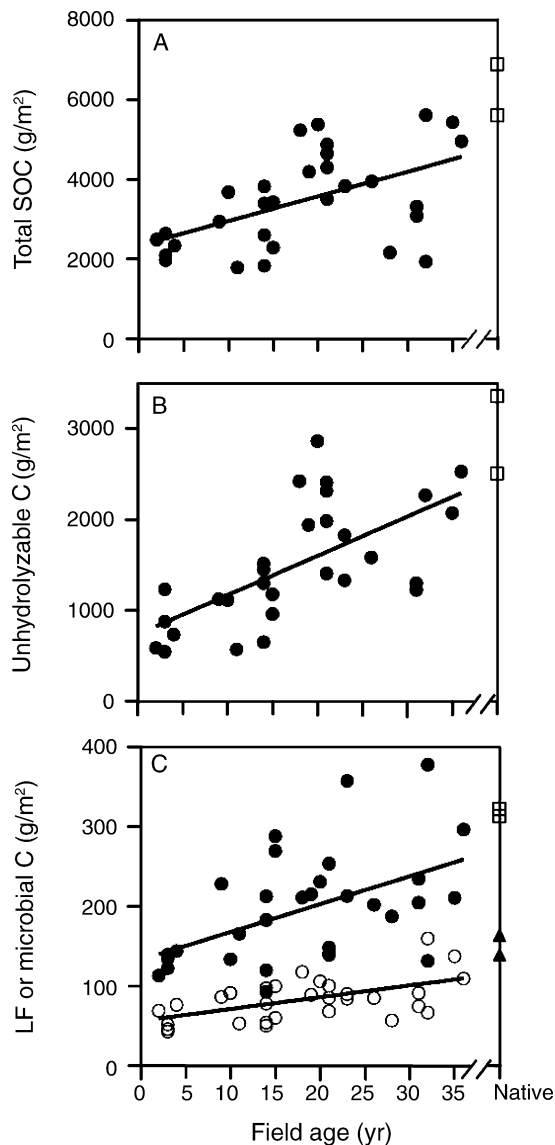

FIG. 1. Soil organic carbon (SOC) content across a chronosequence of former agricultural fields converted to perennial grassland over the past 40 years. (A) Total SOC increases at a linear rate of 62.0 g $C \cdot m^{-2} \cdot yr^{-1}$ ($r^2 = 0.27$, $P < 0.002$; $y = 62.0x + 2337$). (B) Unhydrolyzable C increases with field age ($r^2 = 0.42$, $P < 0.004$; $y = 43x + 743$). (C) Light-fraction C (LF, solid circles) and microbial biomass C (open circles) increase linearly with field age (light fraction $r^2 = 0.25$, $P < 0.005$; $y = 3.5x + 133$; microbial biomass $r^2 = 0.31$, $P < 0.001$; $y = 1.5x + 57$). In two native-prairie sites that were never cultivated, open squares show soil C content, and solid triangles show microbial biomass C. The solid lines indicate a significant linear regression based on formerly cultivated fields.

2A). Aggregate size increased with field age for the duration of the chronosequence (Fig. 2B).

Model

Four combinations of parameters successfully simulated the measured rate of recalcitrant C accumulation
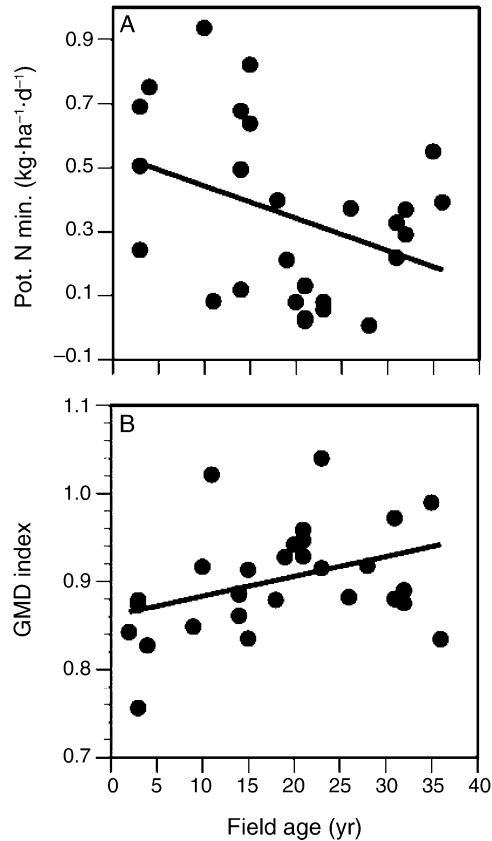
FIG. 2. Effect of time since cultivation, shown as (A) a bivariate linear regression of potential net N mineralization (pot. N min.) against field age ($r^2 = 0.24$, $P < 0.005$); and (B) a bivariate linear regression of geometric mean diameter (GMD, an index of soil aggregate size) against field age ($r^2 = 0.14$, $P < 0.03$). Each point represents a single site.

(Table 1). While large direct inputs from plant material (I_3) and condensation reactions (b) increased the pool size of recalcitrant C, each of these parameters could be set to zero, individually or simultaneously, without affecting the rate of recalcitrant C formation. However, it was necessary that the microbial humification rate (g) be high to attain the observed rates of soil C accumulation (Table 1). The fifth scenario, where microbial humification was set to zero, led to unstable dynamics and unreasonable parameter values. These results support the idea that microbial processing can quickly transform labile C to biochemically recalcitrant C. However, stable C compounds in plant material, such as lignin, may also influence the recalcitrant C pool size.

All three modeled C pools increase in a nonlinear fashion, approaching equilibrium values in approximately the same length of time as linear increases. When initial pool sizes are far from equilibrium and there is a slow decomposition rate, the modeled rate of SOC accumulation is impossible to distinguish from linear in the first few decades of SOC increase. The

equilibrium value of total SOC is 5976 g/m², similar to measured SOC values on native prairie sites.

Effects of vegetation on rate of SOM accumulation

There were differences among grassland vegetation types in aboveground tissue chemistry and productivity that could be summarized with the variable aboveground plant $\delta^{13}C$ (Fig. 3). In particular, C_3 vegetation, indicated by a low $\delta^{13}C$ value, had high concentrations of lignin, soluble cell constituents, and N, and low hemicellulose, compared with C_4 grasses. These characteristics of C_3 vegetation were strongly influenced by the presence of *Medicago sativa*, a legume, which is included in C_3 vegetation. The quantity of *M. sativa* in the vegetation sample at a site significantly predicted the content of lignin in the vegetation sample ($r^2 = 0.53$, $P < 0.005$). C_3 vegetation also had higher belowground net primary productivity (BNPP) than the C_4 vegetation type (Fig. 3C). Overall, aboveground lignin concentrations ranged from 1.0% to 7.3% and aboveground N concentrations ranged from 0.83% to 2.54%.

Despite differences among vegetation types in productivity and tissue chemistry, vegetation characteristics as summarized by aboveground plant $\delta^{13}C$ were unrelated to total SOC or any labile and recalcitrant SOC pool (Fig. 4C). Neither did NPP, tissue N, or lignin concentrations correlate with any SOC pool. Least-squares regression with total SOC as a response variable showed that the interaction term between field age and aboveground plant $\delta^{13}C$ was insignificant. This result also occurred when vegetation was treated as a categorical variable, and regardless of whether the vegetation was classified into two categories (C_3 and C_4 vegetation) or three categories (C_3 grasses, C_3 grasses and legume, or C_4 grasses). Thus, these grassland vegetation characteristics and vegetation types do not predict SOC content, SOC accumulation rate, or type of SOC formed.

The summary vegetation variable, aboveground plant $\delta^{13}C$, was correlated with some soil properties. In particular, vegetation did influence N cycling and soil structure independent of field age (Fig. 4A and B). Soils under C_4 grasses demonstrated significantly lower rates of potential net N mineralization than soils under C_3 grasses. Soils under C_4 vegetation also had significantly larger aggregates than soils under C_3 vegetation.

DISCUSSION

SOM accumulation rates

The rate of soil organic carbon (SOC) accumulation observed in this study, 62.0 g·m⁻²·yr⁻¹ in the top 10 cm of soil, is approximately equal to previously observed rates of SOC increase during the first decade after conversion of agroecosystems to perennial grasslands in the northern Great Plains (USA) (Follett 2001, Mensah et al. 2003). There are differences among ob-
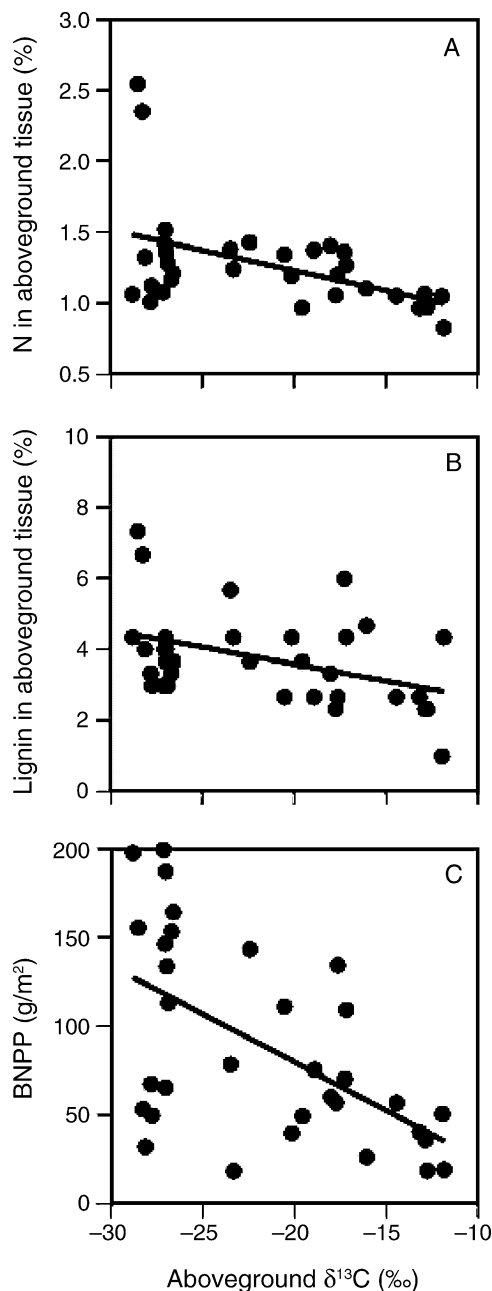
FIG. 3. Traits of vegetation vary with amount of C$_3$ biomass. Bivariate linear regressions of (A) percentage N of aboveground biomass against a summary vegetation variable, aboveground δ^{13}C (r^2 = 0.22, P < 0.006); (B) percentage lignin of aboveground biomass against δ^{13}C of aboveground biomass (r^2 = 0.17, P < 0.02); (C) belowground net primary productivity (BNPP) against δ^{13}C of aboveground biomass (r^2 = 0.35, P < 0.003). Each point represents a single site. Increasing absolute values of δ^{13}C indicate an increasing proportion of C$_3$ biomass.

served rates of grassland SOC accumulation in North America, but is not possible at this point to explain quantitatively these differences with soil-forming factors (Jenny 1941, Post and Kwon 2000). For example, factors that have been postulated to control maximum or equilibrium amount of SOC, such as soil clay content and NPP in grasslands of the Great Plains of North America (Epstein et al. 2002), did not control its rate of accumulation here. Topographic position, which has been shown to explain variation in SOM pool sizes in the shortgrass steppe, may also affect SOM accumulation rates (Hook and Burke 2000). Our estimated rate at the shoulder-slope position is likely to be intermediate between SOM accumulation rates at lower and higher landscape positions. Further chronosequence work that explicitly analyzes the role of soil-forming factors may clarify if these factors quantitatively determine rates of SOC formation.

There are two additional sources of uncertainty about the length of time that former agricultural soils will function as a C sink. First, there is uncertainty about the constancy of the rate of SOC accumulation. Theories of soil development and SOM models, including the one developed here, indicate nonlinear accumulation rates, with SOC accumulation slowing as SOC levels increase (Olson 1963, Jastrow 1996). However, empirical measurements of SOC accumulation during the first decade of grassland establishment exhibit linear rates of increase, similar to those observed in this multidecadal study on these sites in western Minnesota (Baer et al. 2002). It is possible that accumulation rates may slow in the future on these sites as SOC levels continue to increase, approaching those of native prairie, although there is no evidence of changing accumulation rates in the first 40 years of this chronosequence. The former agricultural land in this study is therefore currently a predictable C sink, with a known rate of increase each year, but may saturate in under a century. Second, there is uncertainty in the estimate of maximum SOC content, and it is important to better quantify the determinants of maximum SOC to predict the longevity of C sequestration (Six et al. 2002).

The immediate and rapid increase in recalcitrant C, as measured by unhydrolyzable C and by difference from SOC with light-fraction C and labile C from the laboratory incubation, was unexpected. These findings suggest that, across all 31 sites, a large portion of new C inputs from plants quickly became stabilized in the soil, within years. This contrasts with assumptions of SOM models that most recalcitrant C, which has a long turnover time, takes centuries to millennia to form (Smith et al. 1997). While in many ecosystems unhydrolyzable C is on average older than bulk soil C (Paul et al. 1997), these results indicate that a significant portion of unhydrolyzable C may be formed within the first few decades of grassland establishment. Further investigation into both the age (Trumbore 2000) and stability of this large pool of recalcitrant C may clarify
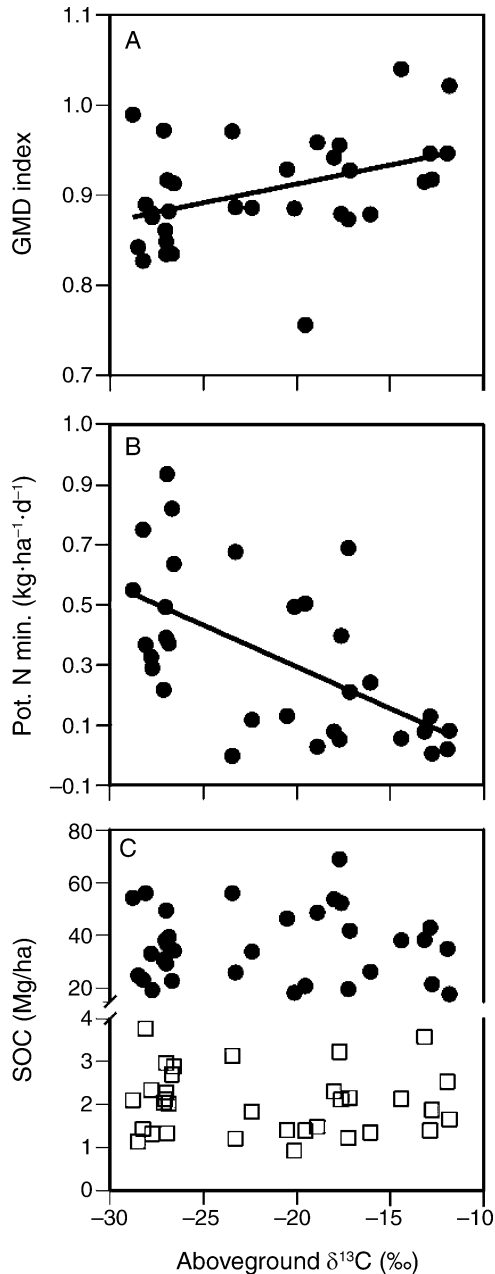
FIG. 4. Bivariate linear regressions between soil variables and vegetation composition: (A) GMD against a summary vegetation variable, $\delta^{13}C$ of aboveground biomass ($r^2 = 0.18$, $P < 0.01$); (B) potential net N mineralization against $\delta^{13}C$ of aboveground biomass ($r^2 = 0.33$, $P < 0.0005$); (C) SOC against $\delta^{13}C$ of aboveground biomass (solid circles, total SOC [NS], open squares, light fraction [NS]). Each point represents a single site.

connections between or independence of these two characteristics.

The immediate and rapid increase in recalcitrant C could be due to biochemical resistance of organic-C molecules contained either in plant material or in microbial metabolic byproducts. Complex, irregular,

heavy organic molecules such as plant lignin are difficult for microbes to decompose, thus contributing to the recalcitrant C pool (Kögel-Knabner 2002). Alternatively, recent studies that examined the composition of humified substances suggest that biochemically recalcitrant microbial byproducts rather than unmodified plant materials are abundant in the stable C pool (Kiem and Kögel-Knabner 2003, Kramer et al. 2003). Both of these alternative mechanisms may produce recalcitrant C that is relatively young in age. Our model indicates that microbial humification is important in forming recalcitrant SOC, and there is no correlation between measured plant lignin and SOC on our sites, suggesting that plant materials are likely microbially modified into recalcitrant SOC.

Other studies have suggested that soil aggregates reach their maximum size around 10 years after cessation of tillage (Jastrow 1996), but here aggregate size continued to increase for the duration of the chronosequence (Fig. 2B). This increase in aggregate size could be driving the increase in the size of the labile C pool over time, as aggregates physically protect labile organic matter from microbial oxidation (Six et al. 1998). However, the increases in labile C over time as measured by light-fraction C, which includes labile C that was inside aggregates, are not sufficient to account for the magnitude of the increase in total C over time. These findings suggest that processes within aggregates may enhance the rate of recalcitrant SOC formation through microbial humification (Guggenberger et al. 1999). The identification of microbial processing as an important component of recalcitrant C formation was also supported by the modeling results.

Soil N pools increase over time on these former agricultural fields, as indicated by gradual increases in soil organic nitrogen with field age. However, N availability or potential net N mineralization declines with time. Other authors have shown, using paired comparisons between tilled and untilled soil, that net N mineralization increases with belowground disturbance from cultivation and low organic-C inputs relative to N inputs (Schimel 1986, Ihori et al. 1995). As soil C levels increased, microbes shifted to immobilizing N rather than mineralizing it and therefore net N mineralization rates decreased. Soil microbes are sensitive to the C:N ratio of inputs, and they immobilize available forms of N when C:N is high (Wedin and Tilman 1996).

Vegetation

Grassland vegetation may affect soil properties through several mechanisms related to plant tissue chemistry, productivity, and morphology. Although it is classically considered that C_4 grasses have lower tissue N concentrations and higher lignin concentrations than do C_3 grasses (Sage and Monson 1999), recent work suggests this may not be universally true (Craine et al. 2002). Rooting depth also differs among

grassland species and hence placement of C inputs between these vegetation types also differs. In this study, we found that C_3 vegetation had higher percentage lignin, percentage N, and BNPP than C_4 vegetation, primarily due to the presence of *Medicago sativa* in the C_3 vegetation.

Vegetation characteristics, particularly NPP, tissue N concentration, and lignin concentration, were uncorrelated with SOC content, SOC accumulation rates, or proportion of recalcitrant C formed. SOC models assume that plant characteristics, specifically lignin concentration, affect the rate and type of SOC formation (Parton et al. 1987, Ogle et al. 2004) and these assumptions have been verified in agricultural systems (Paustian et al. 1992). Our results seem to contradict these ideas. It may be that vegetation characteristics are not very important in determining the rate of SOC increase but are important in determining the equilibrium amounts. Additionally, the differences in tissue chemistry between C_3 and C_4 grassland vegetation types in this study may have been too small to affect the accumulation rate or type of SOC formed within decades in former agricultural fields.

Differences in grassland vegetation did lead to differences in N cycling, verifying the importance of plant traits to N cycling (Wedin and Tilman 1990, Scott 1998). Specifically, the low N concentration of litter produced by C_4 grasses may stimulate microbial immobilization, thereby lowering net N mineralization. Aggregate formation, including aggregate size, abundance, and quality of organic matter incorporated, is tightly linked to root and microbial biomass (Gale et al. 2000), perhaps making aggregate size especially sensitive to vegetation characteristics. It is not clear why increases in aggregate size under C_4 vegetation did not lead to increases in aggregate-protected SOC.

Conclusions

This study shows that soil C accumulates linearly for at least the first 40 years after conversion from agricultural land to grassland. Former agricultural land depleted in SOC can be managed as a C sink at decadal timescales. The results of this study suggest that the capacity of these former agricultural lands in the midwestern United States to store additional soil C will persist for a maximum of 55–75 years after cessation of agriculture, indicating that these soils will not continue to accumulate C more than a century after conversion to grassland. Our data demonstrate that a substantial quantity of recalcitrant C can be stabilized in surface soils within decades, which contradicts the assumption that formation of stable C pools takes centuries to millennia. We found that if stable SOC pools are depleted relative to their maximum potential C content, cessation of tillage combined with increased plant inputs can immediately increase recalcitrant C pool size as measured by several techniques. This indicates that recalcitrant C formed in former agricultural soil can function as an immediate and persistent C sink. A likely mechanism for this immediate increase in soil C pools with intermediate or slow turnover times is the rapid formation of stable microbial byproducts. Finally, while vegetation is certainly an important state factor influencing soil development, it may not be possible to significantly manipulate the storage of SOC in former agricultural fields on decadal timescales by planting C_3 vs. C_4 grasses.

Literature cited

Amundson, R. 2001. The carbon budget in soils. Annual Review of Earth and Planetary Sciences **29**:535–562.

Anderson, T. H., and R. G. Joergensen. 1997. Relationship between SIR and FE estimates of microbial biomass C in deciduous forest soils at different pH. Soil Biology and Biochemistry **29**:1033–1042.

Baer, S. G., D. J. Kitchen, J. M. Blair, and C. W. Rice. 2002. Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecological Applications **12**:1688–1701.

Baer, S. G., C. W. Rice, and J. M. Blair. 2000. Assessment of soil quality in fields with short and long term enrollment in the CRP. Journal of Soil and Water Conservation **55**:142–146.

Burke, I. C., W. K. Lauenroth, and D. P. Coffin. 1995. Soil organic matter recovery in semiarid grasslands: implications for the Conservation Reserve Program. Ecological Applications **5**:793–801.

Christian, J. M., and S. D. Wilson. 1999. Long-term ecosystem impacts of an introduced grass in the northern Great Plains. Ecology **80**:2397–2407.

Craine, J. M., D. G. Tilman, D. A. Wedin, P. B. Reich, M. J. Tjoelker, and J. M. H. Knops. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Functional Ecology **16**:563–574.

Cuevas, E., and E. Medina. 1988. Nutrient dynamics in Amazonian forest ecosystems. II. Fine root growth, nutrient availability, and leaf litter decomposition. Oecologia **76**:222–235.

Davis, R. L., et al. 2003. Nitrogen balance in the Magruder plots following 109 years in continuous cultivation. Journal of Plant Nutrition **26**:1561–1580.

Epstein, H. E., I. C. Burke, and W. K. Lauenroth. 2002. Regional patterns of decomposition and primary production rates in the U.S. Great Plains. Ecology **83**:320–327.

Follett, R. F. 2001. Soil management concepts and carbon sequestration in cropland soils. Soil and Tillage Research **61**:77–92.

Fynn, R. W. S., R. J. Haynes, and T. G. O'Connor. 2003. Burning causes long-term changes in soil organic matter content of a South African grassland. Soil Biology and Biochemistry **35**:677–687.

Gale, W. J., C. A. Cambardella, and T. B. Bailey. 2000. Surface residue- and root-derived carbon in stable and unstable aggregates. Soil Science Society of America Journal **64**:196–201.

Gebhart, D. L., H. B. Johnson, H. S. Mayeux, and H. W. Polley. 1994. The CRP increases soil organic carbon. Journal of Soil and Water Conservation 49:488–492.

Gee, G. W., and J. W. Bauder. 1986. Particle size analysis. Pages 383–412 in A. Klute, editor. Methods of soil analysis. Part I. Physical and mineralogical methods. Second edition. American Society of Agronomy, Madison, Wisconsin, USA.

Gregorich, E. G., R. G. Kachanoski, and R. P. Voroney. 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. Journal of Soil Science 40:649–660.

Guggenberger, G., S. D. Frey, J. Six, K. Paustian, and E. T. Elliott. 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Science Society of America Journal 63:1188–1198.

Hood, G. 2004. PopTools. Pest Animal Control Co-operative Research Center, CSIRO, Canberra, ACT, Australia. ⟨http://www.cse.csiro.au/poptools⟩

Hook, P. B., and I. C. Burke. 2000. Biogeochemistry in a shortgrass landscape: control by topography, soil texture, and microclimate. Ecology 81:2686–2703.

Ihori, T., I. C. Burke, and P. B. Hook. 1995. Nitrogen mineralization in native cultivated and abandoned fields in shortgrass steppe. Plant And Soil 171:203–208.

Jastrow, J. D. 1987. Changes in soil aggregation associated with tallgrass prairie restoration. American Journal of Botany 74:1656–1664.

Jastrow, J. D. 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biology and Biochemistry 28:665–676.

Jenkinson, D. S. 1990. The turnover of organic carbon and nitrogen in soil. Philosophical Transactions of the Royal Society of London, B 329:361–367.

Jenny, H. 1941. Factors of soil formation: a system of quantitative pedology. McGraw Hill, New York, New York, USA.

Kemper, W. D., and W. S. Chepil. 1965. Size distribution of aggregates. Pages 499–510 in C. A. Black, editor. Methods of soil analysis, part I. American Society of Agronomy, Madison, Wisconsin, USA.

Kiem, R., and I. Kögel-Knabner. 2003. Contribution of lignin and polysaccharides to the refractory carbon pool in C-depleted arable soils. Soil Biology and Biochemistry 35:101–118.

Knapp, A. K., and M. D. Smith. 2001. Variation among biomes in temporal dynamics of aboveground primary production. Science 291:481–484.

Knops, J. M. H., and D. Tilman. 2000. Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. Ecology 81:88–98.

Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry 34:139–162.

Kramer, M. G., P. Sollins, R. S. Sletten, and P. K. Swart. 2003. N isotope fractionation and measures of organic matter alteration during decomposition. Ecology 84:2021–2025.

Krull, E. S., J. A. Baldock, and J. O. Skjemstad. 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Functional Plant Biology 30:207–222.

Mann, L. K. 1986. Changes in soil carbon storage after cultivation. Soil Science 142:279–288.

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.

Mensah, F., J. J. Schoenau, and S. S. Malhi. 2003. Soil carbon changes in cultivated and excavated land converted to grasses in east-central Saskatchewan. Biogeochemistry 63:85–92.

Oades, J. M. 1988. The retention of organic matter in soils. Biogeochemistry 5:35–70.

Odum, E. P. 1960. Organic production and turnover in old field succession. Ecology 41:34–49.

Ogle, S. M., D. S. Ojima, and W. A. Reiners. 2004. Modeling the impact of exotic annual brome grasses on soil organic carbon storage in a northern mixed-grass prairie. Biological Invasions 6:365–377.

Olson, J. S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44:322–331.

Parton, W. J., D. S. Schimel, C. V. Cole, and D. S. Ojima. 1987. Analysis of factors controlling soil organic matter levels on Great Plains grasslands. Soil Science Society of America Journal 51:1173–1179.

Paul, E. A., H. P. Collins, and S. W. Leavitt. 2001a. Dynamics of resistant soil carbon of Midwestern agricultural soils measured by naturally occurring 14C abundance. Geoderma 104:239–256.

Paul, E. A., R. F. Follett, S. W. Leavitt, A. Halvorson, G. A. Peterson, and D. J. Lyon. 1997. Radiocarbon dating for determination of soil organic matter pool sizes and dynamics. Soil Science Society of America Journal 61:1058–1067.

Paul, E. A., S. J. Morris, and S. Bohm. 2001b. Determination of soil C pool sizes and turnover rates: biophysical fractionation and tracers. Pages 193–206 in R. Lal, editor. Assessment methods for soil carbon. Lewis Publishers, Boca Raton, Florida, USA.

Paul, E. A., S. J. Morris, J. Six, K. Paustian, and E. G. Gregorich. 2003. Interpretation of soil carbon and nitrogen dynamics in agricultural and afforested soils. Soil Science Society of America Journal 67:1620–1628.

Paustian, K., W. J. Parton, and J. Persson. 1992. Modeling soil organic matter in organic-amended and nitrogen-fertilized long-term plots. Soil Science Society of America Journal 56:476–488.

Post, W. M., and K. C. Kwon. 2000. Soil carbon sequestration and land-use change: processes and potential. Global Change Biology 6:317–327.

Robles, M. D., and I. C. Burke. 1998. Soil organic matter recovery on conservation reserve program fields in southwestern Wyoming. Soil Science Society of America Journal 62:725–730.

Sage, R. F., and R. K. Monson. 1999. C₄ plant biology. Academic Press, San Diego, California, USA.

SAS Institute. 2002. JMP 5.0. SAS Institute, Cary, North Carolina, USA.

Schimel, D. S. 1986. Carbon and nitrogen turnover in adjacent grassland and cropland systems. Biogeochemistry 2:345–357.

Scott, N. A. 1998. Soil aggregation and organic matter mineralization in forests and grasslands: plant species effects. Soil Science Society of America Journal 62:1081–1089.

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.

Six, J., E. T. Elliott, K. Paustian, and J. W. Doran. 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62:1367–1377.

Smith, P., et al. 1997. A comparison of the performance of nine soil organic matter models using datasets from seven long-term experiments. Geoderma 81:153–225.

Staff, S. S. 1997. Soil Survey of Ottertail County, Minnesota, USA. USDA Natural Resources Conservation Service, Washington, D.C., USA.

Tiessen, H., J. W. Stewart, and J. R. Bettany. 1982. Cultivation effects on the amounts and concentrations of carbon, nitrogen, and phosphorus in grassland soils. Agronomy Journal 74:831–835.

Trumbore, S. 2000. Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecological Applications **10**:399–411.

Van Soest, P. J. 1963. Use of detergents in analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. Association of Official Analytical Chemists **46**:829–835.

Wedin, D. A., and D. Tilman. 1990. Species effects on nitrogen cycling: a test with perennial grasses. Oecologia **84**: 433–441.

Wedin, D. A., and D. Tilman. 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science **274**:1720–1723.