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Physical and chemical protection in hierarchical soil aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands

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ABSTRACT

Stabilization offered by physicochemical protection in hierarchical soil aggregates is critical for building and maintaining soil C and N stocks. However, it is unclear if complex stabilization mechanisms can completely recover when native plant communities are re-established on soils depleted of C and N by agriculture. We isolated particulate organic matter (POM) and silt- and clay-sized fractions from four structurally defined locations within soil collected from an agricultural field, prairies restored for 3–33 years, and a never-cultivated remnant prairie. We used aggregate hierarchy to define our four soil locations: non-aggregated material, free microaggregates, macroaggregates (excluding encapsulated microaggregates), and microaggregates-within-macroaggregates. We found that the duration of linear soil C and N accumulation differed among aggregate-occluded pools in relation to the combined influences of soil mass redistribution and increases in C and N concentrations. Silt in microaggregates isolated from within macroaggregates contributed the greatest quantities of C and N to whole soil, yet reached steady state C and N contents that were only 59% (C) and 56% (N) of those observed in the remnant prairie soil. Although the C and N contents of most pools were still well below the amounts in the reference remnant prairie, the overall distribution of C among pools was similar to the remnant within 33 years of restoration, suggesting that SOM stabilization mechanisms do largely recover in the first decades after cessation of tillage and restoration of the plant community. Thus, the pools that fell short of pre-cultivation C and N contents within the timespan of the chronosequence might continue to build C and N even though they appeared to be at steady state at the time of sampling, possibly because not enough time has passed at the current input rate or because of lags in SOM transfer among pools. We hypothesize that several "transient steady states" could occur in some SOM pools along the way to an overall whole-soil steady state that could take centuries to achieve.

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1. Introduction

Soil organic matter (SOM) stabilization and destabilization processes are fundamental for the turnover of nutrients and C flow through terrestrial ecosystems (Sollins et al., 1996). When stabilization outpaces destabilization, soils can be an important component of strategies to mitigate persistent increases in anthropogenic CO_2 emissions by maintaining enormous stored C stocks or potentially offsetting a portion of mounting atmospheric CO_2 (Post and Kwon, 2000; Conant et al., 2001; Ogle et al., 2004). Stabilization mechanisms, such as occlusion in soil aggregates or interactions with mineral surfaces, are highly complex processes whose dynamics are not well understood. Detailed characterization of such mechanisms is therefore crucial for predicting the behavior of soil C and its feedbacks with climate (Sollins et al., 2007).

Carbon-aggrading restored grasslands are a good model system for studying mechanisms of SOM stabilization. Much of the fertile soil that developed under temperate grasslands in North America has been cultivated, reducing surface C stocks by up to 50% (Tiessen et al., 1982; Mann, 1986; Burke et al., 1989, 1995; Schlesinger and Andrews, 2000; Slobodian et al., 2002; Schnitzer et al., 2006). This C depletion occurs through alteration of water regimes (Reicosky et al., 1995), erosion (Pennock et al., 1994; Gregorich et al., 1998), decreased plant inputs to soil (Huggins et al., 1998), and tillage (Davidson and Ackerman, 1993) – all of which impact the soil structure that protects SOM from decomposition (Six et al., 2000; Spohn and Giani, 2011). There is potential for these effects

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to be reversed, however. Dense root biomass, high base saturation, and mixed clay mineralogy typically found in grassland Mollisols (Risser et al., 1981; Brady and Weil, 2008) promote recovery of SOM-protecting aggregate structure and accrual of at least some C during the first decades after the native plant community is re-established (Jastrow, 1987, 1996; Knops and Tilman, 2000; McLauchlan et al., 2006; Kucharik, 2007; Matamala et al., 2008; O'Brien et al., 2010). However, the total soil organic C recovered by a restored ecosystem is difficult to predict because it depends on poorly understood controls on both the rate and duration of C accrual (West and Six, 2007). A better characterization of how such controls operate in grasslands depends on a clear understanding of SOM stabilization mechanisms.

Physicochemical protection offered by aggregate structures and mineral surfaces is critical for building and maintaining soil C and N stocks. Hierarchically organized soil aggregates – a key feature of soils where organic matter is a major aggregate binding mechanism (Tisdall and Oades, 1982; Oades and Waters, 1991) – play an essential role in the accumulation and stabilization of SOM through the physical protection they provide. Aggregates of various sizes exist among and within each other in the soil matrix, held together by a range of organic binding agents. Generally, the collective strength of these binding agents increases as aggregates decrease in size (Tisdall and Oades, 1982; Dexter, 1988). The microscale complexity created by aggregate hierarchy helps to stabilize SOM in several interacting ways: inhibited microbial activity due to limited diffusion of gases or nutrients (Sextone et al., 1985; Horn et al., 1994; Ranjard and Richaume, 2001; Young et al., 2009); separation of enzymes from substrates on mineral and humic surfaces (Mayer, 1994; Ekschmitt et al., 2005; Allison, 2006; Allison and Jastrow, 2006); and limited access of decomposer organisms to SOM occluded within the smallest pores (Mayer, 1994; Ranjard and Richaume, 2001). In addition to occlusion within aggregates, SOM may be stabilized in other ways, such as chemical sorption to mineral surfaces (Baldock and Skjemstad, 2000), polyvalent cation bridging between SOM and minerals (Edwards and Bremner, 1967; Muneer and Oades, 1989), and layered chemical binding to mineral surfaces (Kleber et al., 2007). These mechanisms – independently and synergistically – allow fresh plant inputs to gradually become transformed from recognizable plant residues into SOM, creating a continuum of pools with a range of residence times (Bosatta and Agren, 1991; Baldock and Skjemstad, 2000; Grandy and Neff, 2008).

Recent work in agroecosystems suggests that the duration of C accumulation for a given input level might be constrained by the soil's capacity to protect SOM from decomposition (Six et al., 2002; Stewart et al., 2007). Similar mechanisms likely control soil C recovery in successional ecosystems, such as restored grasslands established on C-depleted soil, and may dictate their return to pre-cultivation conditions and their sink strength for anthropogenic atmospheric CO_2. The C accumulation rate in a chronosequence of restored tallgrass prairies at the Fermi National Accelerator Laboratory (Fermilab) in Illinois, USA, is essentially linear for the first few decades after conversion from agriculture to prairie (Jastrow, 1996; Matamala et al., 2008; O'Brien et al., 2010). This rate of accrual is expected to slow as the prairies mature, C input rates stabilize, and available protection becomes exhausted. Although hierarchical aggregates have been shown to reform rapidly following prairie restoration at this site (Jastrow, 1987, 1996), it is unknown if the full complement of physicochemical protection mechanisms regains complete function within the first few decades after prairie restoration, or if SOM stocks and dynamics can be restored to pre-cultivation conditions. Here, we aimed to explore how SOM stabilization proceeds in C-aggrading grassland soils. Specifically, we investigated (1) how the rates of organic matter accrual in hierarchical aggregates and their components affect recovery and stabilization of whole soil C and N, and (2) whether the distribution of SOM in hierarchical aggregates returns to that observed in remnant prairie soil, which would indicate that physicochemical protection mechanisms can be restored. To address these objectives, we isolated particulate organic matter (POM), and silt- and clay-sized fractions from four structurally defined locations within soil from an agricultural field, a chronosequence of restored prairies, and a never-cultivated remnant prairie.

2. Materials and methods

2.1. Site description

The study utilized a series of restored tallgrass prairies in the National Environmental Research Park (NERP) at Fermi National Accelerator Laboratory (Fermilab) in Batavia, IL, USA (88°14′30″W, 41°50′30″N). Average air temperature was 8.9 °C and average annual precipitation was 975 mm for the period from 1971 to 2000. Beginning in 1975, prairie vegetation was established annually on portions of long-term agricultural fields in and around the site's particle accelerator, forming a chronosequence of restored prairies. To initiate restoration, the land was disked and then seeded with local ecotypes of native prairie species. New prairies were burned 2–3 years after conversion and then annually until the early 1990s. Thereafter, burns were conducted every 1–3 years. Details can be found in Jastrow (1987), Allison et al. (2005), and Matamala et al. (2008).

In addition to restored prairies, active agricultural fields and a never-tilled remnant prairie remain at the site. The agricultural fields were cultivated according to the prevailing practices of the day until Fermilab was established, at which point fields were cropped with continuous corn from the early 1970s until the late 1980s and corn/soybean rotation thereafter. Currently, conservation tillage is used, with fall plowing following corn and no-till following soybean. The remnant prairie, located along a railroad right-of-way, was discovered in 1989 and has been maintained since with a burn schedule similar to that of the restored prairies. The remnant prairie was documented in the 1839 General Land Office survey (Bowles et al., 1998), indicating that it was at least 160 years old in 1999, although it is more likely to be several thousand years old (King, 1981).

2.2. Field sampling

Soil samples were collected in late August through early September of 1999 from five points along 50-m transects in each of six restored prairies and in an agricultural field in the soybean phase of corn-soybean rotation (Table 1). Sampling was carried out in the remnant prairie in the same way in early September of 2001. Four of the prairies were resampled in September 2009 to extend the range of observed ages for the restored prairies. The sampled plots and sampling methods have been described previously (Allison et al., 2005; Matamala et al., 2008). Briefly, at each sampling point, aboveground biomass was clipped to within 2 cm of the soil surface in a 0.1-m^2 circular area. Three cores (4.8 cm in diameter) were then extracted to a depth of 100 cm from the center of each area, cut into increments and pooled in the field by depth. We focused on the 0–5 and 5–15 cm depth intervals (0–5 cm only for 2009 samples) where dense root proliferation promotes rapid, measurable C increases (Matamala et al., 2008; O'Brien et al., 2010), allowing us to examine changes in SOM pools. Samples were restricted to the Drummer soil series (Fine-silty mixed superactive mesic Typic Endoquoll) and were frozen at the end of each day until processing. See Table 1 for plot descriptions and soil characteristics.

Table 1
Characteristics of restored prairies, agricultural field, and never-tilled remnant prairie in the Fermilab chronosequence

Plot	Vegetation	Planting date	Time since disturbance at sampling (years)		Area (ha)	Texture[a] (% silt/ % clay)
			1999	2009		
Ag	Corn/soybean rotation since 1980s	Annual	0.5	NS	120	56/36
A		Spring 1997	3	NS	18	58/32
B		Spring 1992	8	18	4	50/32
C	Restored prairie	Spring 1984	16	NS	13	48/26
D		Autumn 1981	18	28	19	58/26
E		Autumn 1978	21	31	5	50/30
F		Spring 1977	23	33	12	50/28
RP	Remnant prairie	NA	At least 165		5	56/22

NA = Not applicable.
NS = Not sampled.
[a] Measured on a composite sample from the 5–15 cm depth following the methods of Bouyoucos (1962) without removing organic matter. Organic matter oxidation with peroxide yields 54% silt and 36% clay, on average, but the shift from larger particles to clay is most pronounced in the remnant prairie.

2.3. Soil fractionation

Soil cores were thawed in a refrigerator overnight, gently broken apart along natural planes of weakness, and passed through an 8-mm sieve to homogenize the soil and remove roots. After drying at 55 °C, a subsample of 8-mm sieved soil was gently crushed to pass a 2-mm sieve for texture determination (Bouyoucos, 1962). Subsamples of dried 8-mm sieved soil from three sampling locations in each plot were selected for physical fractionation.

Soil organic matter pools were isolated by using a three-step fractionation scheme (Fig. 1). The first step sorted whole soil into water-stable macroaggregates and microaggregates, plus the non-aggregated (NA) silt-sized and clay-sized fractions (hereafter referred to as silt and clay, respectively). Soil samples (50–100 g depending on aggregate size distribution) were slaked in deionized water for 5 min (Kemper et al., 1985). After aspirating away any floating litter, the soil was manually wet sieved for 2 min at 50 oscillations min^{-1} (3-cm movements) on mesh with 1-mm openings (two aliquots were used as necessary to avoid sieve clogging; method adapted from Elliot, 1986). Soil that passed through the 1-mm sieve was gently poured onto a 250-μm sieve for an additional 2 min of sieving. Similarly, soil that passed through the 250-μm sieve was sequentially sieved for 2 min on 150-μm and 53-μm sieves. Material retained on each sieve (aggregates + non-occluded POM of similar size) was dried for 30 min at 105 °C then brushed into aluminum pans to finish drying at 65 °C. Material >250 μm was combined into a single macroaggregate fraction and material 53–250 μm was combined into a single microaggregate fraction (1-mm and 150-μm sieves were employed to avoid sieve clogging and ensure accurate size separations). Silt (NA-silt) and clay (NA-clay) in the rinse water were separated via sequential centrifugation (3 min at 270 × *g* and 22 °C to isolate silt and 10 min at 2000 × *g* with 10 ml 0.25 M $CaCl_2$ + $MgCl_2$ added to flocculate and collect clay).

The second step isolated microaggregates from within macroaggregates. Two 10-g subsamples of the dried >250-μm fraction were subjected to the microaggregate isolator, which disrupts macroaggregates while leaving microaggregates intact (Six et al., 2000). After slaking in deionized water for 10 min, the subsample was poured into a chamber with 50 stainless steel beads (4-mm diameter) in 1 cm of deionized water over a 250-μm mesh on top of an attached funnel. The entire apparatus was mounted on a reciprocating shaker moving at 186 oscillations min^{-1}. Flowing water flushed microaggregates released from within macroaggregates onto a 53-μm sieve in a basin. Coarse POM (>250 μm) that was inside macroaggregates (M(c)POM) and coarse POM not occluded in macroaggregates (Free (c)POM) were retained on the mesh in the chamber. Material collected on the 53-μm sieve was wet sieved as described above to separate water-stable microaggregates from macroaggregate-protected silt and clay (M-silt and M-clay), which were subsequently collected by sequential centrifugation as described above.

Density separation was used to determine the mass of Free (c) POM, which was also mathematically removed from the total coarse POM collected in the microaggregate isolator to estimate the M(c) POM fraction. A separate 3.5-g subsample from the intact 250-μm fraction obtained in the initial wet-sieving step was gently inverted in 45 ml of sodium polytungstate solution (SPT) adjusted to a density of 1.85 g cm^{-3}. The aggregates (in SPT solution) were held under vacuum for 5 min to encourage flotation of non-occluded POM (see Jastrow, 1996). After centrifuging at 1173 × *g* for 30 min, the non-occluded Free (c)POM was collected by using aspiration and filtration on a 20-μm nylon filter and washed with deionized water to remove residual SPT. A similar procedure (excluding the vacuum step and using 5 g of material) was used to isolate fine non-occluded POM from the 53–250 μm fractions from wet-sieving [Free (f)POM] and the microaggregate isolator [M(f)POM].

The third step of the fractionation dispersed microaggregates into their POM, silt, and clay components. After removal of Free (f) POM or M(f)POM, microaggregates were rinsed with deionized water to remove SPT, and then dispersed by shaking in 35 ml deionized water with 20 glass beads (4-mm diameter) on a reciprocating shaker for 16 h. The dispersed soil was washed through a 53-μm sieve. Material remaining on the sieve (POM + sand) was backwashed into an aluminum pan for drying. Silt and clay in the rinse water were isolated as described above. Thus, free microaggregates from wet sieving were fractionated into Fm-POM, Fm-silt, and Fm-clay, and microaggregates-within-macroaggregates (from microaggregate isolator) were fractionated into mM-POM, mM-silt, and mM-clay.

All fractions were dried at 65 °C, weighed, and pulverized in a ball mill (Spex CertiPrep, Metuchen, NJ) or an agate mortar and pestle (for small fractions) in preparation for elemental analysis. Because microaggregates contained within macroaggregates were isolated in a subsequent step, reported values for macroaggregate-C (or N) exclude material from microaggregates occluded within macroaggregates. In addition, 8-mm sieved whole soil (i.e., the starting material) and subsamples of intact sieved fractions were analyzed so that C and N recovery could be calculated. The C and N contents were measured by dry combustion with a Carlo Erba NC2500 elemental analyzer (Milan, Italy). Total mass recovery averaged over all steps of the procedure for both depths was 99.7 ± 0.1%, total C recovery was 93.4 ± 0.4%, and total N recovery was 94.8 ± 0.5%.

Litter (material that floated in water after slaking but before wet sieving commenced) mass, C, and N contents were subtracted from the whole soil values before C and N recovery or any pool sizes were calculated. The NA-POM mass, C, and N contents were determined by mathematically combining Free (c)POM and Free (f)POM mass, C, and N contents, respectively. The M-POM C and N contents were determined by combining the M(c)POM and M(f)POM mass, C, and N contents, respectively.

2.4. Data analysis

Soil C saturation has been documented by experimentally adjusting input rates to create a gradient of fresh SOM inputs (e.g.

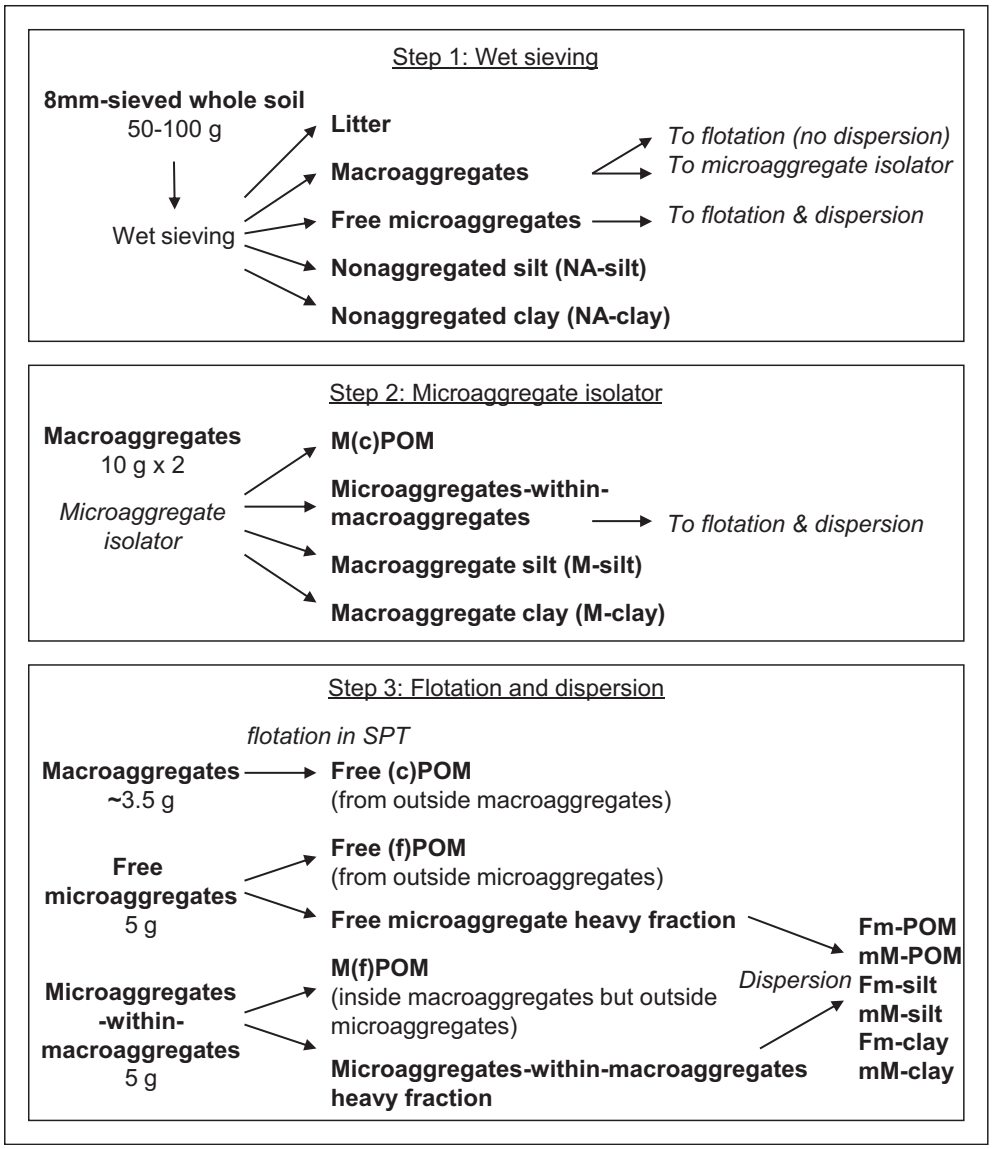

Fig. 1. Fractionation scheme used to isolate particulate organic matter (POM), silt, and clay from four structurally defined locations: non-aggregated (NA) material, free microaggregates (Fm), macroaggregates (M; excluding encapsulated microaggregates), and microaggregates-within-macroaggregates (mM). M(c)POM is coarse POM (>250 μm) inside macroaggregates but outside microaggregates. Free (c)POM is coarse POM not inside any aggregates. M(f)POM is fine POM (53–50 μm) inside macroaggregates but outside microaggregates. Free (f)POM is fine POM not inside any aggregates. Litter mass and C and N contents were subtracted from whole soil mass, C, and N before the C and N contents of other pools were calculated. M(c)POM and M(f)POM were mathematically combined to yield M-POM mass, C, and N contents. Free (c)POM and Free (f)POM were mathematically combined to yield NA-POM mass, C, and N contents.

Gulde et al., 2008). Instead of supplemental OM inputs, build-up of belowground biomass with restoration age in the chronosequence of prairies provided a natural range of OM inputs (Matamala et al., 2008). At this site, asymptotic regression projected that belowground plant C stocks would take 15 years to reach 50% and 64 years to reach 95% of the levels observed in remnant prairies (Matamala et al., 2008). We infer this to reflect a general increase in inputs as the prairies age. Without sustained OM manipulations, we cannot determine the capacity of these soils to accrue C. However, if some pools reach steady state while others continue to accrue C linearly, either (1) more time is required for SOM-transforming processes to deliver C into the steady-state pools or (2) these pools, or portions thereof, are potentially saturated.

Hence, we applied the conceptual framework developed from studies that vary OM inputs to investigate and interpret C accrual dynamics. We plotted the C and N concentrations (g C or N kg^{-1} fraction) and contents (g C or N kg^{-1} whole soil) in each fraction against time since planting, and modeled the relationship with both a linear regression and the asymptotic exponential model:

$$C_t = C_o + C_a\left(1 - e^{-k*\text{time}}\right)$$

where C_o is the initial C determined in the agricultural field, C_a is accrued C, $C_o + C_a$ is the C at steady state, C_t is C at time t, and k is the first-order rate constant for loss of C due to decomposition. Saturated conditions are best represented by an exponential model, whereas a linear model best describes situations that are unconstrained by saturation (Stewart et al., 2007). Linear and exponential models were estimated and plotted using SigmaPlot (version 11.2, Systat Software, Inc., San Jose, CA, USA). Models that had reasonably good fits were further evaluated by comparing their corrected Akaike's information criterion (AICc) scores following the methods of Stewart et al. (2007; Proc nlmixed with trust region optimization,

SAS System version 8.01, SAS Institute, Cary, NC, USA; SAS Institute, 1999). We used three criteria to determine the best-fit model for each pool: (1) the SigmaPlot-generated *P*-value for the model was ≤0.05, (2) the model had the lowest AICc score, and (3) the SAS-generated *P*-value for accrual rate (slope) in linear regression or *k* in exponential regression was ≤0.05. The remnant prairie was not included in the models so we could assess whether pools attained pre-cultivation C and N levels. Furthermore, the C and N stocks in the remnant soil could exert undue leverage on model parameters, and might not necessarily represent the upper limit for soil C and N in this system (Six et al., 2002). One of the replicates from Prairie F was discarded as an outlier because, unlike all other samples, the core was largely occupied by rhizomes and coarse roots of grasses, leading to unusual amounts of detrital material. Means are averages of three replicates and are reported ± standard errors, except for Prairie F where $n = 2$.

3. Results

3.1. Aggregate mass, carbon, and nitrogen

The distribution of soil mass among hierarchical aggregate pools returned to levels similar to the remnant prairie within 3 years of conversion to prairie (Fig. 2). Free microaggregates dominated the agricultural soil, comprising 53 ± 1% of soil mass at 0–5 cm and 51 ± 2% at 5–15 cm depth. Their relative mass quickly declined to 22 ± 3% (0–5 cm) and 27 ± 1% (5–15 cm) in the youngest restored prairie as the proportion of soil mass in macroaggregates and microaggregates-within-macroaggregates increased. The aggregate mass distribution in restored prairies was similar to the remnant prairie and did not change appreciably over the chronosequence, although these patterns were more variable at the 5–15 cm depth.

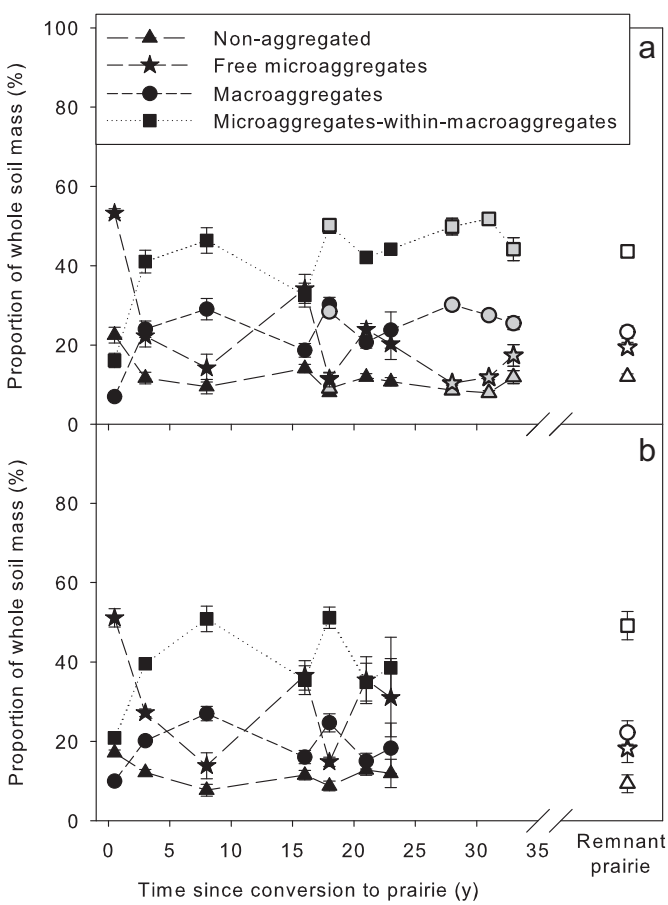

Fig. 2. Redistribution of soil mass in hierarchical aggregates extracted from soils of a prairie restoration chronosequence at 0–5 cm (a) and 5–15 cm (b) depths. Symbols represent plot level means ($n = 3$) and error bars are SE. Black symbols represent restored prairies and a soybean field sampled in 1999, gray symbols represent restored prairies sampled again in 2009, and open symbols represent a never-cultivated remnant prairie. Aggregates were not extracted from the 5–15 cm depth in soil collected in 2009. Lines do not represent regression analyses.

Compared to the mass distribution, aggregate-associated C and N changed more slowly (Fig. 3). Concentrations (mg g^{-1} aggregate) of C and N increased linearly in all aggregates at 0–5 cm depth (Fig. 3a,c). In contrast, only macroaggregates had a linear change in C content (mg g^{-1} whole soil; Fig. 2b). Non-aggregated N, macroaggregate N, and microaggregate-within-macroaggregate C and N contents each fit asymptotic regressions, whereas neither model described other aggregate pools. Notably, the C and N contents of all aggregate pools (as well as NA material) in the remnant prairie were substantially higher than in the restored prairies. At 0–5 cm, macroaggregate-N increased to 95% of the model-predicted steady state 8 years after restoration, but the predicted steady state was only 60% of the N observed in the remnant prairie. Microaggregate-within-macroaggregate-C achieved 95% of the predicted steady state accumulation 36 years after restoration with a predicted C content that was 71% of the C observed in the remnant prairie microaggregates-within-macroaggregates. Nitrogen in this pool was similar, reaching 95% of predicted steady state accrual 33 years after restoration, at about 65% of the remnant mM-N content. No models described the variations in free microaggregate C or N content with time because trends for increasing C and N concentrations were offset by the declining mass of free microaggregates across the chronosequence (Fig. 2). Nitrogen content in non-aggregated material decreased to 45% of the remnant value in less than 1 year. At 5–15 cm, only microaggregates-within-macroaggregates had a significant relationship between C concentration and time since restoration (linear, $P = 0.05$, $r^2 = 0.56$), and no pools had significant regressions for N concentration or for C or N contents (not shown). Overall, microaggregates-within-macroaggregates contributed the most to total soil C and N accrual at both depths. Together, changes in soil mass distribution and C concentration brought the relative allocation of whole soil C and N among aggregate pools to a distribution similar to that of the remnant prairie.

3.2. Carbon and nitrogen in POM, silt, and clay pools defined by aggregate hierarchy

Because the C (or N) *content* of a given SOM pool integrates the C (or N) concentration of that pool with its proportional mass contribution to whole soil, changes following conversion to prairie in the C and N contents of POM, silt, and clay pools within the four structurally defined locations are provided in Figs. 4–7. The dynamics of pool C and N *concentrations* and changes in their proportional mass are available as supplementary data (Figs. S1–S8).

Carbon and N contents of NA-POM increased linearly with prairie restoration at 0–5 cm but reached just 50% of C and 46% of N in the remnant prairie after 33 years of restoration (Fig. 4). This steady accumulation was driven by linear accumulations in NA-POM mass ($P = 0.01$, $r^2 = 0.62$) that outpaced a slow but steady decline in C concentration ($P = 0.01$, $r^2 = 0.56$), and a short exponential decrease in NA-POM N concentration ($P < 0.01$, $r^2 = 0.86$). NA-silt mass decreased exponentially at 0–5 cm ($P < 0.01$, $r^2 = 0.82$), but this mass change did not lead to significant regressions for C or N content. At 5–15 cm, NA-POM N concentration decreased linearly and NA-clay C concentration increased linearly ($P = 0.05$, $r^2 = 0.57$ for both), but these accruals did not lead to

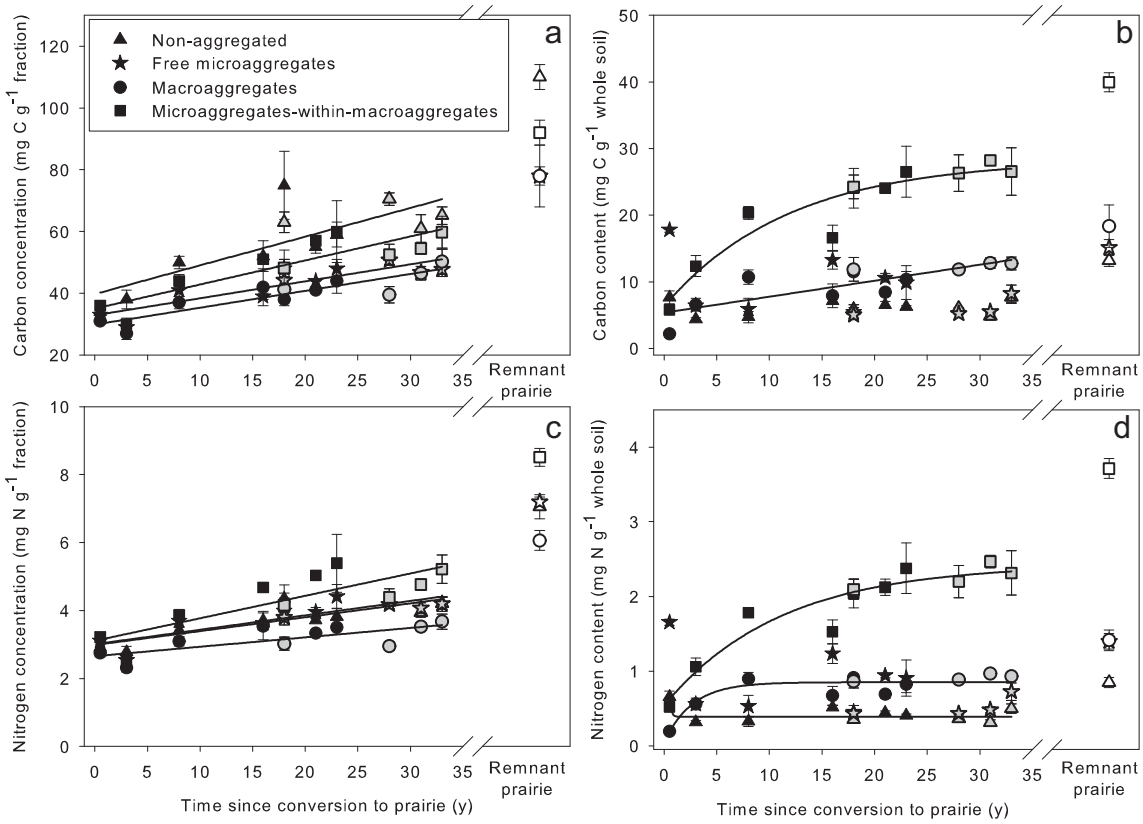

Fig. 3. Recovery of soil C and N in hierarchical aggregates from soils of a prairie restoration chronosequence at 0–5 cm depth. (a) C concentration, (b) C content, (c) N concentration, and (d) N content. Symbols represent plot level means ($n = 3$) and error bars are SE. Black symbols represent restored prairies and a soybean field sampled in 1999, gray symbols represent restored prairies sampled again in 2009, and open symbols represent a never-cultivated remnant prairie that was not included in the regressions. **C concentration** – *Non-aggregated*: Intercept = 39.7 ± 4.9; slope = 0.93 ± 0.24; $P < 0.01$; $r^2 = 0.63$; *Free Microaggregates*: Intercept = 32.9 ± 2.2; slope = 0.55 ± 0.10; $P < 0.01$; $r^2 = 0.75$; *Macroaggregates*: Intercept = 29.9 ± 1.9; slope = 0.55 ± 0.10; $P < 0.01$; $r^2 = 0.80$; *Microaggregates-within-macroaggregates*: Intercept = 35.1 ± 2.9; slope = 0.78 ± 0.14; $P < 0.01$; $r^2 = 0.78$. **N concentration** – *Non-aggregated*: Intercept = 3.0 ± 0.2; slope = 0.04 ± 0.01; $P < 0.01$; $r^2 = 0.73$; *Free Microaggregates*: Intercept = 3.0 ± 0.2; slope = 0.04 ± 0.01; $P < 0.01$; $r^2 = 0.71$; *Macroaggregates*: Intercept = 2.7 ± 0.2; slope = 0.03 ± 0.01; $P = 0.01$; $r^2 = 0.54$; *Microaggregates-within-macroaggregates*: Intercept = 3.1 ± 0.3; slope = 0.07 ± 0.01; $P < 0.01$; $r^2 = 0.69$. **C content** – *Macroaggregates*: Intercept = 5.4 ± 1.2; slope = 0.24 ± 0.06; $P < 0.01$; $r^2 = 0.65$; *Microaggregates-within-macroaggregates*: $C_o = 6.3 \pm 2.5$; $C_a = 22.0 \pm 3.1$; $k = 0.08 \pm 0.04$; $P < 0.01$; $r^2 = 0.89$. **N content** – *Non-aggregated*: $C_o = 7.49 \pm 15.45$; $C_a = -7.10 \pm 15.47$; $k = 6.54 \pm 4.31$; $P + 0.03$; $r^2 = 0.57$; *Macroaggregates*: $C_o = 0.05 \pm 0.16$; $C_a = 0.80 \pm 0.16$; $k = 0.38 \pm 0.17$; $P < 0.01$; $r^2 = 0.82$; *Microaggregates-within-macroaggregates*: $C_o = 0.54 \pm 0.21$; $C_a = 1.89 \pm 0.24$; $k = 0.09 \pm 0.04$; $P < 0.01$; $r^2 = 0.90$.

significant increases in C or N content at the deeper depth. No other non-aggregated pools had significant mass, C, or N relationships with time.

In free microaggregate pools, no trends in C and N contents occurred at either depth (Fig. 5). In the silt and clay pools, mass losses combined with increases in C and N concentrations tended to offset each other, whereas no measured aspect of Fm-POM changed significantly over time. Mass of Fm-silt declined exponentially, reaching a steady state at about 5 years ($P < 0.01$, $r^2 = 0.87$). The C and N concentrations of Fm-silt increased linearly at the 0–5 cm depth (C: $P < 0.01$, $r^2 = 0.75$; N: $P < 0.01$, $r^2 = 0.63$). Fm-clay C and N concentrations also increased linearly at 0–5 cm (C: $P = 0.01$, $r^2 = 0.58$; N: $P = 0.01$, $r^2 = 0.56$). Fm-clay C concentration increased linearly at the 5–15 cm depth ($P = 0.02$, $r^2 = 0.69$), and it was the only significant regression at 5–15 cm. By 33 years after restoration, Fm-silt reached 57% (C) and 55% (N) of remnant concentrations, whereas Fm-clay achieved as much as 84% (C) and 80% (N) of remnant concentrations at 0–5 cm. Agriculture-induced C loss was not as severe in the 5–15 cm layer for Fm-clay, and thus only a similar rate of increase returned the pool to 97% of the remnant prairie C concentration.

In macroaggregates, sustained increases in M-POM C and N contents at 0–5 cm (C: $P < 0.01$, $r^2 = 0.83$; N: $P < 0.01$, $r^2 = 0.82$; Fig. 6) were driven by linear increases in M-POM mass ($P = 0.03$, $r^2 = 0.43$) without any significant temporal trends in M-POM C and N concentrations. Yet, the M-POM C content in the oldest prairie was only 59% of the C observed in the remnant, and the POM-N was only 48% of that in the remnant. Macroaggregate silt reached a steady state for N content ($P < 0.01$) with a predicted value that was 73% of the remnant N content. A similar tendency was observed for C content ($P = 0.01$ but non-significant k). However, there were no significant regressions for M-silt mass, C concentration, or N concentration. Although M-clay mass did not change significantly over time, linear increases in M-clay C and N concentrations (C: $P < 0.01$, $r^2 = 0.68$; N: $P < 0.01$, $r^2 = 0.59$) induced significant increases in M-clay C and N contents (Fig. 6). The C and N contents of the M-clay were best described by linear models (C: $P < 0.01$, $r^2 = 0.62$; N: $P < 0.01$, $r^2 = 0.65$), but the value in the oldest prairie was nearly at the remnant level (93% of remnant C and 91% of remnant N) and exponential models fit the data nearly as well. At 5–15 cm, the only significant changes in any attribute of the macroaggregate fractions were linear increases in M-clay C and N concentrations (C: $P < 0.01$, $r^2 = 0.86$; N: $P = 0.02$, $r^2 = 0.70$).

Most of the SOM pools in microaggregates-within-macroaggregates changed with time. The C and N contents of mM-POM increased linearly at 0–5 cm (C: $P < 0.01$, $r^2 = 0.95$; N: $P < 0.01$, $r^2 = 0.94$) and 5–15 cm (C: $P < 0.01$, $r^2 = 0.83$; N: $P < 0.01$, $r^2 = 0.87$; Fig. 7). This steady increase was driven largely by a linear increase in mM-POM mass (0–5 cm: $P < 0.01$, $r^2 = 0.74$; 5–15 cm: $P < 0.01$, $r^2 = 0.66$), since mM-POM C and N concentrations did not

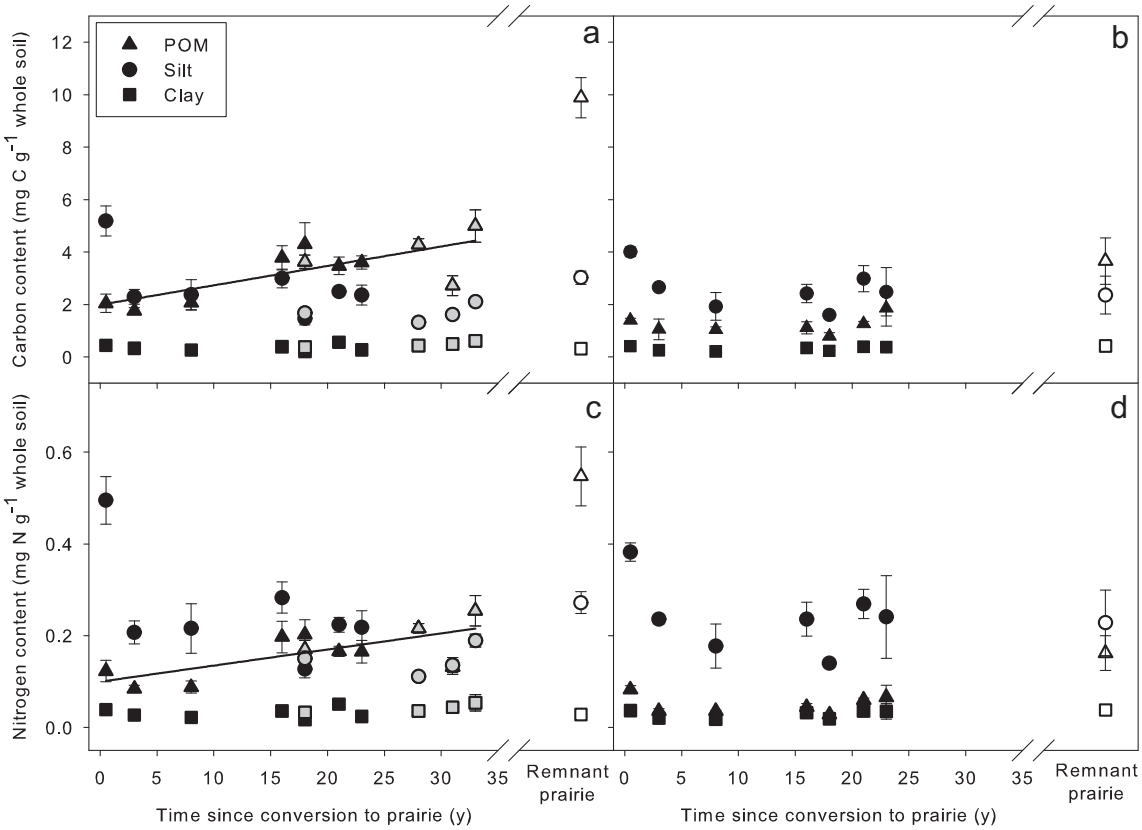

Fig. 4. Dynamics of C (a & b) and N (c & d) associated with non-aggregated particulate organic matter (POM), silt, and clay in soils of a prairie restoration chronosequence at 0–5 cm (a & c) and 5–15 cm (b & d) depths. Symbols represent plot level means ($n = 3$) and error bars are SE. Black symbols represent restored prairies and a soybean field sampled in 1999, gray symbols represent restored prairies sampled again in 2009, and open symbols represent a never-cultivated remnant prairie that was not included in the regressions. Aggregates were not extracted from the 5–15 cm depth in soil collected in 2009. **0–5 cm:** *POM-C*: Intercept = 2.0 ± 0.4; slope = 0.07 ± 0.02; $P = 0.01$; $r^2 = 0.58$; *POM-N*: Intercept = 0.10 ± 0.02; slope = 0.004 ± 0.001; $P = 0.01$; $r^2 = 0.51$.

vary with time. These increases are unlikely to be sustained because the mM-POM C and N contents were similar to the remnant prairie by 33 years (4.8 ± 0.1 mg C and 0.30 ± 0.01 mg N g^{-1} whole soil in Prairie F in 2009 compared to 4.7 ± 0.2 mg C and 0.33 ± 0.01 mg N g^{-1} whole soil in the remnant prairie; exponential regressions also fit reasonably well). At 0–5 cm, mM-silt C and N concentrations increased linearly (C: $P < 0.01$, $r^2 = 0.69$; N: $P < 0.01$, $r^2 = 0.62$), but mass constraints led to asymptotic changes in C and N content (Fig. 7). The mM-silt C and N contents leveled off well below the values measured in the remnant prairie at this depth, reaching 59% and 56% of remnant prairie C and N, respectively. For mM-clay at 0–5 cm, both the C and N concentrations increased linearly (C: $P = 0.04$, $r^2 = 0.40$; N: $P = 0.02$, $r^2 = 0.47$) leading to C and N contents that increased linearly but reached the level of the remnant prairie within the 33-year observation period (Fig. 7). At 5–15 cm, mM-silt and mM-clay both had significant linear increases in C concentration (silt: $P = 0.05$, $r^2 = 0.56$; clay: $P = 0.02$, $r^2 = 0.70$), but these did not lead to significant regressions for C content. No significant changes in mM-silt or mM-clay mass were found at 5–15 cm.

The distribution of soil C among measured pools changed substantially following reestablishment of tallgrass prairie (Fig. 8). A larger percentage of soil C occurred in non-aggregated and macroaggregated POM in the remnant prairie and older restorations compared to the agricultural field and younger restored prairies. An even larger shift occurred in the relative distribution of silt-C, which contributed most to whole soil C. In fact, the majority of soil C was in non-aggregated and free microaggregate silt (15% and 39% of whole soil C, respectively) in the agricultural field, and shifted to macroaggregate and microaggregate-within-macroaggregate silt in the prairies (11% and 31%, respectively).

4. Discussion

4.1. Carbon and nitrogen accrual in hierarchical aggregates

Physicochemical protection appears to first facilitate and then constrain total soil C and N accumulation as restored prairies mature. A dramatic redistribution of soil mass dominated the early stages of SOM recovery (Fig. 2). Free microaggregates were apparently incorporated into new macroaggregates, probably due to physical entanglement in developing root and hyphal networks in the early stages of restoration (Tisdall and Oades, 1982; Miller and Jastrow, 1990; Jastrow et al., 1998). In addition, C and N concentrations increased linearly in nearly all of the aggregate pools at 0–5 cm depth (Fig. 3). Together, these results suggest that sufficient organic materials were available during the early stages of ecosystem restoration to support formation of water-stable aggregates, but that these aggregates still had capacity to accumulate additional SOM. The combined effects of tillage cessation and high perennial belowground biomass allocation replenished root and microbial binding agents in these prairies (Jastrow et al., 1998; Allison et al., 2005; Matamala et al., 2008), encouraging regeneration of a stable hierarchical distribution of macro- and microaggregate structures (Fig. 2), increasing the stability of those structures (Jastrow and Miller, 1998), and promoting C and N accumulation in the early stages of restoration (Fig. 3). Our findings are also consistent with the consensus view that cessation of tillage promotes assembly of

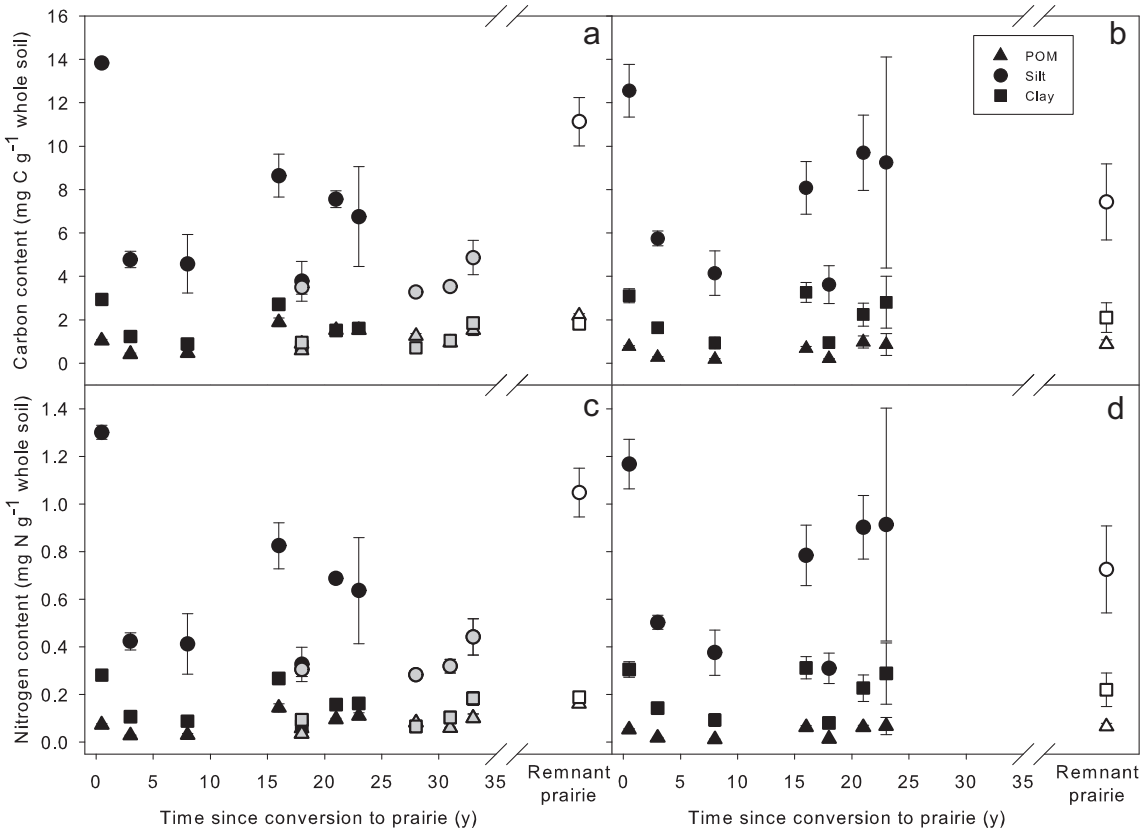

Fig. 5. Dynamics of C (a & b) and N (c & d) associated with free microaggregate particulate organic matter (POM), silt, and clay in soils of a prairie restoration chronosequence at 0–5 cm (a & c) and 5–15 cm (b & d) depths. Symbols represent plot level means ($n = 3$) and error bars are SE. Black symbols represent restored prairies and a soybean field sampled in 1999, gray symbols represent restored prairies sampled again in 2009, and open symbols represent a never-cultivated remnant prairie that was not included in the regressions. Aggregates were not extracted from the 5–15 cm depth in soil collected in 2009.

stable macroaggregates that facilitate formation and stabilization of microaggregates within the larger aggregates, which is a key process for soil C stabilization (Six et al., 2000, 2004).

Sustained increases in aggregate-associated C and N concentrations are unlikely to persist indefinitely because theoretical limits to ecosystem function may eventually slow rates of change in these restored near-surface soils (Jenny, 1941; Olson, 1963; Six et al., 2002; Post et al., 2004). Given that aggregate C and N increased linearly for 33 years from 31–43% to 58–65% of concentrations in comparable pools of the remnant prairie, a slowing of enrichment is likely in the coming decades. Indeed, mass limitations to soil C accrual in the surface 5 cm were already evident in most of the intact aggregate pools; C concentration increased linearly, but C content reached steady state as the aggregate mass stabilized (Fig. 3). Mineral surface area and pore spaces that protect SOM from microbial mineralization are finite (e.g. Hassink, 1997; Kaiser and Guggenberger, 2007; McCarthy et al., 2008). Thus for a given set of inputs and environmental conditions, aggregate-occluded SOM is expected to eventually reach a steady state and limit the duration of linear soil C accrual (Six et al., 2002; Stewart et al., 2007). This potential constraint was particularly evident in the microaggregate-within-macroaggregate pool, which was the greatest contributor to total soil C and N but was already exhibiting a strongly asymptotic change in C and N content by 33 years (Fig. 3).

4.2. Carbon and nitrogen accrual in component pools of hierarchical aggregates

Accrual trajectories of aggregate components (POM, silt, and clay) highlighted stabilization mechanisms in aggregates that impact whole soil C and N. For example, at 0–5 cm the C and N contents of both M-silt and mM-silt reached apparent steady states within the first 33 years of prairie restoration at levels that were well below the remnant prairie (Figs. 6a,c and 7a,c). Matamala et al. (2008) predicted that whole soil C in this same chronosequence might require >400 years to reach 95% of the C stocks in the surface soil of remnant prairie on the basis of regressions that included the first 26 years of restoration and the remnant. However, the rate of whole soil C accrual was essentially linear during the first few decades of restoration (Matamala et al., 2008; O'Brien et al., 2010) whereas the C and N contents of M-silt and mM-silt leveled off within this same period. Given that these pools are the greatest components of total soil C and N, they might eventually limit the expected trajectory of whole soil C and N accrual.

In contrast, POM fraction C and N contents increased linearly in every aggregate pool except free microaggregates, which is consistent with the hypothesis that protection afforded by minerals (either through surface binding or occlusion in aggregates) tends to be exhausted before environmental conditions limit increases in less protected pools (Hassink, 1997; Stewart et al., 2007). Somewhat surprisingly, linear regressions best described changes in C and N content at 0–5 cm for M-clay and mM-clay as well (Figs. 6a,c and 7a,c). Although these observations appear counter to the concept that mineral protection is likely to saturate before POM pools, the M-clay and mM-clay C and N contents in the oldest restored prairies are so similar to those of the remnant prairie that we suspect saturation is imminent. The stability of C and N in these pools suggests that (1) the associated OM was not vulnerable to tillage-induced loss, and (2) that the capacity of these pools to protect organic matter was essentially saturated, given that their C

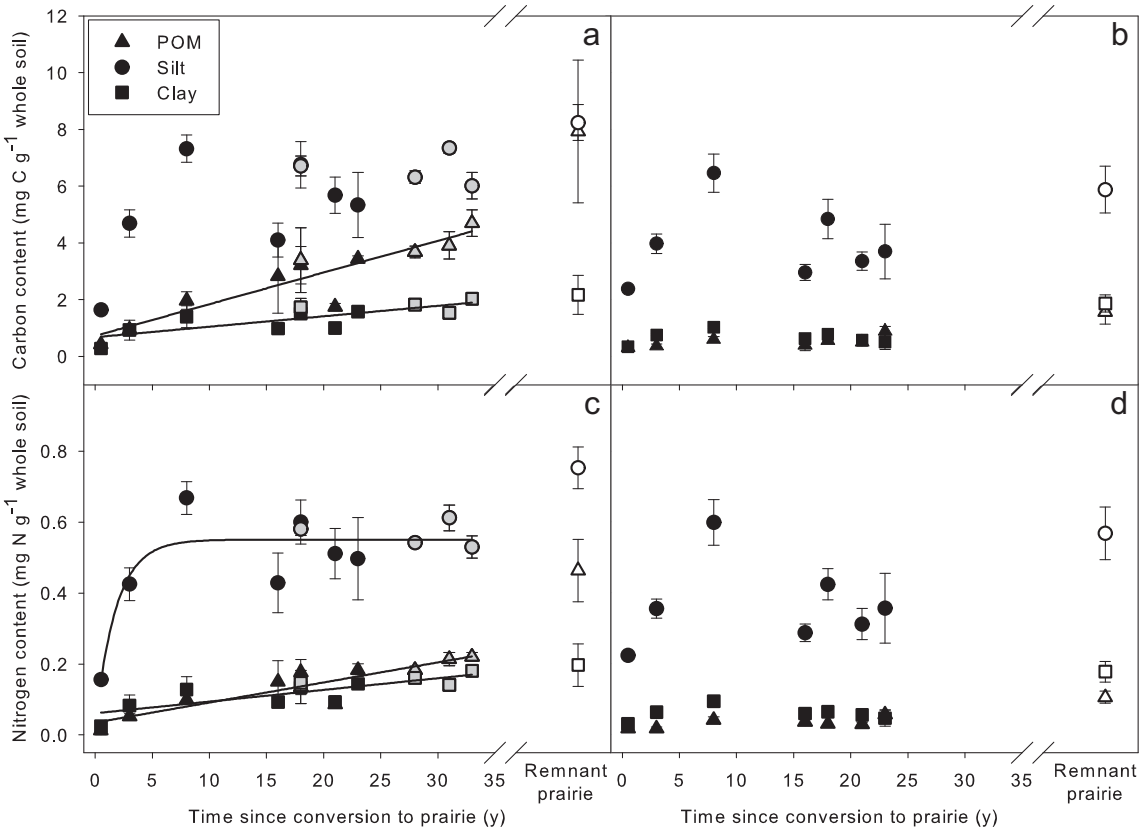

Fig. 6. Dynamics of C (a & b) and N (c & d) associated with macroaggregate particulate organic matter (POM), silt, and clay in soils of a prairie restoration chronosequence at 0–5 cm (a & c) and 5–15 cm (b & d) depths. Symbols represent plot level means ($n = 3$) and error bars are SE. Black symbols represent restored prairies and a soybean field sampled in 1999, gray symbols represent restored prairies sampled again in 2009, and open symbols represent a never-cultivated remnant prairie that was not included in the regressions. Aggregates were not extracted from the 5–15 cm depth in soil collected in 2009. **0–5 cm** – *POM-C*: Intercept = 0.73 ± 0.35; slope = 0.11 ± 0.02; $P < 0.01$; $r^2 = 0.83$; *POM-N*: Intercept = 0.03 ± 0.02; slope = 0.006 ± 0.001; $P < 0.01$; $r^2 = 0.82$; *Silt-N*: $C_o = 0.03 \pm 0.14$; $C_a = 0.52 \pm 0.14$; $k = 0.55 \pm 0.30$; $P < 0.01$; $r^2 = 0.77$; *Clay-C*: Intercept = 0.68 ± 0.20; slope = 0.04 ± 0.01; $P < 0.01$; $r^2 = 0.62$; *Clay-N*: Intercept = 0.06 ± 0.02; slope = 0.003 ± 0.001; $P < 0.01$; $r^2 = 0.65$.

contents in the first decades after restoration were similar to the remnant prairie even though organic inputs were much higher in the remnant (Matamala et al., 2008).

The dynamics of mM-silt, however, were more complicated (Fig. 7). Even though the C and N contents of mM-silt reached an apparent steady state well below the remnant within decades, slow increases in the C and N concentrations of this fraction were sustained for 33 years in near-surface soil. Soil fractionation schemes isolate operationally defined pools that are not as homogeneous as conceptual pools (Olk and Gregorich, 2006; Von Lutzow et al., 2007), and the heterogeneity of silt fractions may explain the seemingly premature steady states. Complex silt-sized aggregates have been shown to play an important role in SOM stabilization (Plante et al., 2006; Virto et al., 2008). In the first decades of prairie development, the dynamics of occluded silt were likely dominated by an increase in C associated with silt-sized aggregates and mineral surfaces following an asymptotic accumulation trajectory as suggested by the saturation conceptual model (Six et al., 2002). However, silt-sized but uncomplexed organic matter probably also accumulated in the mM-silt fraction. This uncomplexed organic matter (which we observed but were unable to isolate effectively) might continue to build up and approach pre-cultivation levels – given a combination of more time, more inputs, and favorable environmental conditions.

One possible mechanism for asymptotic accrual in important pools such as mM-silt is the finite protective capacity provided by submicron-diameter pores in mineral pools. Monreal et al. (2010) characterized complex nanoscale organomineral interactions that stabilized SOM better than association with clays in a soil similar to ours. McCarthy et al. (2008) reported that accumulating C rapidly filled submicron pores within microaggregates as these prairies aged, with some 80% of microaggregate-C occurring in such pores after 24 years of restoration. Although remnant prairie microaggregates contained much more C overall, the proportion of that C occurring in filled submicron pores was lower, suggesting that this mechanism for C accrual had saturated. Thus, they concluded that the additional C in remnant prairie microaggregates occurs in larger sized pores, where its stability is controlled by other mechanisms.

4.3. Potential for carbon recovery and saturation

Although it is possible that the disturbance from agriculture was so profound that internal soil C and N dynamics were irrevocably altered, the distribution of C and N among fractions became similar to the remnant prairie as the restorations matured (Fig. 8), suggesting that internal C and N dynamics begin to recover during the first decades after restoration. Most of the early changes, notably a shift from NA-silt and Fm-silt dominance in the agricultural field to M-silt and mM-silt in prairies, were due to redistribution of soil mass stemming from reassembly of macroaggregates after cessation of tillage. However, concomitant increases in C concentration also contributed to shifts in C distribution among the aggregate-protected pools. Although total C stocks in the older restored prairies were lower than those of the remnant prairie, the proportion of soil C in mineral-associated pools was relatively similar.

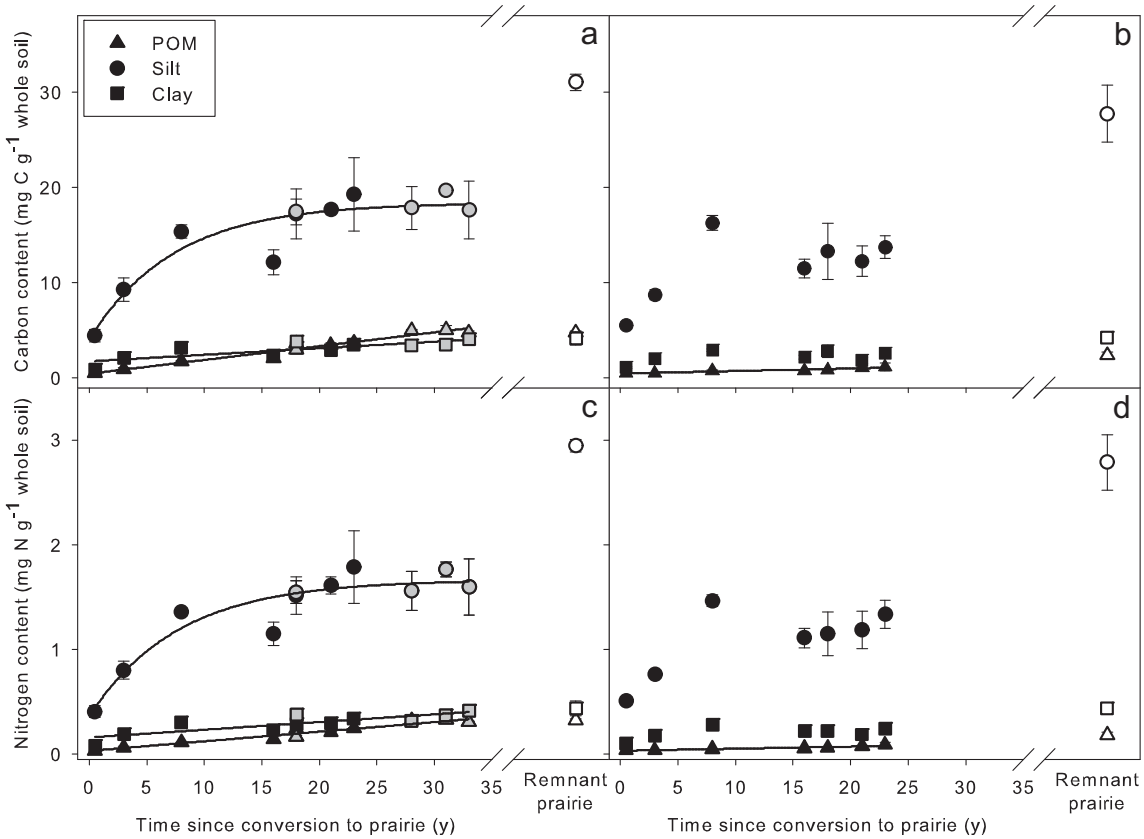

Fig. 7. Dynamics of C (a & b) and N (c & d) associated with microaggregate-within-macroaggregate particulate organic matter (POM), silt, and clay in soils of a prairie restoration chronosequence at 0–5 cm (a & c) and 5–15 cm (b & d) depths. Symbols represent plot level means ($n = 3$) and error bars are SE. Black symbols represent restored prairies and a soybean field sampled in 1999, gray symbols represent restored prairies sampled again in 2009, and open symbols represent a never-cultivated remnant prairie that was not included in the regressions. Aggregates were not extracted from the 5–15 cm depth in soil collected in 2009. **0–5 cm** – *POM-C*: Intercept = 0.46 ± 0.23; slope = 0.14 ± 0.01; $P < 0.01$; $r^2 = 0.95$; *POM-N*: Intercept = 0.03 ± 0.02; slope = 0.009 ± 0.001; $P < 0.01$; $r^2 = 0.94$; *Silt-C*: $C_o = 4.1 \pm 2.1$; $C_a = 14.3 \pm 2.0$; $k = 0.14 \pm 0.06$; $P < 0.01$; $r^2 = 0.87$; *Silt-N*: $C_o = 0.36 \pm 0.17$; $C_a = 1.30 \pm 0.17$; $k = 0.13 \pm 0.05$; $P < 0.01$ $r^2 = 0.88$; *Clay-C*: Intercept = 1.76 ± 0.35; slope = 0.07 ± 0.02; $P < 0.01$; $r^2 = 0.64$; *Clay-N*: Intercept = 0.16 ± 0.03; slope = 0.007 ± 0.002; $P < 0.01$; $r^2 = 0.69$. **5–15 cm** – *POM-C*: Intercept = 0.48 ± 0.08; slope = 0.03 ± 0.01; $P < 0.01$; $r^2 = 0.83$; *POM-N*: Intercept = 0.03 ± 0.01; slope = 0.002 ± <0.001; $P < 0.01$; $r^2 = 0.87$.

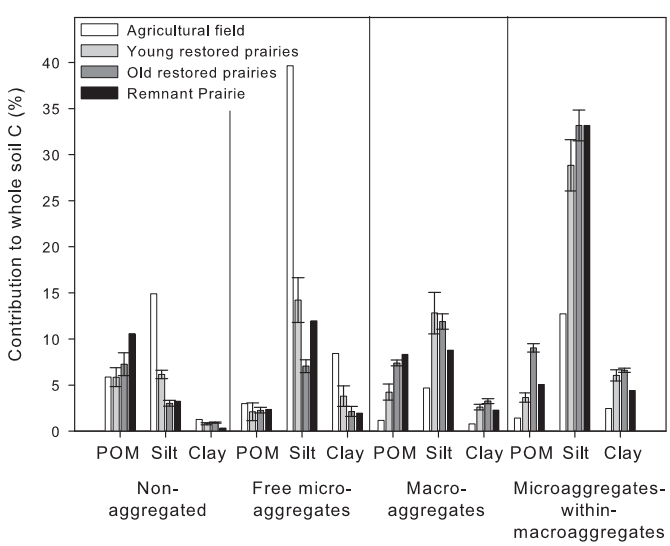

Fig. 8. Carbon distribution among aggregate-occluded particulate organic matter (POM), silt, and clay in an agricultural field (open bars), young restored prairies 3–16 years since planting (light gray bars, $n = 3$, error bars are SE), older restored prairies 28–33 years since planting (dark gray bars, $n = 3$, error bars are SE), and a never-cultivated remnant prairie (black bars).

In contrast, with time an increasingly larger proportion of POM-C was located outside of stable aggregates. Such a distribution is consistent with the hypothesis that increasing contributions from relatively unprotected POM might occur if mineral-associated pools are saturated with C (Six et al., 2002; Stewart et al., 2007).

Carbon accrual in mineral-associated pools appears to be partly attributable to stabilization mechanisms other than simple binding to mineral surfaces. Clay C concentrations were 1.7–5.0 times greater than expected based on the maximal C load method suggested by Feng et al. (2011; assuming that 100% of the clays were 2:1 minerals with a specific surface area of 80 m^2 g^{-1} and a monolayer C load of 1 mg C m^{-2} clay). Such oversaturation indicates that clay-associated SOM was not a monolayer stabilized on clay surfaces, but rather was protected in more complex multi-layer arrangements that probably involved multiple mineral surfaces, i.e., clay-sized aggregates or zonal structures (Chenu and Plante, 2006; Kleber et al., 2007) and organic matter filled pores (McCarthy et al., 2008). In contrast, if silt and clay are considered together, the (mathematically) combined pool has a lower C concentration than expected (predicted was 1.3–2.6 times greater than measured C) except in the remnant prairie (measured was 1.2 times greater than predicted C). The undersaturation of this combined pool is probably due to the inappropriate assumption of a clay-like specific surface area (80 m^2 g^{-1}) for the entire pool even though the silt likely included a substantial proportion of clay bound into silt-sized aggregates (texture analysis was done without removal of organic

matter). Even when these calculations were based on organic matter-free texture determination (the proportion of clay increases from 29% to 36% on average but the proportion of silt is unchanged), both oversaturation of the clay fraction and undersaturation of the silt + clay fraction persisted for the agricultural field and restored prairies. However, organic matter in the remnant prairie soil had a larger effect on texture (by aggregating clay into larger particles). Thus, on the basis of organic matter-free texture, the degree of oversaturation for clay C in remnant prairie was reduced from 5.0 times to 2.8 times greater than expected but predicted C for silt + clay decreased to nearly equal the measured C. Other work at this site has shown that the silt is a rather heterogeneous mixture, with a low-C quartz-dominated component that likely has a small surface area and few reactive surfaces (O'Brien et al., 2011), indicating that the assumption of a high surface area with a high potential for SOM stabilization is indeed inappropriate for the silt-sized pool.

Several recent studies have shown that mechanisms other than sorption to mineral surfaces are critical for stabilizing SOM, especially physical protection in soil aggregates (Kong et al., 2005; Kool et al., 2007; Chung et al., 2008; Gulde et al., 2008; Stewart et al., 2008). It is possible that the protection offered by adsorption to mineral surfaces remained relatively intact during cultivation and then saturated quickly following restoration. If so, then continued increases in C concentrations might be sustained by other mechanisms, such as accumulation of uncomplexed organic matter (Turchenek and Oades, 1979), filling of submicron pores with organic matter (McCarthy et al., 2008), and the formation of silt- and clay-sized aggregates (Chenu and Plante, 2006; Virto et al., 2008). It is also possible that the mineral surfaces themselves were not actually saturated, but that the mechanisms that deliver organic matter of the right size and reactivity to mineral surfaces were not yet fully developed after only 33 years.

Several "transient steady-states" could occur along the way to an overall soil steady state due to the heterogeneity and phasing of stabilization mechanisms and time constraints on the transfer of SOM among pools in surface soils (Fig. 9). SOM pools or stabilization mechanisms that achieve steady state because of saturation cannot accrue additional C even when exposed to increased organic inputs (Six et al., 2002). Although we did not manipulate inputs, belowground biomass (and presumably inputs) increased with restoration age and was projected to require more than 60 years to fully recover the near-surface standing stocks present in remnant soils (Matamala et al., 2008). However, even with a gradient of increasing inputs, SOM pools that reached an apparent steady state below pre-cultivation levels during our study are not necessarily saturated, and active C and N building might resume over a longer timespan. For example, a mineral-associated pool with available reactive surface areas might appear saturated across a gradient of inputs if there is limited delivery of SOM of appropriate size and chemistry for sorptive stabilization, e.g., if there is a lag between when the observation is made and the time it takes for fresh inputs to be sufficiently rendered to interact with minerals or for such inputs (or resulting microbial residues) to simply encounter available mineral surfaces. In other words, inputs to a particular pool might be limited for some time period even if the total inputs to the soil are in excess. On the other hand, because measured SOM pools are typically not homogenous, some pools could continue to gain C in the form of uncomplexed organic matter or organic agglomerations even if the surfaces of mineral particles in that pool are entirely occupied by SOM. Either of these mechanisms might be at work in the mM-silt, where asymptotic regressions for C and N content and linear regressions for C and N concentration suggest that the mechanisms controlling accrual could be shifting over time. In addition, few patterns were apparent at the 5–15 cm interval, probably because inputs at this depth were not great enough and/or too variable to drive any SOM pools to steady state within the time frame we observed. Given sufficient time, we expect the unprotected pools of surface soil and pools in the deeper soil to reach steady state as well.

A phased multi-steady-state hypothesis might also explain pools with observed linear accrual rates. These pools should ultimately level off given that we expect whole soil C to follow an asymptotic function that predicts our system will require hundreds of years to achieve steady state (Matamala et al., 2008). Furthermore, linear increases that stop abruptly at an assumed climax

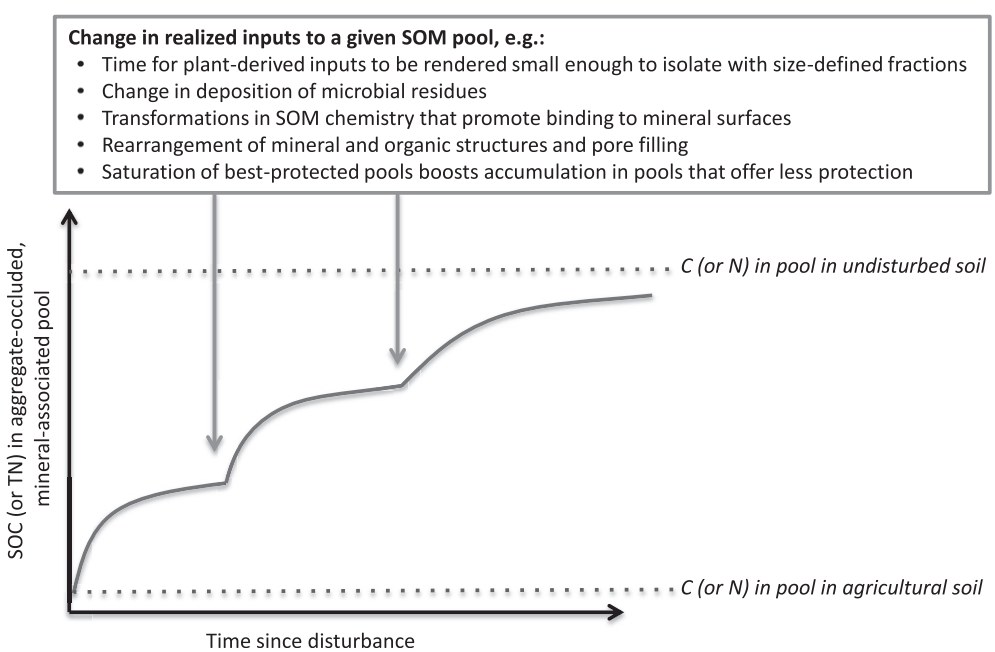

Fig. 9. Hypothesized carbon (or nitrogen) accrual in a pool that exhibits serial steady states. Under such a scenario, the total inputs to the soil are constant over time, but the inputs to a particular fraction change as a result of other mechanisms that control intra-soil C and N cycling and movement of organic matter between different pools.

condition are ecologically unrealistic (Jenny, 1941; Olson, 1963), and asymptotic curves appear linear in their early stages or over short ranges. Therefore, it is likely that C and N accrual rates in pools with non-saturating behavior during the first 33 years of the restoration process are actually on a path to a steady state. For example, the amount of unprotected POM will eventually be controlled by the interplay between organic inputs, decomposer activity, and environmental constraints (Stewart et al., 2008), and aggregate-occluded POM will also be affected by aggregate turnover rates (Plante and McGill, 2002; Yoo et al., 2011). Over time, POM will become a more important component of total soil C and N as observed for the remnant soil (Fig. 8), making the total SOM pool, on average, more labile (see Baldock and Skjemstad, 2000).

In conclusion, the processes and SOM stabilization mechanisms promoting C and N accrual in restored ecosystems are complex. Intricacies arise from many sources: among aggregates (different size classes within the hierarchy), within aggregates (POM, silt, and clay pools), and even within aggregate-occluded pools (e.g., mM-silt is probably itself comprised of pools that vary in SOM-stabilizing importance; see Plante et al., 2006; Virto et al., 2008; O'Brien et al., 2011). SOM does not accumulate at the same pace in all pools, and some pools (or portions of them) may be essentially saturated while others are still building C, even within an aggregate class. Despite this heterogeneity, the overall SOM distribution returned to near pre-cultivation conditions within our chronosequence time frame (Fig. 8). Thus, even though some pools arrived at an initial steady state C or N content below what is presumably possible based on the remnant prairie, physicochemical protection mechanisms can be largely restored in temperate grasslands. However, changes in environmental conditions or ecosystem processes as the restorations continue to mature might still prevent complete recovery of the SOM stocks observed in the remnant. Hence, SOM that builds up during the earlier stages of restoration probably has a better opportunity to become well stabilized, making restored grasslands more important for C sequestration early in their development.

Further research is necessary to more directly observe the small-scale heterogeneity that drives the mechanisms that regulate SOM stabilization. For example, investigation of the chemistry of aggregate-associated SOM pools and of the constraints on microbial mineralization/stabilization of SOM in aggregate-occluded pores could help explain time lags and mechanisms behind the multi-steady-state behaviors hypothesized for SOM pool dynamics in these restored grasslands.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.01.031.

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