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ORIGINAL ARTICLE

Subsurface Soil Carbon and Nitrogen Losses Offset Surface Carbon Accumulation in Abandoned Agricultural Fields

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Abstract

Abandoned agricultural fields (old fields) are thought to accumulate soil organic matter (SOM) after cultivation cessation. However, most research on old fields soil carbon (C) and nitrogen (N) sequestration has focused on the surface (10 or 30 cm depth) and overlooked their dynamics below 30 cm. This study quantified C and N stock change in both the surface and subsurface with repeated inventories over 13 years. We conducted repeated soil surveys in 8 old fields that form a 64-year chronosequence at Cedar Creek Ecosystem Science Reserve (CCESR), Minnesota in 2001 and 2014. On average, soil C and N accumulated by 16.5 ± 14.5 g C m^{-2} y^{-1} and 1.0 ± 1.1 g N m^{-2} y^{-1} in the surface (0–20 cm). In contrast, we found soil C and N decreased by 78.9 ± 26.3 g C m^{-2} y^{-1} and 12.9 ± 2.42 g N m^{-2} y^{-1} in the subsurface (20–100 cm). The C and N losses in the subsurface soil were correlated with low deep root biomass; the majority of roots are located in the top 20 cm of soil. Such root distribution may be attributed to the continuing dominance of nonnative and shallow-rooted C3 grasses and the lack of legumes after field abandonment. This study shows that agriculture has a long legacy effect after abandonment on subsurface soil C and N. Some abandoned agricultural fields can continue to lose C and N because surface C and N accumulation does not offset the ongoing deeper soil C and N losses.

Key words: abandoned agricultural fields; grassland; carbon cycling; nitrogen cycling; carbon sequestration; subsurface soil; species composition; long-term soil survey.

Highlights

- Agricultural abandonment led to net losses of C and N in the 0–100 cm soil profile
- Soil C and N accumulated in the surface yet decreased in the subsurface in old fields
- Land-use change had long legacy effects on subsurface soil C and N

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Author contributions YY and JK contributed equally on designing the study, performing the research, analyzing the data, and writing the paper.

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Introduction

Abandoned agricultural fields have received growing research interest because an increasing number of degraded agricultural lands are abandoned due to decreasing agricultural profitability (Ustaoglu and Collier 2018) and conservation purposes (USDA 2020), especially in developed countries (Rey Benayas 2007; Lark and others 2020). These lands have great potential for soil C sequestration (Knops and Tilman 2000; Post and Kwon 2000; Knops and Bradley 2009). When lands are used for agricultural production, extensive soil disturbance tends to accelerate SOM decomposition (Knops and Tilman 2000; Conant and others 2001), leading to greenhouse gas emissions (Hutchinson and others 2007). Once agricultural lands are abandoned, and soil disturbance ceases, the soil C stocks can recover to the previous level (Post and Kwon 2000; Jones and Donnelly 2004). However, widely varying rates of C accumulation have been reported (McLauchlan and others 2006).

To date, much of the research on C sequestration in abandoned agricultural fields has focused on the surface soil (Jia and others 2020), and much less is known about deeper depths (Rumpel and Kögel-Knabner 2011). The dynamics of subsoil C may even be more important in terms of C storage than surface soil C (Rumpel and Kögel-Knabner 2011). About 50% of the C stocks in the first 1 m of soil in temperate grasslands are stored between 30 and 100 cm (Jobbágy and Jackson 2000). Any change in this amount of soil C can have significant impacts on the fluxes of atmospheric CO_2 in the abandoned agricultural fields. Yet, our knowledge of the dynamics of subsurface SOM, as well as the factors influencing subsoil C, are limited.

Our understanding of the factors that control C dynamics in surface soil might not apply to the subsurface soil, as C inputs and SOM stability are different between the surface and subsurface soil (Wordell-Dietrich and others 2017). Roots are an important source of the C input (Rumpel and Kögel-Knabner 2011) for both surface and subsurface soil. However, carbon flux from roots to the soil is much lower in the subsurface soil as compared to the surface soil, because the root biomass decreases sharply with depth, especially in grassland ecosystems (Jackson and others 1996). Previous research also suggests subsurface SOM stabilization is influenced by the availability of labile substrates, that is, the priming effect (Fontaine and others 2007). However, whether the priming effect is positive or negative, as well as its controlling factors, are still poorly understood (Kuzyakov 2010; Pausch and Kuzyakov 2018). In addition, changes in temperature and nutrient availability also impact the subsoil C dynamics (Fierer and others 2003). This variability highlights the need for improving our understanding of the dynamics of the C stocks in the soil below 20 cm to obtain accurate estimates of the total soil C storage potential of abandoned agricultural areas.

To understand the dynamics of both surface and subsurface soil C after agricultural abandonment, we conducted repeated soil surveys in old fields at Cedar Creek Ecosystem Science Reserve (CCESR), Minnesota, which has a long history of monitoring soil C and N dynamics after agricultural abandonment from crop cultivation. Knops and Tilman (2000) using a repeated survey of the surface soil of 21 old fields which include the 8 old fields in this study, demonstrated that soil C stocks decreased by 89% and soil N decreased by 75% during the agricultural period, and predicted that it required 180 years for C and 230 years for N to recover to pre-agricultural levels. They also concluded that plant functional group composition significantly affects the accumulation rate of both soil C and N. However, the study of Knops and Tilman (2000) was based on only the top 10 cm of soil depth. Knops and Bradley (2009) subsequently conducted a one-time inventory of soil C and N stocks in the same old fields down to 1 m with six depth intervals, and they found C and N accumulated in the surface soil and did not find significant trends in the subsoil. This could be attributed to the fact that the one-time inventory had to use a chronosequence approach, and inherent spatial variability among fields in soil conditions at the time when the old fields were abandoned and slow C and N change rates in the subsoil, which make it difficult to detect changes (Kravchenko and Robertson 2011; Maillard and others 2017). Finally, a recently published study (Yang and others 2019) of a fenced and species richness controlled experiment at CCESR demonstrated that soil C accumulated over a 21-year period in both 0–20 cm and 20–60 cm depths, and the C accumulation is positively associated with species richness and the abundance of legume and C4 grasses. However, in the experiment site of Yang and others (2019), large herbivores, such as deer and pocket gophers, were excluded from the field, and vegetation species were planted and controlled. Therefore, we still lack the understanding of how soil C and N stocks change in subsurface soil in unmanaged grasslands reestablished after agricultural abandonment.

In this study, we report the soil C and N stocks and vegetation surveys in 2001 and 2014 in 8 old fields at CCESR. With repeated surveys, our study directly compares soil C and N stocks changes in the same locations. We hypothesized that (1) C and N accumulate in the surface and the subsurface soil with higher rates in the surface after agricultural abandonment and (2) the soil C and N accumulation rates are positively related to species richness and the abundance of C4 and legume species.

Materials and Methods

Site Description

The study site (CCESR) is located in East Bethel, Minnesota (USA) (42° 25′ N, 93° 10′ W). The growing season is from May through August, with mean annual temperature of 6 °C and mean annual precipitation of 750 mm (Hijmans and others 2005). The soils at CCESR were formed from glacial outwash and are well-sorted fine (Sartell and Zimmerman series) and medium (Nymore series) sands (> 85% sand in upland soils) (Grigal and others 1974). These soils are characterized by low N and organic matter content and are well-drained and aerated (Grigal and others 1974).

CCESR natural vegetation is a mix of oak savanna, perennial grasslands, upland deciduous forest, and lowland marshes (Cook and Allan 1992). European settlement started in the late nineteenth century and most fields were first cultivated between 1900 and 1910 (Pierce 1954). Common crops grown in this area were corn, potatoes, wheat, rye, and soybeans (Inouye and others 1987). Over the past 100 years, most of the agricultural fields have been abandoned and a natural vegetation succession has occurred. With increasing age after field abandonment, the species composition has shifted from early dominance by annual forbs to C3 grasses to C4 grasses (Inouye and others 1987). In most of the abandoned fields, woody species are rare and consist of *Quercus macrocarpa* and *Quercus ellipsoidalis* (Inouye and others 1987).

We conducted repeated soil surveys in 8 old fields at CCESR. The old fields are a part of a long-term observation experiment set up in 1983 to document secondary vegetation succession and soil C and N dynamics. In 2014, the abandonment age (years after abandonment) of the 8 fields ranged from 23 to 87 years. Within each field, four permanent, parallel transects (40 m long and 25 m apart) were set up for vegetation composition sampling and soil C and N content. Each transect contains 25 permanently marked plots of 1 × 0.5 m plots (Inouye and others 1987). Each field has four, 3 × 3 m plots by one end of each transect that is set up for annual aboveground vegetation biomass and composition sampling.

Soil and Vegetation Sampling and Analyses

This study used soil and vegetation samples collected in the 3 × 3 m plots in 2001 and 2014. (n = 4 per field and per depth interval). For soil sampling, 2.5-cm-wide cores were taken at six depth intervals: 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm. The corer was inserted into each depth interval separately. Samples were dried and sifted with a 2 mm sieve to remove roots. No stones were found in all soil samples. In 2001 and 2014, bulk density was measured by collecting 5-cm-wide soil cores with the same depth intervals as the soil samples in each field. Soils from the 5-cm-wide soil cores at each depth were dried and weighed to calculate bulk density.

For vegetation samples, we first collected aboveground biomass and litter by clipping a 10 × 300 cm strip at one end of each transect in each field (n = 4 per field) in mid-August 2001 and 2014. Samples were first sorted into species and litter, dried, and weighed. The aboveground vegetation samples were subsequently aggregated into functional groups (C3 grass, C4 grass, litter, legume, forb, sedge, wood, moss, lichen, and pine needles) before combining for C and N analyses. Root samples were collected in the clip strips with a 5-cm-wide corer at the same depths as soil sampling (n = 4 per field and per depth interval). The collected soil cores were washed over 1 mm screens. Then the roots were dried and weighed. All soil and vegetation samples were dried at 55 °C. Soil samples were ground with coffee mills, and the roots, shoots, and litter were ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ). The ground samples were analyzed for total C and N content with a Costech ECS 4010 (Costech Analytical Technologies, Valencia, CA). A subset of the soil samples was tested with acid for inorganic C and none was detected. We calculated the soil C and N stocks using the equivalent soil mass method presented in von Haden and others (2020) with the measured C and N concentrations and bulk density and used the soil samples at each depth collected in 2001 as the reference masses.

Data Analyses

The differences of C and N stock at different depths in 2001 and 2014 were compared with linear mixed-effect models with sampling year, soil depth, and their interactions as the fixed effect and the field and plot nested in each field as the random effects. This model structure ensures that sample values are compared in the same field, plot, and depth between the two sampling years. Linear mixed-effect models with sampling year, soil depth, field age (as a continuous variable), and their interactions as the fixed effects and field as the random effect were used to compare the soil C and N stocks across the chronosequence. This model structure ensures the sample values are compared across fields with different field ages. The differences of soil C and N in pooled depths (0–20 cm, 20–100 cm, and 0–100 cm) were computed using linear mixed-effect models with year, pooled depths, and their interactions as the fixed effect and the field and plot nested in each field as the random effects. Soil C and N stock values were log-transformed when performing the statistical analyses and back-transformed for creating the figures. The differences between aboveground biomass and litter between two sampling years and the difference in root biomass at each depth between two samplings were each tested with nested two-way ANOVA. Linear mixed-effect models were used to investigate the relationship between soil C and N stock changes (stocks in 2014 minus stocks in 2001) at the surface (0–20 cm) and the subsurface (20–100 cm) with vegetation composition, species richness, and above- and below-ground biomass. All statistical analyses were performed using R version 4.1.1 (R Core Team, 2021). 'lme4' (Bates and others 2015) was used to perform linear mixed-effect models and nested ANOVA; 'emmeans' (Russell 2019) was used to perform pairwise comparison for the linear mixed-effect models and nested two-way ANOVA. 'ggplot2' (Wickham, 2016) was used to create the figures.

RESULTS

13-Year Changes in Soil C, N, and C:N Ratio in Old Fields

On average, the repeated soil surveys show that the soil of 100 cm depth lost 812.4 ± 485.3 g m^{-2} of C and 155.3 ± 42.3 g m^{-2} of N over the 13 years between 2001 and 2014. In the surface soil, the C and N stock significantly increased in 0–10 cm, while soil stocks did not differ in 10–20 cm (Figure 1a and b and Tables 1 and 2). Combining the data from these two depths, C and N stocks increased in the top 20 cm at average rates of 16.5 ± 14.5 g C m^{-2} y^{-1}, and 1.0 ± 1.1 g N m^{-2} y^{-1}, respectively. In contrast to the surface, the soil C and N stocks decreased significantly at all depths below 20 cm, and the combined soil layers had lost at the rate of 78.9 ± 26.3 g C m^{-2} y^{-1} and 12.9 ± 2.4 g N m^{-2} y^{-1} (Figure 1a, b and Tables 1 and 2). The soil C: N ratios also had a decreasing trend in deeper depths, which was statistically significant at 40–60 cm, 60–80 cm, and 80–100 cm (Supp. Figure 1).

The 8 old fields also form a chronosequence because they were abandoned at different times ranging from 1927 to 1991. Investigating the soil C and N dynamics with the chronosequence approach, we found that the values in 2001 showed an increase in C stocks with field age in 0–10 cm and 10–20 cm depth, whereas the values of soil C stocks in 2014 did not show a positive relationship in any of the depths (Figure 2). N stocks showed similar trends that the chronosequence only shows a significant positive relationship in 0–10 cm in 2001 values (Figure 3). These results showed the C and N stock in surface soil occurs more in younger fields and the older fields lost more C and N in the subsurface soil.

Biomass and Vegetation Composition in Relation to Soil C and N Stock Changes

To investigate the mechanisms that lead to the observed C and N dynamics in these 8 old fields, we pooled the soil C and N stock changes (stocks in 2014 minus stocks in 2001) into two depths: 0–20 cm and 20–100 cm, then examined the effect of above- and below-ground biomass, species richness and vegetation composition on the change of soil C and N stock. A linear mixed-effect model (Supp. Table 1) showed soil C stock changes at the surface and subsurface were not associated with vegetation species richness, average aboveground biomass between 2001 and 2014, average root biomass between 2001 and 2014 at the surface and subsurface soil, or functional groups (C3, forb, legume, and sedges). The abundance of the C4 functional group is negatively correlated with soil C stock changes, which indicates that fields with more C4 grass lost more C. There were interactions of forbs with soil C stock changes at the two depths, which indicates that fields with a higher presence of forbs tend to lose less C in the subsurface soil. The same patterns hold for soil N (Supp. Table 2).

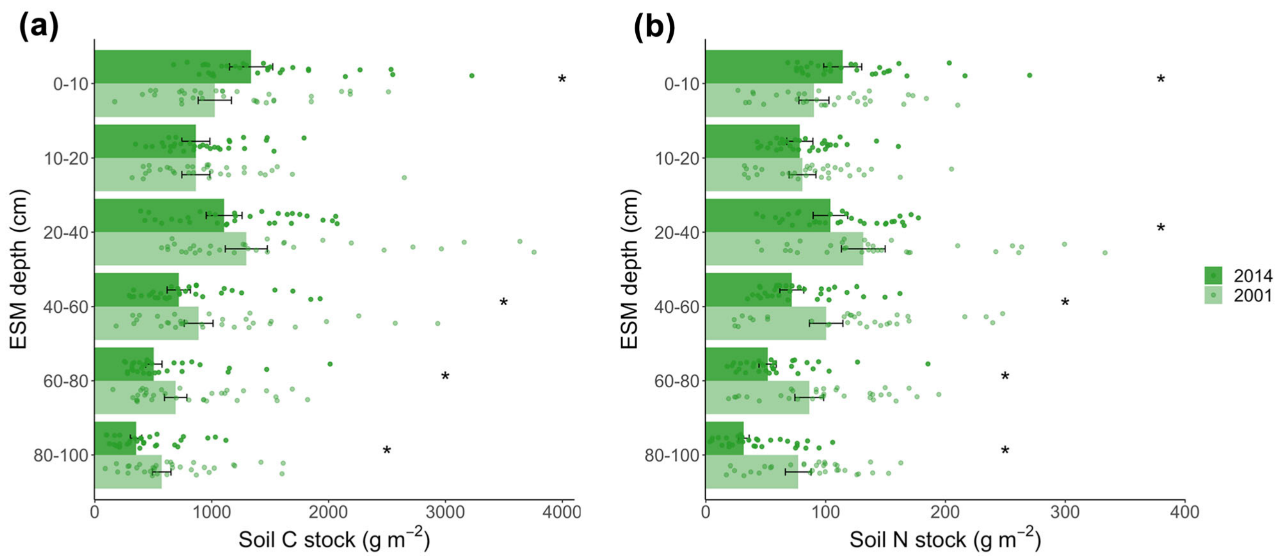

Figure 1. Soil C stocks (**a**), and N stocks (**b**) at different depths in the 8 old fields. The soil C and N stocks are calculated with the equivalent soil mass (ESM) method using 2001 samples as the reference masses and depths. Dark green bars represent data from 2014, and light green bars represent data from 2001. Data shown are the means ± 1 standard error (SE). The circles are the actual soil C and N stocks. The asterisks indicate that the differences between data from the two sampling years and within the same depth are statistically significant at $p < 0.05$. From 2001 to 2014, both C and N accumulated in the 0–10 cm soil but declined below 40 cm.

Soil C and N Inputs

In both 2001 and 2014, aboveground biomass and root biomass did not vary with field age, which implies that organic matter inputs to the soil from both above- and below-ground biomass are similar across the 8 old fields. The average live aboveground biomass in 2014 was significantly higher as compared with 2001, whereas the average litter in 2014 was lower as compared to 2001 (Figure 4a). Root biomass significantly increased in 0–10 cm ($p < 0.0001$), 10–20 cm ($p < 0.0001$), and 20–40 cm ($p < 0.0001$) (Figure 4b). This suggests that root biomass, and thus the root C stock, is accumulating in these old fields.

Discussion

Old fields at CCESR are still losing C and N even up to 88 years after abandonment. The repeated soil surveys showed that the 8 old fields lost on average 812.4 ± 485.3 g m^{-2} of C and 155.3 ± 42.3 g m^{-2} of N between 2001 and 2014 in the 0–100 cm soil profile. The surface (0–20 cm) soil C and N stocks significantly increased by 16.5 ± 14.5 g C m^{-2} y^{-1} and 1.0 ± 1.1 g N m^{-2} y^{-1}, respectively, between 2001 and 2014. However, the subsurface (20–80) soil C and N stocks decreased by 78.9 ± 26.3 g C m^{-2} y^{-1} and 12.9 ± 2.4 g N m^{-2} y^{-1} (Figure 1). The 2014 chronosequence did not show any significant relationship of soil C and N with field age in any of the depths (Figures 2 and 3). These findings suggest that the older fields with longer abandonment history did not accumulate as much of C and N in the surface soil as the younger fields between 2001 and 2014, likely due to the surface soil C and N in the older fields approaching the equilibrium level. The inclusive results in the subsurface soil in both 2001 and 2014 chronosequences showed that the variability of C and N is still substantial, even though the glacial outwash soils at CCESR have relatively similar characteristics, such as topography, soil texture, and soil moisture.

The repeated soil surveys results showed that soil C sequestration rates from surface soil surveys, which is a common practice in abandoned agriculture fields (McLauchlan and others 2006; Foote and Grogan, 2010; Preger and others 2010; Li and others 2017; Zethof and others 2019) are not representing total soil C and N dynamics across the soil profile. Had we only collected soil samples from the surface to monitor long-term C dynamics, we would draw erroneous conclusions. Long-term repeated inventories of C and N stocks at soil depths below 30 cm are rare, yet there is accumulating evidence showing that under land-use change or different management, subsoil C and N react differently compared to the surface soil (Mobley and others 2015; Tautges and others 2019). A recent

Table 1. Results of the Linear Mixed-Effect Model Testing the Impact of Sampling Year, Soil Depth, and Their Interactions on Soil C Stocks

Predictors	Chisq	*Df*	*p*
Intercept	2501.6	1	< **0.0001**
Year	6.6	1	**0.01007**
Depth	78.5	5	< **0.0001**
Year: Depth	31.9	5	< **0.0001**
Random effects			
Residual			0.17
SD (Residual)			0.41
Field			0.11
SD (Field)			0.33
Plot: field			0.04
SD (Plot: Field)			0.19
Number of groups (Field)			8
Number of groups (Plot: Field)			32
Number of observations			384

Post hoc tests (Year: Depth)	**Contrast**	*Df*	*t*	*p*
Depth				
0–10 cm	2001/2004	365	− 2.573	**0.0105**
10–20 cm	2001/2004	365	0.004	0.9972
20–40 cm	2001/2004	365	1.540	0.1245
40–60 cm	2001/2004	365	2.073	**0.0389**
60–80 cm	2001/2004	365	3.066	**0.0023**
80–100 cm	2001/2004	365	4.708	< **0.0001**

The post hoc tests show the contrast of soil C stocks at each depth between 2001 and 2014.
Bold fonts indicates statistical significance

Table 2. Results of the Linear Mixed-Effect Model Testing the Impact of Sampling Year, Soil Depth, Field Age, and Their Interactions on Soil N Stocks

Predictors	Chisq	*Df*	*p*
Intercept	1048.3	1	< **0.0001**
Year	4.9	1	**0.02765**
Depth	32.6	5	< **0.0001**
Year: Depth	66.0	5	< **0.0001**
Random effects			
Residual			0.18
SD (Residual)			0.42
Field			0.10
SD (Field)			0.32
Plot: Field			0.02
SD (Plot: Field)			0.15
Number of groups (Field)			8
Number of groups (Plot: Field)			32
Number of observations			384

Post hoc tests (Year: Depth)	**Contrast**	*Df*	*t*	*p*
Depth				
0–10 cm	2001/2004	365	− 2.202	**0.0283**
10–20 cm	2001/2004	365	0.263	0.7924
20–40 cm	2001/2004	365	2.185	**0.0296**
40–60 cm	2001/2004	365	3.137	**0.0019**
60–80 cm	2001/2004	365	4.802	< **0.0001**
80–100 cm	2001/2004	365	8.284	< **0.0001**

The post hoc tests show the contrast of soil N stocks at each depth between 2001 and 2014.
Bold fonts indicates statistical significance

soil C survey across the conterminous United States showed that land in Conservation Research Program, where row crop agricultural lands were converted to perennial systems, had higher soil C stock in the surface (0–5 cm) but lower soil C stock in the subsurface (30–100 cm) as compared with adjacent croplands on average (Yang and others 2022a). These findings highlight the importance of including the inventories of subsoil when quantifying C and N dynamics in abandoned agricultural fields.

Mechanisms of C and N increase in the surface soil

The C accumulation rates in surface soil at CCESR are comparable with previously reported C sequestration rates of 30 to 60 g C m^{-2} y^{-1} in post-agricultural fields (Schlesinger 1986; Post and Kwon 2000; McLauchlan and others 2006). However, such increases are not uniform, and several studies have reported that even in relatively shallow depths (10–30 cm), C loss still occurs after field abandonment (Baer and others 2002; Kucharik 2007; Steinbeiss and others 2008; O'Brien and

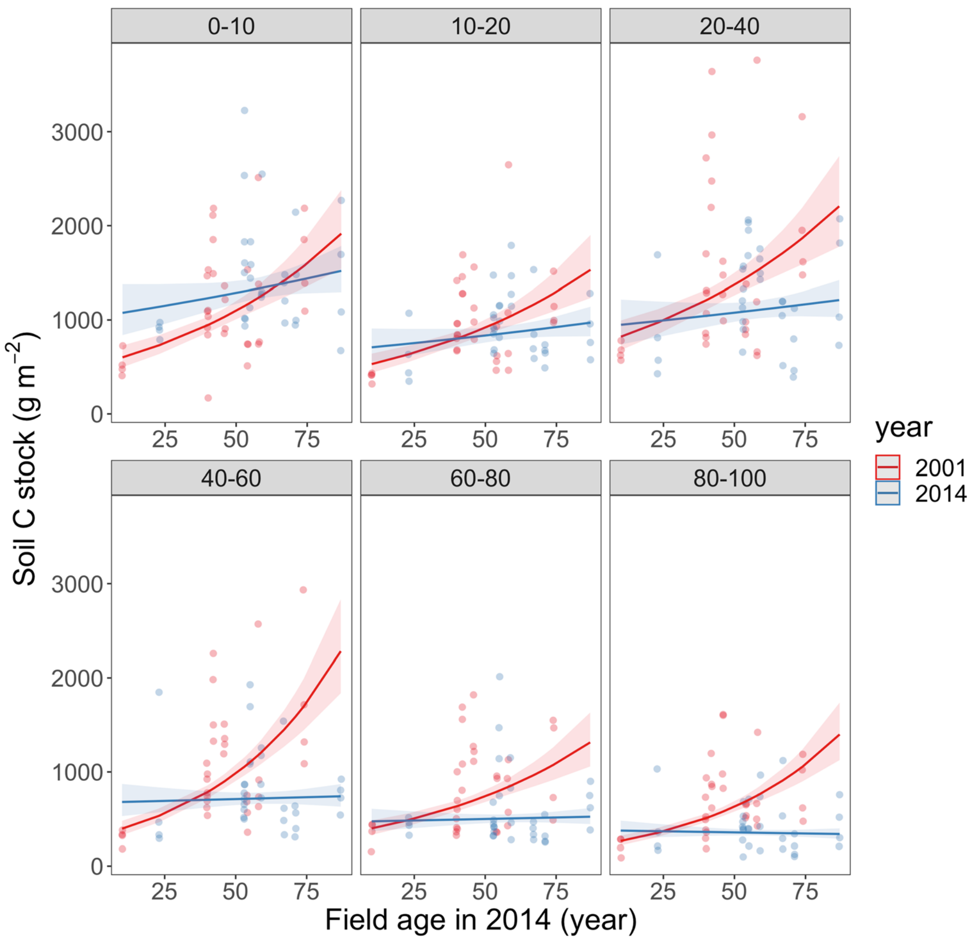

Figure 2. Soil C stock across the chronosequence at the soil depth of 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm. The soil C stocks are calculated with the equivalent soil mass (ESM) method using 2001 samples as the reference masses and depths. The circles represent the soil C stock at each sampling plot in 2001 (red) and 2014 (blue). The 2001 data are shifted 13 years earlier to show the actual age of these samples. The regression lines and the confidence interval show the relationship between soil C stock and field age (years after abandonment in 2014). The relationships were modeled by a linear mixed-effect model. The confidence intervals are modeled SEs.

others 2010). Surface C accumulation has been explained because of ecosystem changes after land abandonment. Grassland vegetation produces higher below-ground biomass in surface soil than crop species, thus increasing soil C input (Bronson and others 2004) and aboveground litter is not removed (Knops and Bradley 2009). Soil disturbance decreases after agricultural cessation, which promotes the formation of soil aggregates and SOM (Six and others 2002). However, this is likely less important in sandy soils (more than 85% sand, see Supp. Table 4) at CCERS (Grigal and others 1974; Plante and others 2006), because the lack of clay in the soil impedes the formation of soil aggregates (Kristiansen and others 2006; McLauchlan, 2006).

Evidence showed that the soil C accumulation rate is controlled by the N accumulation rate. The average N accumulation over the 13-year survey period was 1.0 ± 1.1 g N m^{-2} y^{-}1 in the surface. The annual atmospheric N deposition during the soil surveys was about 1.6 g N m^{-2} y^{-1} (Wall and Pearson 2013), which is comparable to the N accumulation in the surface soil. Thus, almost, if not all, atmospheric deposited N is currently retained in the surface soil (Wedin and Tilman 1996), likely because of the strong vegetation (Tilman, 1984) and microbial N limitation (Laungani and Knops 2012). The long-term soil C accumulation rate in temperate grassland during Holocene was estimated at 2.2 g C m^{-2} y^{-1} (Schlesinger 1990), which is much lower than the current C accumulation rate at CCESR, at 16.5 ± 14.5 g C m^{-2} y^{-1}. The current rates of soil C accumulation in the surface at CCESR predict a recovery to pre-settlement levels within two centuries (Knops and Tilman 2000). Therefore, the slow, past Holocene

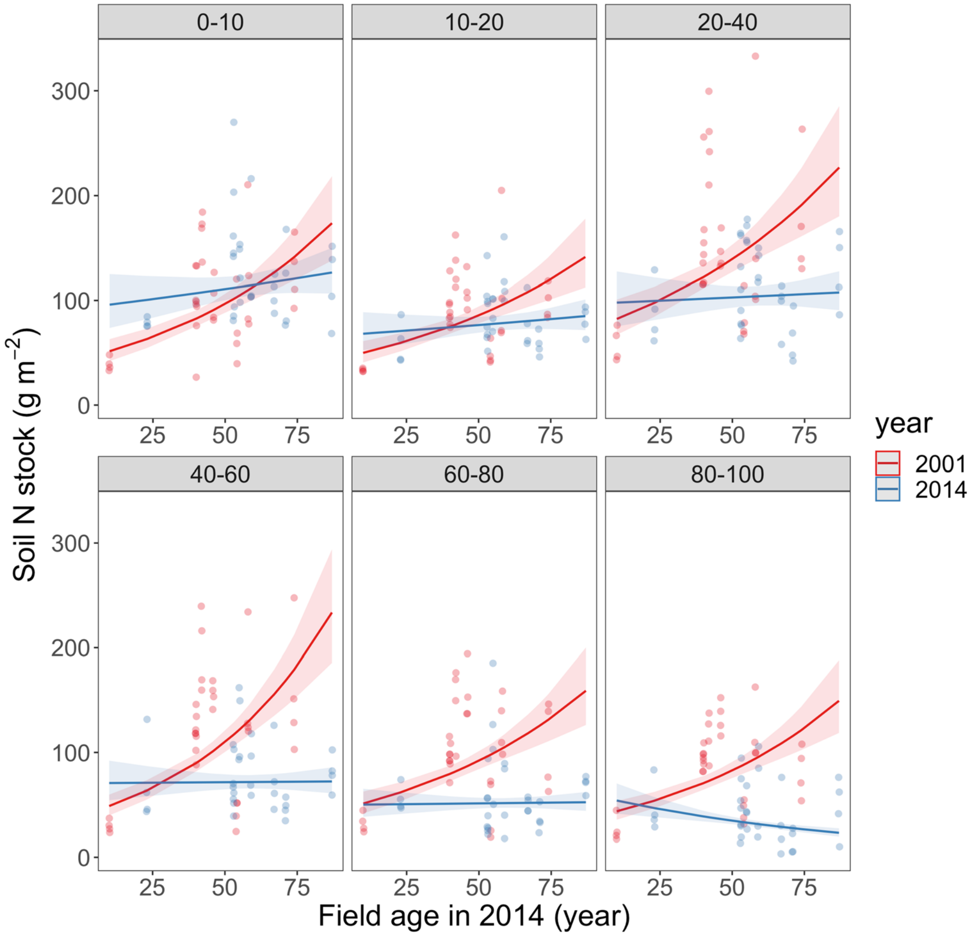

Figure 3. Soil N stock across the chronosequence at the soil depth of 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm. The soil N stocks are calculated with the equivalent soil mass (ESM) method using 2001 samples as the reference masses and depths. The circles represent the soil N stock at each sampling plot in 2001 (red) and 2014 (blue). The 2001 data are shifted 13 years earlier to show the actual age of these samples. The regression lines and the confidence interval show the relationship between soil N stock and field age (years after abandonment in 2014). The relationships were modeled by a linear mixed-effect model. The confidence intervals are modeled SEs.

rates of C accumulation imply the high SOM content in virgin grasslands accumulated over thousands of years, because N deposition was insignificant during the pre-settlement period as compared to nowadays with N deposition from fossil fuel combustion and fertilizer production (Kanakidou and others 2016). The current surface soil C dynamics differ from pre-settlement grasslands because N input has strongly increased.

Mechanisms of C and N Losses in Subsurface Soil

Subsurface soil C losses have to be caused by losses being larger than C inputs. The losses of C and N in the subsurface can be contributed to the well-aerated sandy soil at CCESR (Grigal and others 1974; Plante and others 2006) which enables SOM mineralization and efficient diffusion of products from decomposition. Deep roots are the main soil C inputs in subsurface soil (Rumpel and Kögel-Knabner 2011). On average, we found in this study, that 85% of the roots are in the top 20 cm depth in the old fields at CCESR (Figure 4b). Therefore, the soil C input from root turnover and exudates were mostly located in the surface soil. In addition, we found no difference in root biomass distribution across the chronosequence, indicating that once agricultural fields are transformed into perennial grasslands, the stratification of roots formed quickly and remained similar on a decadal time scale. We did observe root biomass increase between 2001 and 2014. However, the majority of the increases were still in the surface soil. Therefore, current C inputs in the subsurface soil from roots are low. The decrease in the C:N ratios (Supp. Figure 1) we

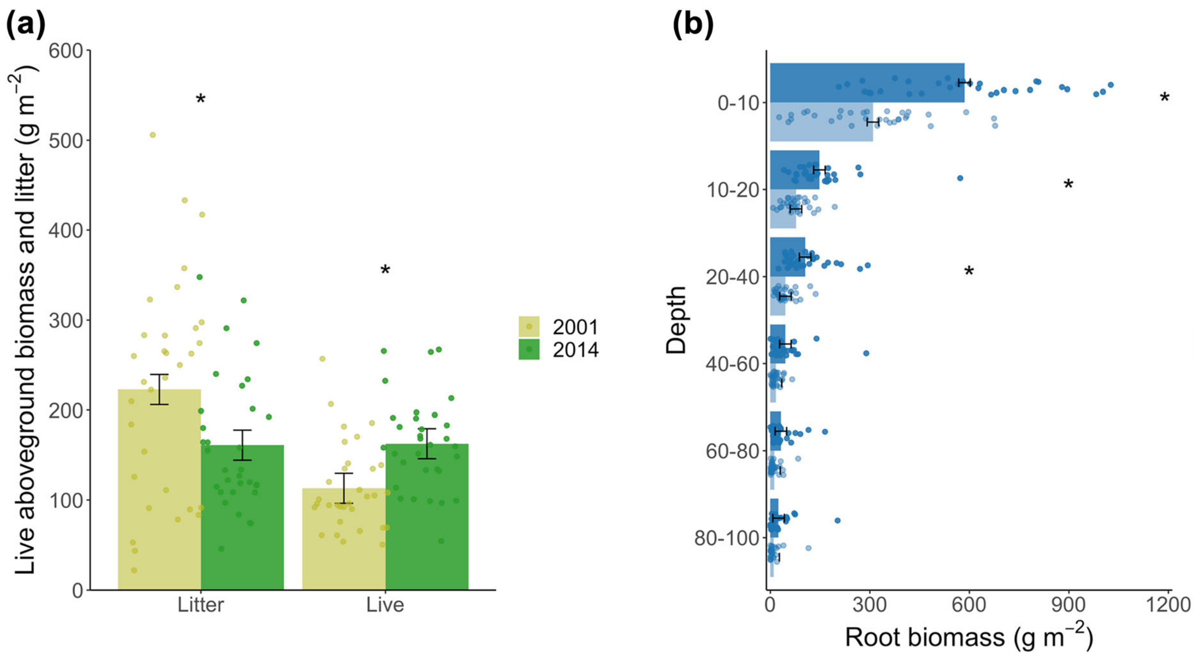

Figure 4. Aboveground living biomass and litter (**a**) and root biomass in each depth (**b**) in 2001 and 2014. In (**a**), each bar represents the average aboveground biomass of all 8 old fields. The green bars are the living biomass, and the yellow bars are the litter. The data shown are the means ± 1 SE. The asterisks represent significant differences at $p < 0.05$ of living biomass and litter between two sampling years. In (**b**), the light blue and dark blue bars are the average root biomass of 8 old fields at each depth in 2001 and 2014, respectively. The data shown are the means ± 1 SE. The asterisks indicate that the differences between data from the two sampling years and within the same depth are statistically significant at $p < 0.05$. Root biomass increased from 2001 to 2014 at 0–10 cm, 10–20 cm, and 20–40 cm.

found in the subsurface soil also suggests the lack of fresh carbon input from roots in the deep soil. The root biomass is largely distributed in the surface soil and does not supply much organic matter input into the subsurface soil, which may be attributed to the persistent C3 grass dominance in these old fields (Clark and others 2019). C3 grasses have less root biomass and higher decomposition rates than C4 grasses and perennial forbs (Yang and others 2019). Most of the C3 grasses (for example, *Poa pratensis*, *Agropyron repens,* and *Bromus inermis*) present at CCESR were introduced in the last 150 years and were not present before the European settlement. The plant species with deep roots (C4 grasses, perennial forbs, and legumes with taproots) were the dominant species before the cultivation. After agricultural abandonment, C3 grasses gained an increased competitive advantage because of the absence of fire (Li and others 2014) and increasing rates of atmospheric N deposition (Dijkstra and others 2004). The subsurface soil C may come from deep-rooted trees, as old fields at CCESR were savannah before agricultural settlement. However, we are not able to address this hypothesis at CCESR without historical data. Overall, the rate of C accumulation in the surface soil is controlled by the rate of atmospheric N deposition. With all additional N being retained in the surface soil, there is likely little addition of new C and N into the subsurface soil. The soil C inputs into deep soil can also be bioturbation and dissolved organic C (DOC) leaching (Rumpel and Kögel-Knabner 2011). Bioturbation is unlikely at CCESR, because pocket gophers, the main bioturbator present, have the majority of their burrows in the surface soil (Yang and others 2022b). We also attempted to collect DOC samples in 2017. However, we could not obtain conclusive results on the contribution of DOC to the C storage in the deep soil from the limited samples we collected.

Vegetation Composition Impact on Soil C and N

We did not find that soil C change between 2001 and 2014 in either the surface or subsurface was associated with species composition or species richness (Supp. Table 1). Many studies (for example, Baer and others 2002; Cahill and others 2009; O'Brien and others 2010), including studies conducted at CCESR (Fornara and Tilman 2008; Yang and others 2019), have shown that C4 grass abundance promotes soil C accumulation, as C4 grasses produce higher root biomass and have lower decomposability than other plant functional groups, such as C3 grass, forb, and legume. The discrepancy between our research and previous studies (Fornara and Tilman 2008; Yang and others 2019) is likely because the 8 old fields have highly variable soil C and N stock changes and only a small range in species richness and C4 abundance. In contrast, the experiment site of Fornara and Tilman (2008) and Yang and others (2019) was located in one old field, the soil was homogenized before experimental establishment and seeded with combinations of 1, 2, 4, 8, and 16 grassland species. Comparing the results of Yang and others (2019) with this study provides further evidence that deep root biomass is likely a key controlling factor for C and N stocks in the subsurface soil. With repeated soil surveys, Yang and others (2019) found soil C accumulates in both surface and subsurface in treatments with high species richness (8–16), especially with the combination of legume and C4 grasses. The positive interaction of legumes and C4 grasses increases the production of root biomass, likely due to facilitation and niche differentiation (Fornara and Tilman 2008). Supporting this hypothesis, Yang and others (2019) found that plots with a combination of legumes and C4 grasses had about 35% more total root biomass and 45% more deep root biomass (60–100 cm) than the old fields in our study (Supp. Figure 2). These findings, combined with old field correlational patterns reported in (Knops and Tilman 2000), indicate that legume abundance, in addition to atmospheric N deposition, may be a key factor in increasing C accumulation after agricultural abandonment. However, as a preferred food source for large herbivores (deer and gophers), legumes usually have a low abundance in unmanaged grasslands. The experimental site of Yang and others (2019) was fenced to exclude deer, and pocket gophers are actively trapped, resulting in a high abundance of legumes (for example, *Lupinus perennis*). In addition, herbivores can also limit root growth (Hummel and others 2007; Walter and Hummel 2008). The lack of such an effect may also have contributed to the high root biomass in plots with high diversity in Yang and others (2019).

Conclusions

This study used repeated soil surveys in 8 old fields to evaluate C and N stocks change following the re-establishment of perennial grasslands after agricultural abandonment. This study demonstrates that the subsurface soil is dynamic and ecologically relevant, and adds to the increasing evidence that soil C sequestration in unmanaged abandoned agricultural fields has limited, if any, potential to mitigate current CO_2 released into the atmosphere (Fissore and others 2010). Old field soils at CCESR are losing C and N because the losses in the subsurface soil are much higher than the surface soil gains. The root biomass and its accumulation are largely located in the surface soil and do not supply enough organic matter input into the subsurface soil, which can be a result of the ongoing dominance of C3 grasses and the lack of dominance of legumes and C4 grasses in these old fields. These findings indicate that the subsurface soil C and N stocks are a legacy from before settlement when the grasslands were dominated by native species with deep roots. Based on this hypothesis, the subsurface soil C and N will likely further decline, unless the vegetation shifts to species that have deeper roots (that is, C4 grasses, forbs, legumes with deep taproots, or trees).

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