Statistical assessment of a paired-site approach for verification of carbon and nitrogen sequestration on Wisconsin Conservation Reserve Program land

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ABSTRACT: The threat of global climate change has provoked policy-makers to consider plausible strategies to slow the accumulation of greenhouse gases—especially carbon dioxide (CO_2)—in the atmosphere. One such idea involves the sequestration of atmospheric carbon (C) in degraded agricultural soils as part of the Conservation Reserve Program (CRP). While the potential for significant C sequestration in CRP grassland ecosystems has been demonstrated, the paired-site sampling approach traditionally used to quantify soil C changes has not been evaluated with robust statistical analysis. In this study, 14 paired CRP (> 8 years old) and cropland sites in Dane County, Wisconsin, were used to assess whether a paired-site sampling design could detect statistically significant differences (ANOVA) in mean soil organic C and total nitrogen (N) storage. We compared 0 to 10 cm (0 to 3.9 in) bulk density and sampled soils (0 to 5 cm, 5 to 10 cm, and 10 to 25 cm [0 to 2 in, 2 to 3.9 in, and 3.9 to 9.8 in]) for textural differences and chemical analysis of organic matter (OM), soil organic C (SOC), total N, and pH. The CRP contributed to reducing soil bulk density by 13% ($p < 0.0001$) and increased SOC and OM storage (kg m^{-2} [lb ft^{-2}]) by 13% to 17% in the 0 to 5 cm (2 in) layer ($p = 0.1$). We tested the statistical power associated with ANOVA for measured soil properties and calculated minimum detectable differences (MDD). We concluded that 40 to 65 paired sites and soil sampling in 5 cm (2 in) increments near the surface were needed to achieve an 80% confidence level ($\alpha = 0.05$; $\beta = 0.20$) in soil C and N sequestration rates. Because soil C and total N storage was highly variable among these sites (CVs > 20%), only a 23% to 29% change in existing total organic C and N pools could be reliably detected. While C and N sequestration (247 kg C ha^{-1} yr-1 and 17 kg N ha^{-1} yr^{-1} [220 lb C ac^{-1} and 15 lb N ac^{-1}]) may be occurring and confined to the surface 5 cm (2 in) as part of the Wisconsin CRP, our sampling design did not statistically support the desired 80% confidence level. We conclude that usage of statistical power analysis is essential to insure a high level of confidence in soil C and N sequestration rates that are quantified using paired plots.

Keywords: Agricultural land management, carbon sequestration, CRP, soil organic matter, Wisconsin

There is now an ever-increasing interest in restoring agricultural landscapes in the U.S. Midwest and Great Plains to native prairie and grassland ecosystems for multiple environmental benefits, including improved water retention, reduced runoff of agrochemicals, reduced soil erosion, and—most recently—carbon (C) sequestration. Clearly, a focus on developing sustainable systems for future human growth is moving to the forefront of agricultural land management, with a recent emphasis placed on maintaining or enhancing soil organic matter (SOM). For example, 14 million hectares (34.6 million acres) of cropland in the United States has been converted to grassland since 1985 under the federal Conservation Reserve Program (CRP) (Paul et al., 1997; Metting et al., 2001). Agricultural land use during the last century has led to a significant (~20% to 60%) depletion of SOM and subsequent release of soil CO_2 to the atmosphere (Mann, 1986; Paul et al., 1997; Kucharik et al., 2001). However, there are signs that this trend may be reversed with increased crop production and residue return, with more widespread adoption of conservation tillage practices, or through programs such as the CRP (Buyanovsky and Wagner, 1998; Schlesinger, 1999).

Several studies taking place on land set-asides as part of the CRP in Kansas, Oklahoma, Colorado, Texas, and Wyoming (Gebhart et al., 1994; Burke et al., 1995, Reeder et al., 1998; Robles and Burke, 1998; Potter et al., 1999) have shown that the re-establishment of native perennial grasses on previously tilled soils has helped increase soil C levels and improve other soil-quality indicators (Staben et al., 1997; Reeder et al., 1998; Robles and Burke, 1998; Potter et al., 1999; Baer et al., 2000). It has been reported that soil C sequestration potential of the CRP varies from 110 to 3,040 kg C ha^{-1} yr^{-1} (98 to 2,714 lb C ac^{-1} yr^{-1}) (Bruce et al., 1999; Potter et al., 1999; Post and Kwon, 2000; Metting et al., 2001), but Bruce et al. (1999) argue that an average rate of accumulation is about 800 kg C ha^{-1} yr^{-1} (714 lb C ac^{-1} yr^{-1}) during the first decade after revegetation, with a decline thereafter. While C sequestration is a timely, relevant, and attractive issue, maintenance of SOM is essential to overall soil tilth and long-term sustainability of other ecosystem services because it is a storage pool of important plant nutrients (N, P, and S).

Because SOC has a long residence time (centuries to millennia), only small changes occur over short time periods (e.g. three to five years). Because these changes are much smaller than the actual C pool sizes (1% to 10%), reliable detection and verification of C pool changes is particularly difficult. The majority of studies that have quantified SOM recovery as part of the CRP used paired plots and stratified soil cores in 2 to 10 cm (0.75 to 3.9 in) increments in the top 20 cm (7.9 in) to detect changes in soil C and total N pools as a function of depth (Gebhart et al., 1994; Potter et al., 1999; Follett et al., 2001). Although the majority of these studies have

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Table 1. Site descriptions for paired CRP / cropland sites in Dane County, Wisconsin. Distance refers to the approximate number of meters between soil sampling transects on adjacent CRP / cropland sites.

Site ID	Year planted	Management	Elevation (m)	Slope %	Hectares	CRP soil series	Soil subgroup	Adjacent crop	Crop elevation (m)	Slope %	Crop soil series	Distance (m)
1	1991	mixed grass / forb	267.6	1-4	2.14	Troxel Silt Loam	Pachic Argiudolls	Soybeans	274.6	6-12	Dodge Silt Loam	207
2	1988	mixed grass / forb	255.4	2-6	15.50	Dresden Silt Loam	Mollic Hapludalfs	Soybeans	251.8	2-6	Dresden Silt Loam	86
3	1987[*]	mixed grass / forb	247.2	2-6	10.52	Dodge Silt Loam	Typic Hapludalfs	Maize	245.7	2-6	Dodge Silt Loam	30
4	1991	mixed grass / forb	254.5	12-20	3.24	McHenry Silt Loam	Typic Hapludalfs	Alfalfa	255.1	12-20	McHenry Silt Loam	111
5	1987	mixed grass / forb	305.4	6-12	5.34	Dunbarton Silt Loam	Lithic Hapludalfs	Soybeans	317	6-12	Dunbarton Silt Loam	8
6	1993	mixed grass / forb	267.9	6-12	2.31	Dunbarton Silt Loam	Lithic Hapludalfs	Maize	270.5	6-12	Dunbarton Silt Loam	40
7	1988	mixed grass / forb	298.7	6-12	3.04	Griswold loam	Typic Argiudolls	Maize	297.5	6-12	Griswold Loam	55
8	1988	mixed grass / forb	284.4	2-6	2.91	Plano silt loam	Typic Argiudolls	Alfalfa	360	2-6	Plano Silt Loam	49
9	1988	mixed grass / forb	322.8	12-20	4.86	McHenry Silt Loam	Typic Hapludalfs	Alfalfa	317	6-12	McHenry Silt Loam	77
10	1988	mixed grass / forb	344.4	6-12	5.50	Ringwood Silt Loam	Typic Argiudolls	Soybeans	339.8	2-6	Plano Silt Loam	150
11	1988	mixed grass / forb	341.4	2-6	4.09	Ringwood Silt Loam	Typic Argiudolls	Alfalfa	342.9	2-6	Ringwood Silt Loam	38
12	1987	mixed grass / forb	269.1	12-20	12.55	Basco Silt loam	Mollic Hapludalfs	Alfalfa	266.1	12-20	Dubuque Silt Loam	60
13	1987	Switchgrass	263.3	12-20	11.13	Dubuque Silt Loam	Typic Hapludalfs	Alfalfa	266.1	12-20	Dubuque Silt Loam	41
14	1991	mixed grass / forb	298.7	12-20	4.45	Seaton Silt Loam	Typic Hapludalfs	Alfalfa	285.6	12-20	Seaton Silt Loam	73

[*] Site 3 was fallow with grass from 1987-1997, enrolled in CRP 1998.

used ANOVA to detect significant changes ($p < 0.05$) in soil properties as a result of land-use change or differences in climate regime, they did not determine whether the number of sampling locations was adequate to achieve good statistical power (e.g. 80%). These previous studies did not calculate minimum detectable differences (MDD) or the smallest change in soil C (or N) that can be attributed to land-use (or climate) differences once the significance level (e.g. $p = 0.05$), statistical power, and sample size are determined (Garten and Wullschleger, 1999).

There is an urgent need to develop more comprehensive field-level methodology to ensure that C pool changes can be detected with acceptable levels of precision (Garten and Wullschleger, 1999; Post et al., 2001). Garten and Wullschleger (1999) suggest that measures of variation or confidence limits in SOC storage pools are rarely reported in the literature, and future implementation of C-crediting programs will require a much better understanding of the type of sampling procedure and frequency of measurements that will be necessary to yield scientifically defensible hypotheses. For example, *in situ* observations of terrestrial C pools and the impacts of vegetation on C cycling will form part of the backbone for the future international Terrestrial Carbon Observation (TCO) initiative (Cihlar et al., 2002). Moreover, Post et al. (2001) iterate that reliable monitoring methods and programs will be key to assessing compliance with future laws and regulations and assigning C credits or offsets. Because of this, farmers, stakeholders, utility companies, and policy-makers all have vested, economic interests in quantifying C sequestration as a function of climate, land-use practice, soil type, and vegetation type.

Since 1986, almost 255,400 hectares (631,084 acres) in Wisconsin have been a part of the CRP (USDA, 2001). About 78% of the acreage enrolled in the Wisconsin CRP has been planted with some type of dominant grass cover (introduced, native, or established), but diverse seed mixes of grasses and forbs are generally used in restorations and are favored by landowners for aesthetic reasons. Therefore, vegetative cover type, density, and diversity in the Wisconsin CRP vary considerably across the landscape. Only a small percentage of landowners plant monocultural stands of perennial grasses (e.g. switchgrass). This management approach appears to differ from past studies of the CRP in states west of Wisconsin.

In this study, the general hypothesis was that CRP land would demonstrate improved soil bulk density, soil pH levels that were comparable to cropped land, and increased SOM after a period of 8 years or more, contributing to soil C and N sequestration. We examined soil organic C (SOC) and total N, total OM, soil textural fractions, and soil pH as a function of fixed soil depths to 25 cm (9.8 in), and surface (0 to 10 cm [0 to 3.9 in]) bulk density. This project differs from previous studies of C sequestration on CRP land for several reasons: 1) a significant number of paired sites (14) were delineated in a localized area (county) on similar silt loam soils, 2) sand, silt, and clay fractions were quantified for all soil samples as a function of depth to gauge similarity of sample variance among paired sites, and 3) MDD were calculated. A main objective of this study was to determine MDD (Garten and Wullschleger, 1999) for SOC, N, OM, and bulk density based on sample size and variance. Our goal was to determine whether C and N sequestration had occurred and how the paired-site approach may need to be modified (e.g. number of sampling locations needed) in southern Wisconsin to produce scientifically defensible results for future carbon-crediting and emissions-reduction registry programs. Because measurements of C and N pool sizes on Wisconsin CRP land are not usually available from the onset of enrollment, paired sites or chronosequences will continue to be used as a space-for-time substitution in the near future. We envision that if a standardized soil-sampling protocol were developed, Wisconsin landowners could eventually be educated and integrated into a comprehensive monitoring program.

Methods and Materials

Study area. In spring 2001, 14 paired cropland / CRP study sites were chosen, in coordination with the U.S. Department of Agriculture's Natural Resources Conservation Service (USDA-NRCS), from a database of more than 1,200 landowners currently having contracts with the CRP. Paired sites were selected based on several criteria: 1) CRP land had been enrolled in either CP1 (permanent introduced grasses and legumes) or CP2 (establishment of permanent native grasses) for at least eight years (1993) as of

Table 2. Cumulative statistics for all quantities measured as a function of land cover and soil depth. The mean square error (MSE), P-value, and variance values were calculated from a one-way analysis of variance (ANOVA). SE and CV refer to the standard error and coefficient of variation, respectively.

	0-5 cm				5-10 cm			
Quantity	**CRP**	**Crop**	**MSE**	**P-value**	**CRP**	**Crop**	**MSE**	**P-value**
Bulk density (g cm^{-3})[1]	1.41	1.59	0.012	< 0.0001				
SE	0.03	0.03						
CV	0.08	0.07						
Variance	0.01	0.01						
Organic Matter (%)	3.95	3.10	0.603	0.008	2.66	2.66	0.369	1.000
SE	0.23	0.18			0.16	0.17		
CV	0.22	0.21			0.22	0.23		
Variance	0.77	0.43			0.35	0.39		
Organic C (%)	2.70	2.06	0.379	0.010	1.86	1.76	0.366	0.653
SE	0.19	0.14			0.19	0.13		
CV	0.26	0.25			0.38	0.28		
Variance	0.50	0.26			0.49	0.24		
Organic N (%)	0.26	0.20	0.004	0.020	0.18	0.17	0.002	0.668
SE	0.02	0.01			0.01	0.01		
CV	0.27	0.25			0.28	0.23		
Variance	0.01	0.00			0.00	0.00		
Total OM (kg m^{-2})	2.76	2.44	0.262	0.108	1.90	2.09	0.161	0.219
SE	0.15	0.12			0.11	0.11		
CV	0.21	0.18			0.21	0.19		
Variance	0.33	0.20			0.16	0.16		
Total Organic C (kg m^{-2})	1.89	1.62	0.177	0.105	1.30	1.38	0.156	0.607
SE	0.13	0.10			0.12	0.09		
CV	0.25	0.22			0.36	0.23		
Variance	0.23	0.13			0.22	0.10		
Total Organic N (g m^{-2})	179.23	158.70	1624.0	0.189	122.71	132.62	815.1	0.367
SE	11.74	9.71			8.55	6.59		
CV	0.25	0.23			0.26	0.19		
Variance	1929.06	1320.50			1022.60	607.60		
Soil pH	6.67	6.73	0.186	0.722	6.67	6.78	0.189	0.528
SE	0.47	0.39			0.49	0.37		
CV	0.07	0.06			0.07	0.06		
Variance	0.22	0.15			0.24	0.14		
% sand	24.09	28.86	170.950	0.264	25.68	27.38	21.660	0.663
SE	2.12	2.64			1.98	2.41		
CV	0.33	0.34			0.29	0.33		
Variance	158.61	105.07			139.54	83.73		
% silt	57.60	52.19	219.270	0.171	56.10	54.34	23.210	0.656
SE	2.61	2.40			2.33	2.41		
CV	0.17	0.20			0.16	0.21		
Variance	142.82	79.57			152.49	76.95		
% clay	18.32	18.95	3.000	0.686	18.22	18.28	0.030	0.964
SE	1.12	0.91			1.10	0.80		
CV	0.23	0.18			0.16	0.16		
Variance	22.97	13.04			15.99	11.12		
C/N	10.56	10.26	1.030	0.445	10.48	10.34	1.360	0.757
SE	0.29	0.25			0.36	0.26		
CV	0.10	0.09			0.13	0.09		
Variance	1.20	0.86			1.80	0.92		

[1] Bulk density is reported as an average for the 0- to 10-cm surface layer.

2001, 2) each CRP site was directly adjacent to a cropped field that has been in production for at least 50 years, 3) individual paired sites were initially identified on the same soil series or a comparable silt loam soil using digitized soil surveys for Dane County, Wisconsin, 4) CRP and cropland had similar elevation and slope. Based on the above criteria, it was not possible to identify 14 paired sites on the same soil series. The soils are classified as Typic Hapludalfs, Mollic Hapludalfs, Typic Argiudolls, and Lithic Hapludalfs (Table 1). Sites were not chosen based on seed mixtures used to re-establish the prairies because this information was not available with older contracts (e.g. initial enrollment in 1987). Agricultural fields have generally been in production since 1940 or earlier.

At three of the study sites, adjacent pastureland soil was also sampled for comparison. While one of the original goals of this study was to compare CRP and cropland with pasture, we quickly found that grazing land is quite scarce in this county, and land-use history for pastures is vague. We sampled three pastures at sites 1, 3, and 5 (as identified in Table 1) that have been in pasture for at least 50 years or longer. We only used these data to point to potential differences for other land-use practices on similar soils in this region.

Mean annual precipitation in this region is 784 mm (30.9 in), with a mean annual minimum temperature of 1.5˚C (34.7˚F) and mean annual maximum of 13.1˚C (55.6˚F).

Soil sampling. Soil samples for chemical analysis were collected during summer 2001 at each site in 0 to 5, 5 to 10, and 10 to 25 cm (0 to 2, 2 to 3.9, and 3.9 to 9.8 in) fixed soil-depth intervals. According to field observations and official soil series descriptions of the USDA-NRCS, this design permitted sampling that extended below the Ap horizon, which is typically between 12 and 23 cm (4.7 and 9.1 in) in this region. A global positioning system (GPS) was used in coordination with digitized soil surveys to establish 50 m (164 ft) transects in adjacent CRP and cropped fields on either the same soil series or similar soil types. The average distance between the CRP and cropland soil sampling transects was 73.2 m (240 ft) (Table 1). Fifteen 2 cm (0.79 in) diameter cores were collected along each transect at each independent depth; then they were well mixed and combined into a composite sample. Therefore, in this study, no measure of within-field variability was assessed. Soil sampling took place in a manner to form an average for each site; thus, a random placement of the soil probe was performed for each sample location along each transect. Each paired CRP and cropland site was completely sampled within a 3 to 6 hr period on the same day. No distinction or preference was made to sample soils under grasses vs. between plants (Robles and Burke, 1998).

Five bulk density (Mg m^{-3} [lb ft^{-3}]) cores were collected for each land-use type for the 0 to 10 cm (0 to 3.9 in) surface soil layer using an 184 cm^3 (11.2 in^3) stainless steel cylinder (4.8 cm [1.9 in] inside diameter) inserted within a gravity-driven hammer attachment (Blake and Hartge, 1986; Elliott et al., 1999).

Laboratory analysis. Soil samples were dried in a force-draft dryer for 48 hr at 33˚C (91.4˚F). Dried soil samples for SOC, total N, OM, and pH analysis were ground to pass through a 2 mm (0.079 in) mesh screen and ground by hand with a mortar and pestle until they passed through a 150 µm (0.00591 in) sieve. Percent soil C and N was determined on finely ground soil subsamples (5 g [0.18 oz] and < 150 µm [< 0.00591 in]) using a Carlo-Erba Model NA 1500 CN autoanalyzer (Carlo Erba Instruments, Milan, Italy). While these soils are calcareous in origin, no removal of inorganic soil C took place before SOC and total N were determined because free carbonates are typically confined below 50 cm (19.7 in) based on sampling at a few sites to a 1 m (3.3 ft) depth. Percent SOM was determined by loss on ignition (Nelson and Sommers, 1996).

Bulk density samples were dried for 48 hrs at 33˚C (91.4˚F) and weighed to determine the mass of soil on a dry weight basis. All concentration data (g kg dry $soil^{-1}$ [oz lb dry $soil^{-1}$]) were multiplied by mean bulk density values and fixed sampling depth increments (soil layer thickness) to convert C, N, and SOM to an area basis (kg m^{-2} [lb ft^{-2}]) for fixed-depth comparisons (Ellert and Bettany 1995). Soil pH was measured using a 1:1 soil: deionized water mixture at each depth after equilibrating for 1 hr. Soil textural analysis was performed on each sample using the hydrometer method (Bouyoucos, 1962; Ashworth et al., 2001).

Field analysis. A catalog of vegetation growing at each CRP site was assembled, but frequency (area covered) was not quantified in a standardized manner. Species were categorized according to those that are part of the seed mixtures used in the Wisconsin CRP and those that are not.

Statistical analysis. Statistical differences between the means of each soil property in CRP and cropped fields were made using one-way analysis of variance (ANOVA) as a function of soil depth. A p-value of less than 0.05 was regarded as being statistically significant. Normality of data was confirmed both visually (histograms) and more rigorously by the Shapiro-Wilk test (Shapiro and Wilk, 1965; Parkin and Robinson, 1994). Each pair of the CRP and cropland sites was treated as a replicate for the overall t-test for silt loam soils within Dane County. The within-group mean square error (e.g. denominator of F-ratio) was the error term. The mean, standard error (SE), coefficient of variation (CV), and variance (mean square error from ANOVA) of measurements are reported in Table 2.

We used the approach described by Garten and Wullschleger (1999) and Homann et al. (2001) to calculate MDD (Zar, 1984; Homann et al., 2001) for several soil properties measured in this study, given the variation across the 14 paired study sites. In this study, the MDD is defined as the smallest difference that could be detected between similar quantities exposed to different land management with 80% confidence ($1-\beta = 0.80$) and $\alpha = 0.05$. Following Garten and Wullschleger (1999), the MDD was calculated as

$$\mathrm{MDD} = \mathrm{SQRT}\ \{[(2k)(\sigma^2)(\phi^2)]/n\}, \qquad (1)$$

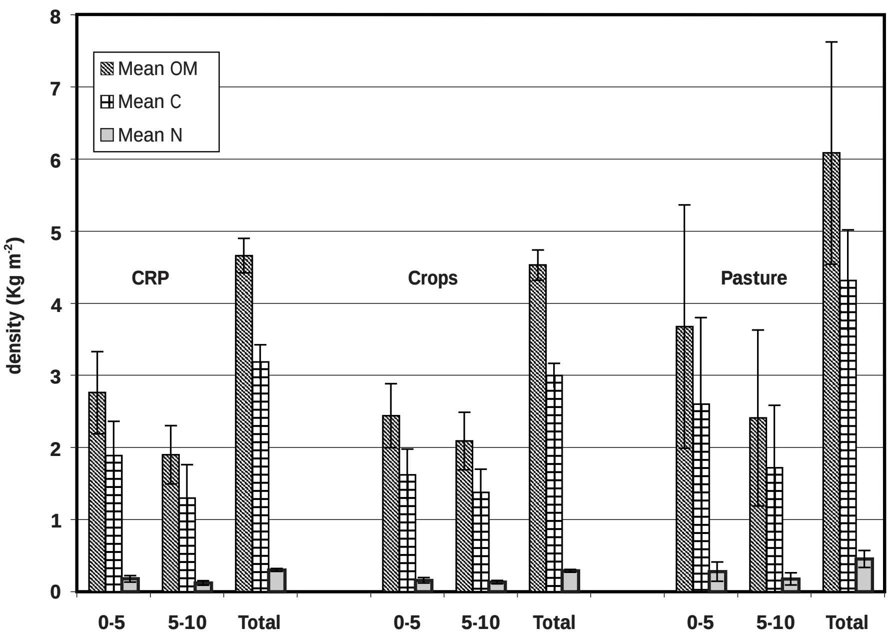
where,
k is the number of groups sampled (e.g. land-cover types = 2),
n is the number of paired sites (14),
ϕ is a critical value assigned to be 2.06 based on $\alpha = 0.05$, $\beta = 0.20$, $\nu_1 = 1$ (k-1), and $\nu_2 = 26$ [k(n-1)] (Zar 1984).

Results and Discussion

Percent sand, silt, and clay. Soil textural fractions of paired sampling sites should be analyzed for potential differences in sample variances. Past studies of C sequestration in CRP soils have failed to test for soil-texture variance among paired sites. Because of the relationship between SOM accumulation and soil textural makeup, conclusions that are made about the impacts of land-management change on soil C or N sequestration may be invalid unless variances are similar. In addition, while soil surveys may prove useful to delineating paired sites that are situated on

Figure 1

Mean (and SE) SOM, C, and N as a function of depth (cm) for CRP, cropland, and pasture sites in Dane County, Wisconsin. Total refers to the summation for the 0 to 10 cm layer.



similar soil types, these surveys may not be accurate at smaller scales used in field sampling. In our study region, we did not detect any significant differences in soil textural fractions between CRP and cropped sites in this study (Table 2). The results from ANOVA conducted showed that both sample means and variances of sand, silt, and clay fractions in the 14 paired CRP and cropped sites were similar in the 0 to 5 cm (0 to 2 in) and 5 to 10 cm (2 to 3.9 in) layers. In both surface layers, the CRP sites were 24% to 26% sand, with a CV of 29% to 33%, whereas cropped systems contained 27% to 29% sand and had a CV of 34%. Clay content was about 18% to 19% in the paired sites within the surface 10 cm (3.9 in). The textural makeup of these soils is consistent with the silt loam classification designated by previous USDA soil sampling surveys.

Bulk density. Surface (0 to 10 cm [0 to 3.9 in]) bulk density was 13% lower in CRP soils than in adjacent crop soils and statistically significant ($p < 0.0001$) (Table 2). The average bulk density was 1.41 g cm^{-3} (88.0 lb ft^{-3}) (SE 0.03; range 1.22 - 1.63 g cm^{-3} [76.2 - 101.8 lb ft^{-3}]) in CRP and 1.59 g cm^{-3} (99.3 lb ft^{-3}) (SE 0.03; range 1.47 - 1.82 g cm^{-3} [91.8 - 113.6 lb ft^{-3}]) in cropped sites. The CV was generally low in each set of replicates across the county, as it was 8.1% in CRP and 6.6% in crops.

Soil C, total N, and OM. The percent organic C (mass basis; g kg^{-1} [oz lb^{-1}]) within the 0 to 5 cm (0 to 2 in) layer in the CRP (27 g kg^{-1} [0.43 oz lb^{-1}]) was significantly greater ($p = 0.01$) than in the crop soils (21 g kg^{-1} [0.34 oz lb^{-1}]). The range was 12 to 37 g kg^{-1} (SE 1.9) (0.19 to 0.59 oz lb^{-1}) for CRP and 13 to 30 g kg^{-1} (SE 1.4) (0.21 to 0.48 oz lb^{-1}) for cropland. However, percent SOC was not significantly different between the two land-use types in the 5 to 10 cm (2 to 3.9 in) or 10 to 25 cm (3.9 to 9.8 in) layers ($p > 0.5$). The percent organic N (g kg^{-1} [oz lb^{-1}]) within the 0 to 5 cm (0 to 2 in) layer in the CRP (2.6 g kg^{-1} [0.04 oz lb^{-1}], range 1.1 to 3.5 g kg^{-1} [0.018 to 0.056 oz lb^{-1}]) was significantly greater ($p = 0.02$) than in the adjacent crop soils (2.0 g kg^{-1} [0.032 oz lb^{-1}], range 1.2 to 2.9 g kg^{-1} [0.019 to 0.046 oz lb^{-1}]), but no significant differences were found in the other two soil layers sampled. The total percent OM (g kg^{-1} [oz lb^{-1}]) within the 0 to 5 cm (0 to 2 in) layer was significantly greater ($p = 0.008$) in the CRP (39.5 g kg^{-1} [0.63 oz lb^{-1}], range 22 to 51 g kg^{-1} [0.35 to 0.82 oz lb^{-1}]), compared with the crop soils (31 g kg^{-1} [0.50 oz lb^{-1}], range 20 to 41 g kg^{-1} [0.32 to 0.66 oz lb^{-1}]) but were almost identical below that surface layer.

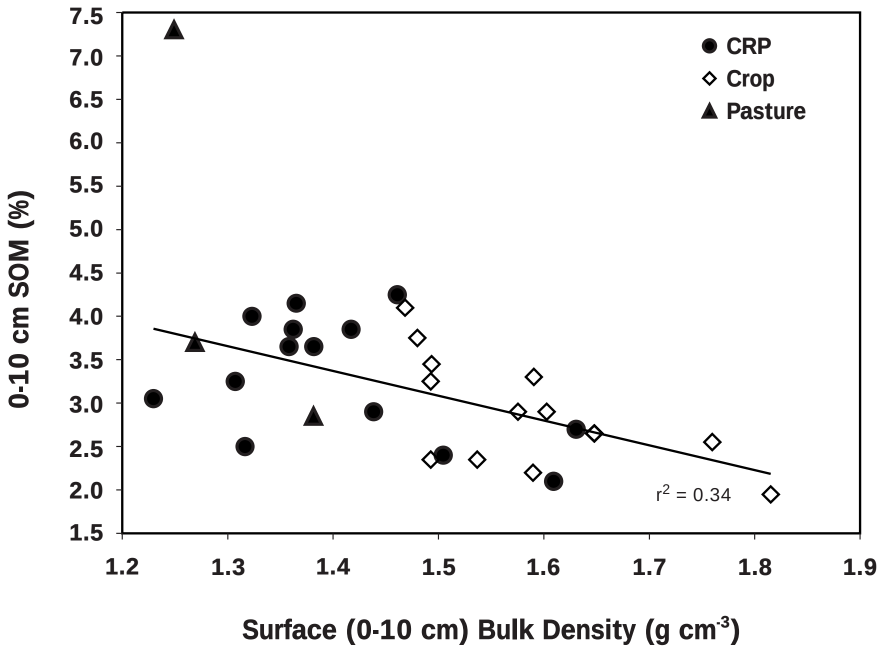
Total C, N, and SOM storage (area basis; kg m^{-2} [lb ft^{-2}]) were compared for the 0 to 5 cm (0 to 2 in) and 5 to 10 cm (2 to 3.9 in) layers individually and as a total for the surface 10 cm (3.9 in) (Figure 1). We did not extend these computations to the 10 to 25 cm (3.9 to 9.8 in) layer because we did not collect subsurface (> 10 cm [> 3.9 in]) bulk density data. Total soil organic C, N, and OM were greater in the CRP 0 to 5 cm (0 to 2 in) layer ($p = 0.105, 0.189,$ and 0.108) than cropped systems, but these were not significant at the probability level (0.05) we chose. Below the top 5 cm (2 in), no significant differences were noted between these data as a function of land-management practice.

The average total SOC in the top 10 cm (3.9 in) was 3.2 kg m^{-2} (0.66 lb ft^{-2}) for CRP (SE 0.24 and range of 1.8 to 5.1 kg m^{-2} [0.37 to 1.045 lb ft^{-2}]) and 3.0 kg m^{-2} (0.61 lb ft^{-2}) for crop sites (SE 0.17, range 2.0 to 4.1 kg m^{-2} [0.41 to 0.84 lb ft^{-2}]), and average total organic soil N was 0.30 kg m^{-2} (0.061 lb ft^{-2}) (SE 0.02, range 0.18 to 0.41 kg m^{-2} [0.037 to 0.084 lb ft^{-2}]) and 0.29 kg m^{-2} (0.059 lb ft^{-2}) (SE 0.02, range 0.21-0.39 kg m^{-2}) for CRP and cropped sites, respectively. Total SOM in the surface 10 cm (3.9 in) was only slightly higher (4.66 kg m^{-2} [0.95 lb ft^{-2}], SE 0.24, range 3.4-6.2 kg m^{-2} [0.70 to 1.27 lb ft^{-2}]) in CRP soils compared with the cropped sites (4.5 kg m^{-2} [0.92 lb ft^{-2}], SE 0.21, range 3.5 to 6.0 kg m^{-2} [0.72 to 1.23 lb ft^{-2}]). While only three pastures adjacent to CRP and cropland (at sites 1, 3, and 5) were sampled in this study, these companion sites on average had 20% to 40% higher soil C, N, and OM in the surface 10 cm (3.9 in) when compared with crop sites (Figure 1). We also note that surface bulk density in these pasture sites was 1.29 g cm^{-3} (SE 0.06) (80.5 lb ft^{-3}), about 19% lower than crop sites, and 9% lower than the CRP sites (Figure 2).

As has been shown in earlier studies (Adams, 1973; Rawls, 1983), organic matter content and soil texture significantly impact soil bulk density. In this study, we performed linear regression analysis to examine the contribution of increasing SOM on decreasing the soil bulk density (Figure 2). The composite analysis for CRP and crop data yielded an $r^2 = 0.34$, suggesting a somewhat weak correlation between bulk density and OM across all sites. While pasture data is shown in Figure 2, we excluded it from the composite regression analysis because these soils were previously untilled (according to landowners) and the relationship (slope) between soil bulk density and OM is probably different than similar silt loam soils that have experienced

Figure 2

Relationship between 0 to10 cm SOM (%) and surface (0 to 10 cm) bulk density. The best-fit line (linear regression) was made using CRP and cropland soil data.



mechanical disturbance (Adams, 1973). While the results of regression analysis performed on the three pasture sites supports this idea ($r^2 = 0.56$), this dataset is limited because of the low number of replicates. When linear regression was performed on CRP and crop soils independently, a stronger correlation between bulk density and OM in cropped soils was noted ($r^2 = 0.42$) compared with CRP sites ($r^2 = 0.18$). The fact that the CRP had significantly reduced bulk density but did not significantly increase OM in the 0 to 10 cm (0 to 3.9 in) layer suggests that physical processes other than OM formation may be contributing to differences in soil bulk density. Total root biomass, distribution with depth, and longevity are different between managed and grassland ecosystems in this region (Kucharik et al., 2001). Bulk density values may change during the growing season because of soil moisture changes, machinery traffic, and fine root dynamics (Elliott et al., 1999). Thus, it may be necessary to conduct sequential coring for bulk density to reduce associated sampling errors and improve generalizations about the effect of organic matter accumulation on soil bulk density.

In general, the CVs for soil C, total N, and OM data across the county were between 18% and 28%, with a tendency for higher variation among CRP land use in the two surface layers. This is not surprising considering that cropped soils in this region are continually being tilled and mixed to a depth of about 15 cm (6 in) while supporting a typical rotation of maize and soybean. In addition, significant variation in vegetation structure among the 14 CRP sites is potentially contributing to variable NPP and C inputs to soils. At several sites, we estimated that 20% to 50% of the ground area lacked vegetative growth eight or more years after re-establishment of prairie vegetation.

Soil C:N and C:OM. Soil C:N (g C kg^{-1} soil / g N kg^{-1} soil [oz C lb^{-1} soil / oz N lb^{-1} soil]) as a function of fixed soil depth was not significantly different between CRP and crop-management scenarios. The largest difference was apparent in the surface 0 to 5 cm (0 to 2 in) (10.56 and 10.26 for CRP and crops, respectively), but was not statistically significant ($p = 0.445$). Similarly, there were no significant differences in the organic C:OM (g C kg^{-1} soil / g OM kg^{-1} soil [oz C lb^{-1} soil / oz N lb^{-1} soil]) values between land-management practices. Organic C generally was between 64% to 70% of SOM in the CRP and 61% to 66% in crop systems in the top 25 cm (9.8 in). However, CVs were much higher for the CRP (range 22% to 30%) than in crop soils (9% to 17%). Based on soil cores collected below 25 cm (9.8 in) to a depth of 1 m (3.3 ft) at 5 of the 14 paired sites, C:SOM decreased to 48% and 55% for the 25 to 50 cm layer in CRP and cropped soils, respectively. These values decreased further to 43% and 46% at 50 to 100 cm (19.7 to 39.4 in) in CRP and cropped soils, respectively.

Soil pH. In general, there were no statistically significant differences in soil pH between land-management practices in this study as a function of soil depth. Averages were 6.67 and 6.73 in the 0 to 5 cm (0 to 2 in) layer for the CRP and crop systems, respectively, with a slight increase with soil depth. Generally, CVs were low, between 5% and 8% in both CRP and crop systems, although there was a tendency for pH to be slightly more variable in CRP based on variance calculations (Table 2). The soil pH at all three pasture sites showed a significant upward trend with depth, and pastures sampled at sites 1 and 5 had the only sub 6.0 pH values encountered in this study (5.65 and 5.66, respectively, for the 0 to 5 cm [0 to 2 in] layer). This is probably the result of agricultural (crop) soils having been limed in the past, whereas pastures most likely were not. Additionally, the USDA-NRCS uses soil testing on land entering the CRP in Wisconsin to identify acidic soils that should be limed so pH can be taken to a value near 6.5. Our data collected across Dane County obviously reflects this management goal in these restoration projects.

Vegetation. In general, the most frequent species found in these sites included smooth bromegrass *(Bromus inermis)*, red clover *(Trifolium pratense)*, timothy *(Phleum pratense)*, showy goldenrod *(Solidago speciosa)*, switchgrass *(Panicum virgatum)*, and Kentucky bluegrass *(Poa pratensis)*. Species encountered that were not part of CRP seed mixtures included pasture thistle *(Cirsium odoratum)*, daisy fleabane *(Erigeron annuus)*, yarrow *(Achillea millefolium)*, junegrass *(Koeleria macrantha)*, milkweed *(Asclepias syriaca)*, and St. John's Wort *(Hypericum perforatum)*.

We attribute some of the observed variability in soil properties to significant variation in vegetation structure, diversity, and coverage across the CRP sites. In some fields (e.g. sites 2, 7 through 11), bare soil was completely absent as the landscape was dominated by grasses and legumes with dense rooting structures (e.g. red clover, smooth bromegrass, and timothy). At other sites (e.g. 3 and 6), even after 10 years, a significant amount of bare soil (> 30% ground area) was still visible. These

Table 3. Calculation of minimum detectable differences (MDD) for several soil quantities in the top 5 cm. We assumed $\alpha = 0.05$, $\beta = 0.20$ ($1-\beta = 0.80$), and the critical parameter (ϕ) was 2.06 (Zar, 1984). The *% Diff (measured)* is the percent change in CRP values relative to cropland ([CRP-crop] / crop); *% Diff MDD* is the percent change for the MDD values relative to cropland (MDD / crop); *Obs needed* refers to the number of sampling sites necessary to achieve $p < 0.05$ and a confidence level of > 80% based on measured sample variance; the column for *ϕ (meas. diff)* is calculated by substituting the measured mean difference between CRP and crop sites as the MDD and using the MSE (within group) to compute statistical power ($1-\beta$) of our sampling technique for soil quantities.

Variable (units)	MSE Between	MSE Within	MDD	CRP measured	Crop measured	Difference (CRP-crops)	% Diff measured	% Diff MDD	Obs needed	ϕ (Measured difference)	Power $1-\beta$
Bulk density (g cm^{-3})	0.221	0.012	0.121	1.407	1.585	-0.178	-11.23	7.61	6	3.04	> 98%
Organic C (%)	2.887	0.379	0.678	2.701	2.059	0.642	31.18	32.92	16	1.95	76%
Organic N (%)	0.022	0.0036	0.066	0.258	0.202	0.056	27.72	32.71	19	1.75	67%
SOM (%)	5.058	0.6028	0.855	3.950	3.100	0.850	27.42	27.58	14	2.05	80%
Total soil C (kg m^{-2})	0.499	0.177	0.463	1.886	1.619	0.267	16.49	28.61	42	1.19	< 40%
Total soil N (g m^{-2})	2960.9	1624	44.374	179.233	158.701	20.532	12.94	27.96	65	0.95	< 40%
Total SOM (kg m^{-2})	0.725	0.262	0.564	2.761	2.439	0.322	13.20	23.11	43	1.18	< 40%

The value for ϕ based on v1=1 and v2=26 and 80% level of confidence was 2.06 (Zar, 1984).

sites were generally absent of large, dense expanses of grasses and dominated by forbs.

Calculation of minimum detectable differences. Table 3 depicts some of the key statistics used in the equation presented by Garten and Wullschleger (1999) for MDD, along with actual values for MDD. We analyzed the following for the 0 to 5 cm (0 to 2 in) layer: (a) the MDD for soil properties, (b) percentage change we measured relative to cropland, (c) the smallest percentage change we could expect to measure for MDD, (d) the number of observations that would be required to achieve 80% confidence in our results based on the measured variance, and (e) a calculation of what the actual statistical power was for our measured differences.

The results suggest that for quantities that had significant observed differences between the two land-use types (Table 2; $p < 0.05$; C, N, OM [mass basis; g kg^{-1}], and bulk density), only bulk density had a statistical power of > 80% (98%). While the differences in concentration data (mass basis) for C, total N, and OM between CRP and cropland were significant at the $p = 0.05$ level, they had statistical power at or slightly below the critical threshold (range 67% to 80%). While we measured relative (to cropland) differences of 27% to 31% in those quantities between land-cover types, we would have needed about two to five more paired sites to achieve an 80% confidence level for all concentration data on a mass basis.

When total C, N, and OM storage was analyzed on an area basis (kg m^{-2} [lb ft^{-2}]), we detected relative (to cropland) changes of 13% to 17% in inventories of C, total N, and OM for the surface 5 cm (2 in). To detect this small change in storage pools with high statistical power, we would have needed to increase the number of observations (paired sites) to about 42 to 65 to attain an 80% confidence level (Table 3). As in the study of Garten and Wullschleger (1999), the CVs of > 20% for SOC inventories in this study warrant the need for about 40 or more sample sites to achieve good statistical power.

Analysis of C and N sequestration. The underlying assumption in forming estimates of C and N sequestration using paired sites or chronosequences is that the rehabilitated sites, at inception of the restoration, possessed similar soil C, total N, SOM, and textural profiles as the adjacent cropped sites. In this study, we confined our sequestration analysis to using data from the top surface (5 cm [2 in]) layer where detectable (but not significant) differences (p=0.1) in soil C and total N (kg m^{-2} [lb ft^{-2}]) were found. Minimal sequestration below the surface layer is in accordance with general relationships found in many other studies (Conant et al., 2001). However, based on our calculation of MDD, the rates of C and N sequestration are not statistically supported because the statistical power associated with ANOVA was well below the 80% confidence level (Table 3). Thus, results presented here may not be completely representative of prairie ecosystems restored as part of the CRP in Wisconsin.

We calculated soil C and N sequestration by taking the observed differences in soil C and total N between CRP and cropped sites and divided the change by the number of years that the land has been enrolled in the CRP. This was done for each individual site for the surface 5 cm (2 in) layer. Table 4 shows that 10 of the 14 sites may have been soil C sinks, and 4 sites slight C sources, over the past 8 to 14 years. The same number of sites exhibited similar patterns for N sequestration. The range in C sequestration rates was -26.9 g C m^{-2} yr^{-1} to 80.2 g C m^{-2} yr^{-1} (-240 to 716 lb C ac^{-1} yr^{-1}). The average rate of C sequestration was 24.7 g C m^{-2} yr^{-1} (220 lb C ac^{-1} yr^{-1}), but the CV of 146% was indicative of the high variability of these rates across field sites. Uncertainty in these results is also attributed to the poor statistical power associated with detecting differences in soil C storage between land-use types (Table 3). Follett et al. (2001) suggested that when paired sampling is implemented and CRP and cropped fields have different ownership (as was the case in the current study), soils might have experienced different historical management regimes (e.g. tillage) that contribute to variability, even though sites are well-matched in terms of slope and texture.

Rates of N sequestration ranged from -2.8 to 9.8 g N m^{-2} yr^{-1} (-25 to 88 lb N ac^{-1} yr^{-1}. The average N sequestration rate was 1.7 g N m^{-2} yr^{-1} (15 lb N ac^{-1} yr^{-1}), but there was a high degree of variability among the 14 sites (CV = 239%). Again, uncertainty is supported by the low statistical power associated with detecting differences in soil N storage between land-use types (Table 3). Carbon and N sequestration in this study was occurring, on average, with a C:N of 14.4.

Table 4. Changes in surface layer (0 to 5 cm) soil Carbon (C) and Nitrogen (N) as a result of the CRP compared to adjacent cropland. Rates of C and N sequestration were calculated as total change in storage by the number of years since restoration of prairie vegetation as part of the CRP.

Paired site	Year planted	0-5 cm ΔC g-C m^{-2}	0-5 cm ΔN g-N m^{-2}	C seq rate g-C yr^{-1}	N seq rate g-N yr^{-1}
1	1991	258.42	20.93	25.84	2.09
2	1988	913.96	88.44	70.30	6.80
3	1987	-254.60	-21.92	-18.19	-1.57
4	1991	131.87	-19.82	13.19	-1.98
5	1987	-377.09	-36.94	-26.94	-2.64
6	1993	588.13	-17.57	73.52	-2.20
7	1988	207.69	52.21	15.98	4.02
8	1988	171.45	36.52	13.19	2.81
9	1988	369.78	37.94	28.44	2.92
10	1988	913.86	98.54	70.30	7.58
11	1988	-112.10	-4.73	-8.62	-0.36
12	1987	302.22	-4.94	21.59	-0.35
13	1987	-177.63	-39.12	-12.69	-2.79
14	1991	802.18	97.92	80.22	9.79
Average		267.01	20.53	24.72	1.72
SE		112.36	13.11	9.67	1.10
CV (%)		157.00	239.00	146.00	239.00

Compared with previous assessments of C sequestration by the CRP in the surface 5 or 10 cm (2 or 3.9 in), the statistically unsupported C accumulation rates we have calculated are conservative estimates. Follett et al. (2001) examined C sequestration by the CRP for 14 sites at least five years old in nine Great Plains and Midwest states but excluded sites from Wisconsin and did not test statistical power. The soil-sampling strategies (paired site and depth increments) were nearly identical between our study and this recent comprehensive study, and the majority of sites in both studies were found on fine-textured soils. Based on their data, Follett et al. (2001) suggested that the CRP sequesters between 57 and 74 g C m^{-2} yr^{-1} (508 and 661 lb C ac^{-1} yr^{-1}) in the 0 to 5 (0 to 2 in) and 0 to 10 cm (0 to 3.9 in) depth increments, respectively. It is difficult to determine whether the differences between our results and data from that study were due to sampling errors, species diversity, climate, soils, or past land management.

Unlike the study of Follett et al. (2001), sequestration rates in our composite 0 to 10 cm (0 to 3.9 in) layer were undetectable. Our data suggest that differences in sampling protocol near the soil surface might impact calculations of soil C and N sequestration. We used the composite analysis of soil C, N, and OM storage (on an area basis; kg m^{-2} [lb ft^{-2}]) for the entire 0 to 10 cm (0 to 3.9 in) layer to show that differences in soil C, total N, and OM storage in the 0 to 5 cm (0 to 2 in) layer would have been masked if using a 0 to 10 cm (0 to 3.9 in) fixed sampling depth. In this scenario, results from ANOVA on differences between paired sites yielded $p = 0.53$ for SOC, $p = 0.67$ for soil total N, and $p = 0.69$ for SOM. Although CRP quantities for the pooled 0 to 10 cm (0 to 3.9 in) layer were higher in each case than cropland, they were not statistically significant. These results support a sampling protocol that uses soil-core increments less than 10 cm (3.9 in) near the surface (Post et al., 2001). In many previous studies, a surface sampling depth of 7.5 cm (3 in) has been used (Karlen et al., 1999; Gilley et al., 2001). Our study suggests that a 5 to 7.5 cm (2 to 3 in) surface layer might be essential in detecting small differences in C pools between land-use types. However, we are aware that changes in specific C pools (e.g. active vs. passive) might be more detectable rather than the entire SOC pool.

Soil sampling for SOC of just a few pastures used for grazing in Dane County suggests that if prairies and the CRP were to replace these land-cover types, net C sequestration would probably be diminished or possibly undetectable, similar to a previous study (Bransby et al., 1998).

Summary and Conclusion

It is likely that future feasibility studies will help determine whether the costs associated with sampling and processing of samples outweigh any future C-credit payments that may be given to CRP participants. Utility companies and other industry will have a significant interest in the future of C crediting for economic and regulatory reasons, but also because it can help enhance their public image through support of land-conservation programs. Because it will be impossible to track individual landowners for C sequestration as part of a program such as the CRP, utilities may be persuaded to offer payments for large areas of land as part of the program based on an average result, rather than individual results, because individual site data probably will not exist. Thus, based on statistical analysis such as that used in this study, all CRP enrollees in a small region or county on similar soil types might receive an "average" payment for C sequestered, making no effort to base payments on individual field results. We cannot rely upon *in situ* measurements (e.g. component measurements of C cycling or eddy-covariance techniques) to provide verification of C sequestration occurring for each individual project.

In this study, statistical analysis showed that we did not sample a sufficient number of paired CRP and cropland sites to achieve high statistical power for SOC, total N, and OM differences on an area basis (kg m^{-2} [lb ft^{-2}]) in the surface 5 cm (2 in) layer ($p < 0.1$), with the exception of surface bulk density. We detected relative ([CRP-cropland]/cropland) changes of 27% to 31% for soil C, total N, and OM concentration data on a mass basis (g kg^{-1} [oz lb^{-1}]), and 12% to 17% on an area basis (kg m^{-2} [lb ft^{-2}]). We concluded that these silt loam soil types in southern Wisconsin possessed a high degree of spatial variability in C and N storage (CVs > 20%). Subsequently, about 40 to 45 paired sites will need to be identified and sampled to achieve good statistical power to verify that the CRP is contributing to C and N sequestration using a paired-site sampling approach. This type of information is valuable for proper planning and budgeting of future initiatives. We conclude that soils should be sampled in fairly small increments near the surface (e.g. 5 cm [2 in]); otherwise, differences between sites may be undetected.

In future assessments of C and N sequestration as part of the CRP, more attention will need to focus on (1) producing statistically defensible results using power analysis to help establish a standardized sampling protocol, (2) assuring that soil textural compositions possess similar variance between paired-site locations, and (3) investigating the connection between species composition, restoration protocol, and C cycling. Relatively few studies have examined the impact of vegetation biodiversity on C sequestration rates (Knops and Tilman, 2000; Tilman et al., 2001), but more are needed because previous studies have suggested that an overabundance of forbs in prairie restoration projects may lead to lower net primary production (NPP) (Knops and Tilman, 2000; Kucharik et al., 2001). Moreover, soil-erosion control may also be affected because of differences in rooting depth and total root length between forbs and grasses. Because scientists generally favor quantification and improvement of soil physical (e.g. bulk density), chemical (increased SOM), and biological (microbial biomass) properties and landowners set their goals in terms of increasing aboveground biodiversity (e.g. insects, birds, and other wildlife) and enhanced visual appeal, some controversy is likely to abound from private citizens in regard to scientific hypotheses. The Wisconsin CRP might differ from other states in that a high diversity of forbs were generally found and thus seeded from the onset of restoration in the majority of sites. At this time, it is unclear what role increased species diversity in the Wisconsin CRP plays in soil C and N sequestration.

Acknowledgments

The authors wish to thank Kevin Connors, J.B. Martin, and Patrick Sutter with the USDA Natural Resources Conservation Service in Madison, Wisconsin; Carmela Diosana, Joe Helkowski, and Mary Sternitzky. for assisting with field work; and the landowners in Dane and Iowa Counties in Wisconsin for their cooperation, insightful discussion, and valuable land-use information essential for this study. Extensive comments and suggestions from three anonymous reviewers and the editor contributed significantly to the final version of this manuscript. The Barker Fund, through the University of Wisconsin Foundation, supported this project.

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