

# Prairie restoration and carbon sequestration: difficulties quantifying C sources and sinks using a biometric approach

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**Abstract.** We investigated carbon cycling and ecosystem characteristics among two prairie restoration treatments established in 1987 and adjacent cropland, all part of the Conservation Reserve Program in southwestern Wisconsin, USA. We hypothesized that different plant functional groups (cool-season C<sub>3</sub> vs. warm-season C<sub>4</sub> grasses) between the two prairie restoration treatments would lead to differences in soil and vegetation characteristics and amount of sequestered carbon, compared to the crop system.

We found significant ( $P < 0.05$ ) differences between the two prairie restoration treatments in soil CO<sub>2</sub> respiration and above- and belowground productivity, but no significant differences in long-term (~16-year) carbon sequestration. We used a biometric approach aggregating short-term observations of above- and belowground productivity and CO<sub>2</sub> respiration to estimate total net primary production (NPP) and net ecosystem production (NEP) using varied methods suggested in the literature. Net ecosystem production is important because it represents the ecosystem carbon sequestration, which is of interest to land managers and policymakers seeking or regulating credits for ecosystem carbon storage. Such a biometric approach would be attractive because it might offer the ability to rapidly assess the carbon source/sink status of an ecosystem.

We concluded that large uncertainties in (1) estimating aboveground NPP, (2) determining belowground NPP, and (3) partitioning soil respiration into microbial and plant components strongly affect the magnitude, and even the sign, of NEP estimates made from aggregating its components. A comparison of these estimates across treatments could not distinguish differences in NEP, nor the absolute sign of the overall carbon balance. Longer-term quantification of carbon stocks in the soil, periodically linked to measurements of individual processes, may offer a more reliable measure of the carbon balance in grassland systems, suitable for assigning credits.

**Key words:** aboveground; belowground; carbon cycling; carbon sequestration; carbon sink; Conservation Reserve Program; grassland; net ecosystem production; net primary production; southwestern Wisconsin, USA; uncertainty analysis.

## INTRODUCTION

Grasslands are an important biome across the planet, covering 20% of the terrestrial surface, storing 10–30% of global soil carbon stocks, and accounting for as much as 20% or more of total terrestrial production (Schlesinger 1977, Eswaran et al. 1993, Scurlock and Hall 1998). Despite their importance, grasslands have not received as much attention as forests in the context of global change, although belowground allocation patterns in grasslands contribute to significant carbon

storage potential (Scurlock and Hall 1998). Provisions in the Kyoto Protocol and other emissions reductions frameworks allow carbon uptake and accumulation in vegetation and soils of terrestrial ecosystems to be used to meet greenhouse gas reduction targets. However, no standardized, widely adopted protocol for verifying carbon credits yet exists. Therefore, further analysis of available methodologies to quantify ecosystem carbon storage is necessary.

Net carbon gain in plant tissues above and below ground is termed net primary production (NPP), an important ecosystem property that determines carbon available for sequestration (see Fig. 1; Jenkinson et al. 1992, Scholes et al. 1997). Although NPP is widely measured and modeled, several different methodologies are commonly used to calculate grassland NPP, and these can produce widely varying results with important implications for estimating ecosystem carbon balances. Most published reports of NPP do not explicitly state which components of NPP have been included, so

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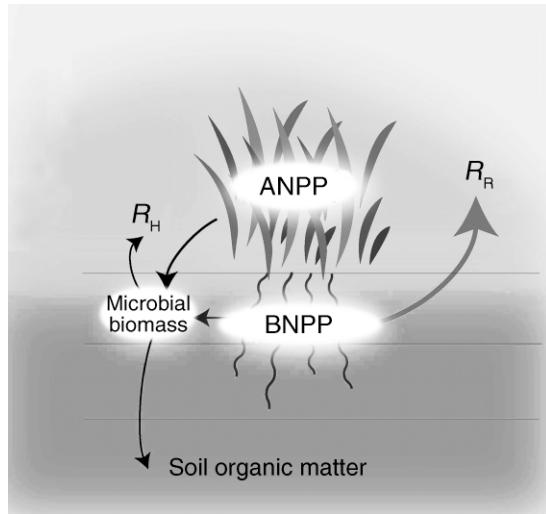


FIG. 1. Conceptual model of the carbon cycle. NPP is net primary production, the net vegetation carbon gain from photosynthesis. ANPP is aboveground NPP; BNPP is belowground NPP;  $R_R$ , root respiration;  $R_H$ , heterotrophic or microbial respiration; and  $R_R$ , autotrophic root respiration. Soil respiration consists of  $R_R + R_H$ . Net ecosystem production = ANPP + BNPP -  $R_H$ . Net ecosystem production (NEP) is total ecosystem carbon gain or loss.

comparisons among studies can be difficult (Chapin et al. 2002). While NPP is often thought to be underestimated in grasslands (Scurlock et al. 2002), much debate on this topic remains (Kelly et al. 1974, Singh et al. 1984, Vogt et al. 1986, Biondini et al. 1991, Defosse and Bertiller 1991, Scurlock et al. 2002).

The annual net carbon balance of an ecosystem (net ecosystem productivity, or NEP) includes NPP from plants as well as carbon cycling changes in soil and microbial biomass. Here we will adopt the convention that NEP is positive when ecosystem gains exceed losses (i.e., a carbon sink from atmosphere to ecosystem) and negative when losses exceed gains (a carbon source from ecosystem to atmosphere). NEP is the balance between two large terms with opposite signs: carbon inputs originating from plants and  $\text{CO}_2$  released from decomposition. We identify three basic approaches to estimate NEP and carbon sequestration at the ecosystem scale, each of which has advantages and drawbacks. First, whole-ecosystem  $\text{CO}_2$  flux measurements may be made using eddy covariance towers or Bowen energy ratio systems to measure meteorological conditions and atmospheric gradients of  $\text{CO}_2$  and moisture to determine the exchange rate of  $\text{CO}_2$  between the atmosphere and plant canopy (Dugas 1993, Dugas et al. 1999, Suyker and Verma 2001, Baldocchi 2003). This approach captures the overall net exchange of ecosystem  $\text{CO}_2$  but is subject to errors when measuring heterogeneous landscapes under variable atmospheric conditions and at night with weak mixing due to low wind speeds. Furthermore, the equipment is expensive to install and maintain (Baldocchi 2003). Second, repeated measures

of soil and plant carbon stocks and their change through time can be used to estimate NEP (Brown 2002, Garcia-Oliva and Masera 2004). This approach can accurately quantify stocks if replicated over heterogeneous landscapes, but soil carbon accumulation may be slow and difficult to detect in a short time frame. Third, the biometric approach aggregates elements of a simplified ecosystem carbon budget for grasslands that considers only carbon inputs from above- and belowground plant production and the largest source of carbon losses from soil respiration (e.g., Brye et al. 2002). This approach is increasingly employed to estimate components of ecosystem carbon balance and has the potential to be relatively rapid and cost-effective, although methods are still being refined (Norman et al. 1992, 1997, Canadell et al. 2000, Rustad et al. 2000, Barford et al. 2001, Law et al. 2001, Curtis et al. 2002, Franzluebbers et al. 2002).

In this paper, we have two goals. First, we report short-term field measurements obtained in restored Wisconsin, USA, Conservation Reserve Program (CRP) grasslands to illustrate difficulties in implementing the biometric approach to measure individual processes contributing to NEP. Observational studies reporting NEP commonly estimate the individual components or apply values from the literature from other sites using different methods, and we want to explore how this may affect the resulting report of source or sink by applying a factorial range of estimates to one site. In Kucharik et al. (2006), we applied the belowground productivity estimates reported in detail here to briefly report a range of belowground NPP estimates in highly managed native and restored prairies. We are not aware of any study that focuses on how the uncertainties of the aboveground, belowground, and respiration components interact when attempting to close an ecosystem carbon budget on CRP land.

Second, we report ecosystem properties for three land-use treatments to contribute to an improved understanding of the factors that make ecosystem carbon sequestration so highly variable in space and time. For example, while a number of grasslands enrolled in the CRP west of Wisconsin have reported significant carbon sequestration rates (Gebhart et al. 1994, Barker et al. 1995, Follett et al. 2001, Paustian et al. 2001), several studies have found Wisconsin grassland sequestration rates to range from negligible to 50% less than those reported for the Great Plains, USA (Brye et al. 2002, Kucharik et al. 2003). Numerous studies of restoration from agriculture to grassland, many using the Conservation Reserve Program (CRP) as a model, have demonstrated that restoration enhances carbon sequestration (Gebhart et al. 1994, Burke et al. 1995a, b, Robles and Burke 1997, Karlen et al. 1999, Wang et al. 1999, Baer et al. 2000, Kucharik et al. 2003). Several studies suggest that  $\sim 50$  yr is adequate for recovery of restored agricultural soils to regain active carbon pools (Burke et al. 1995a, Kucharik et al. 2001), but further study is needed to understand the intensity and type of

management necessary to sustain long-term carbon sinks. Few estimates have quantified the effects on carbon cycling of plant functional groups (i.e., C<sub>3</sub> grasses, C<sub>4</sub> grasses, nitrogen (N)-fixing species, and forbs). The higher photosynthetic rates and higher nitrogen and water-use efficiencies of C<sub>4</sub> vs. C<sub>3</sub> grasses have been correlated with greater productivity, and may have important consequences for ecosystem function and global change (Epstein et al. 1998, Owensby et al. 1999, Wang et al. 1999, Knops and Tilman 2000, Kucharik et al. 2001, Derner et al. 2003).

## MATERIALS AND METHODS

### *Site description*

The study site was located in the unglaciated Driftless Area of southwestern Wisconsin, USA (43°4'12" N, 89°49'12" W). The underlying soil was Dunbarton silt-loam (a clayey, smectitic, mesic Lithic Hapludalf) with a 70 cm depth. Soil erosion has been a serious and widespread problem in the area due to the steep slopes (>12%) of cultivated land (Hole 1976). Growing degree-day (GDD) accumulation was 1908 GDD (base 8°C) and precipitation was 778 mm in 2002 (Kucharik et al. 2006). The study site had been in continuous maize cropping from the 1940s to 1987, when it was enrolled in the Conservation Reserve Program (CRP) and converted to three land-cover treatments: the C<sub>4</sub>-dominated treatment (seeded with *Andropogon gerardii*, switchgrass; *Panicum virgatum*, big bluestem; and *Sorghastrum nutans*, Indiangrass); the C<sub>3</sub>-dominated treatment (seeded with *Bromus inermis* Leyss., smooth brome grass; *Phleum pratense* L., timothy; and *Medicago* spp., alfalfa); and the crop treatment, a disk planted, unfertilized, continuous no-tillage rotation of maize (in odd years) and soybeans (in even years, including during this study).

### *Experimental design*

The three land-use treatments were applied in long, thin strips, which varied in slope and aspect over much of the hilly terrain. The three ~1 ha study plots were selected at the one location where all three treatments were adjacent and shared a flat slope, to eliminate the variables of landscape position in comparing treatments. Three lines of evidence support the assumption that the three treatments were very similar in terms of landscape position, previous land use, and soil properties at the time of restoration, so that observed differences can be attributed to land management change. First, study plots were selected for their shared slope/aspect and proximity. Second, government and landowner records demonstrate homogenous management under the same owner before restoration for all of the area now studied under different land management treatments. Finally, since soil texture is slow to change compared with soil C and N, the very similar proportions of silt, sand, and clay for the three land-use treatments demonstrate that the soil types were similar among the three treatments (Table 1).

### *Vegetation and soil measurements*

Measurements were initiated in the two grass-dominated treatments in mid-April 2002, and in early June 2002 in the crop treatment, after soybeans had been planted to avoid disturbance to buried sensors. Leaf area index (LAI) was measured with a LI-COR LAI 2000 plant canopy analyzer (LI-COR, Lincoln, Nebraska, USA) every 7–10 days throughout the growing season. Species composition and abundance was estimated using the method of Jorgensen and Tunnell (2001). Soil samples for pH, texture, bulk density, and carbon (C), nitrogen (N), and organic matter (OM) were collected in May 2003 from 2 × 2 m subplots randomly located along a ~200 m transect in each treatment. Soil C and N were determined by high-temperature catalytic combustion, OM by loss on combustion, texture using the hydrometer method (Bouyoucos 1962), and pH was determined using a soil slurry. Bulk density samples were taken using a 4.67 cm diameter corer, dried at 40°C for 48 h, and weighed. A Campbell Scientific CR-10X data logger (Campbell Scientific, Logan, Utah, USA) hooked up to thermocouples and ECH<sub>2</sub>O dielectric aquameters (Decagon Devices, Pullman, Washington, USA) was used to continuously monitor air temperature, soil temperature at 2, 5, 10, 30, and 70 cm depth, and soil moisture at 15 and 30 cm depth in each treatment.

### *Estimates of aboveground net primary production*

We measured aboveground biomass monthly by weighing the dried matter collected from 0.25-m<sup>2</sup> clip plots. Aboveground NPP (ANPP) in grasslands is most commonly estimated by peak (maximum) live biomass. However, this method may underestimate NPP by two to three times in humid temperate grasslands (Gill et al. 2002, Scurlock et al. 2002) because it fails to account for the dynamics of simultaneous vegetation growth, mortality, and decay which transfer plant matter among the live, standing dead, and litter pools during the growing season. Additionally, some early- or late-developing species may not be captured by sampling at a single time point (Kelly et al. 1974, Brye et al. 2002). Even repeated seasonal measurements such as those we undertook may underestimate ANPP by failing to account for growth changes between sampling intervals and for insect herbivory (Kelly et al. 1974). Given the continuing debate over the methodology to best estimate grassland ANPP, we present results from three approaches applied to our aboveground biomass data for comparison of the two prairie restoration treatments.

1) Peak live (PL) biomass only. This assumes that each increment increase in live biomass between sampling dates was significant, and that all live growth represents the current season and is equal to the sum of live increments from all previous months (Scurlock et al. 2002).

2) Significant increments (SI). Peak live biomass plus statistically significant increases in either standing dead

TABLE 1. Soil properties (mean  $\pm$  SE) of three land-cover treatments of our study site in the Conservation Reserve Program (CRP) in southwestern Wisconsin, USA.

Soil depth and property	Treatment		
	C <sub>3</sub> dominated	C <sub>4</sub> dominated	Crop
0–20 cm			
Silt (%)	41 <sup>a</sup> $\pm$ 4	42 <sup>a</sup> $\pm$ 3	44 <sup>a</sup> $\pm$ 3
Sand (%)	41 <sup>a</sup> $\pm$ 3	43 <sup>a</sup> $\pm$ 4	43 <sup>a</sup> $\pm$ 3
Clay (%)	18 <sup>a</sup> $\pm$ 2	15 <sup>a</sup> $\pm$ 2	13 <sup>a</sup> $\pm$ 2
0–10 cm			
<i>D<sub>b</sub></i> (g/cm <sup>3</sup> )*	1.39 <sup>a</sup> $\pm$ 0.02	1.34 <sup>a</sup> $\pm$ 0.04	1.54 <sup>b</sup> $\pm$ 0.03
0–6 cm			
VWC (m <sup>3</sup> water/m <sup>3</sup> soil)*	0.28 <sup>a</sup> $\pm$ 0.01	0.31 <sup>b</sup> $\pm$ 0.01	0.24 <sup>c</sup> $\pm$ 0.01
0–5 cm			
pH*	5.99 <sup>a</sup> $\pm$ 0.19	6.01 <sup>a</sup> $\pm$ 0.19	6.44 <sup>b</sup> $\pm$ 0.13
SOM (g/kg)*	3.66 <sup>a</sup> $\pm$ 0.16	3.37 <sup>a</sup> $\pm$ 0.24	2.63 <sup>b</sup> $\pm$ 0.11
SOC (g/kg)*	2.12 <sup>a</sup> $\pm$ 0.10	2.11 <sup>a</sup> $\pm$ 0.19	1.58 <sup>b</sup> $\pm$ 0.08
TN (g/kg)*	0.19 <sup>a</sup> $\pm$ 0.01	0.18 <sup>ab</sup> $\pm$ 0.01	0.16 <sup>b</sup> $\pm$ 0.01
5–10 cm			
SOM (g/kg)	2.91 <sup>a</sup> $\pm$ 0.16	2.60 <sup>a</sup> $\pm$ 0.21	2.47 <sup>a</sup> $\pm$ 0.11
SOC (g/kg)	1.50 <sup>a</sup> $\pm$ 0.09	1.46 <sup>a</sup> $\pm$ 0.19	1.41 <sup>a</sup> $\pm$ 0.08
TN (g/kg)	0.14 <sup>a</sup> $\pm$ 0.01	0.13 <sup>a</sup> $\pm$ 0.01	0.14 <sup>a</sup> $\pm$ 0.01
10–20 cm			
SOM (g/kg)	2.57 <sup>a</sup> $\pm$ 0.18	2.19 <sup>a</sup> $\pm$ 0.17	2.19 <sup>a</sup> $\pm$ 0.09
SOC (g/kg)	1.13 <sup>a</sup> $\pm$ 0.08	1.27 <sup>a</sup> $\pm$ 0.21	1.16 <sup>a</sup> $\pm$ 0.05
TN (g/kg)	0.11 <sup>a</sup> $\pm$ 0.00	0.10 <sup>a</sup> $\pm$ 0.01	0.11 <sup>a</sup> $\pm$ 0.01

Notes: Values that are significantly different are distinguished with different letters within the same row and with an asterisk after the property. *D<sub>b</sub>* is bulk density, VWC is volumetric water content, SOM is soil organic matter, SOC is soil organic carbon, and TN is total soil nitrogen; *n* = 10 samples per treatment; for bulk density, *n* = 30 samples per treatment.

\* *P* < 0.05.

or litter pools when the total biomass increased from the previous sample date. This assumes that after the peak total biomass was reached, increases in the two dead compartments originated from the live pool (Paustian et al. 1990).

3) All increments (AI). Peak live biomass plus any increases from previous measurements in standing dead or litter regardless of statistical significance (e.g., Brye et al. 2002).

#### Estimates of belowground net primary production

Belowground biomass was estimated by hand picking roots from 6.67 cm diameter root cores taken from the 0–50 cm soil layer in 10 cm increments; these were oven dried at 70°C for 24 h, weighed, then dry ashed to correct for mineral soil contamination (Steele et al. 1997). Belowground NPP (BNPP) is critical to understanding ecosystem dynamics and carbon sequestration potential, but the challenge of removing root tissue from soil samples and identifying live and dead roots is substantial (Silver and Miya 2001, Gill et al. 2002). Fewer than 10% of studies that report ecosystem NPP actually measure components of belowground production (Clark et al. 2001), so field observations are needed for a range of biomes.

There is considerable debate regarding methodologies for estimating grassland root turnover and associated errors (Scholes et al. 1997, Gill et al. 2002). Although

isotopic techniques have been developed (Milchunas and Lauenroth 2001), the most common approach to estimate BNPP remains direct biomass sampling. This approach employs either sequential belowground biomass sampling (similar to aboveground estimates) or static biomass estimates corrected with turnover coefficients to estimate annual productivity (Gill et al. 2002). Root turnover has been defined as an annual proportion describing the death and subsequent detachment of roots (Gill et al. 2002), the production of new roots and the disappearance of old ones (Rice et al. 1998), the result of dividing annual root increment by peak belowground biomass (Sims and Singh 1978), and finally, production divided by maximum standing crop (Hayes and Seastedt 1987).

Although the technique of multiplying maximum live root biomass by a turnover coefficient to estimate BNPP may be sensitive to variability in the data and sampling bias (Singh et al. 1984, Gill et al. 2002), this approach is believed to be more accurate (Gill et al. 2002). Because belowground production is influenced by available water, soil properties, fire history, plant species composition, and other factors that may vary significantly among sites (Rice et al. 1998), direct fine-root sampling generally represents a more accurate estimate than using a modeled relationship based on aboveground productivity (Gill et al. 2002) or relying on very few estimates published elsewhere.

TABLE 2. Reported values for grassland heterotrophic respiration ( $R_H$ ).

Author	Ecosystem studied	Reported $R_H$
Bond-Lamberty et al. (2004)	algorithm for estimating $R_H$ fraction developed for forests but also recommended for prairies	53–56%
Brye et al. (2002)	Wisconsin prairie	25%
Buyanovsky et al. (1987)	$C_4$ -dominated tallgrass prairie	75–80%
Herman (1977)	$C_4$ -dominated tallgrass prairie	60–83%; recommended 70%
Hungate et al. (1997)	annual coastal California grassland	55%
Kucera and Kirkham (1971)	$C_4$ -dominated tallgrass prairie	60%
Millard et al. (2008)	Texas savannah	51–57%

We used five different estimates of root biomass turnover to derive BNPP. These turnover estimates are expressed as proportions of annual turnover and are multiplied by our static fine root biomass data to estimate BNPP in units of  $\text{g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ . The first three estimates were modeled relationships for grassland root turnover proposed by Gill et al. (2002) for live root biomass: (1) a direct (DIR) function of ANPP,  $0.0009 \text{ g/m}^2 \times (\text{ANPP}) + 0.25 \text{ yr}^{-1}$ , which yielded turnover coefficients between 0.56 and 1.21 depending on ANPP; (2) a constant (CON) function of 0.65 regardless of ANPP; (3) an exponential (EXP) function of mean annual temperature (MAT, °C),  $0.2884e^{0.046\text{MAT}}/\text{yr}$ , which yielded a turnover of 0.43.

The final two estimates for root biomass turnover values came from observational studies. (1) A total (live + dead) root biomass turnover value of 2.5 in a restored,  $C_4$  grass-dominated Wisconsin prairie reported by Brye et al. (2002) using minirhizotrons (MRH). (2) Root turnover of 0.40 for live and 0.26 for dead root biomass, which is the mean of two years of data reported by Hayes and Seastedt (1987) using root windows (RW).

Estimation of crop ANPP used the peak live-soybean biomass at maturity, a common assumption for crop species (Brye et al. 2002) because the soybeans completed their life cycle in the one growing season measured; there was no initial standing dead matter, and the only litter was corn stubble from the previous year. Similarly, we adopted an assumption for agricultural crops that BNPP equaled peak root biomass, since the majority of roots present were produced during the current growing season (Gower et al. 1999).

#### Soil respiration

Soil surface  $\text{CO}_2$  flux ( $R_S$ , total soil respiration, consisting of both autotrophic [plant] and heterotrophic [soil animals and microbes] respiration) was measured in each treatment using a LI-COR 6400 portable photosynthesis and infrared gas analyzer system equipped with a 1-L soil respiration chamber (Norman et al. 1992, 1997). Measurements at 10 randomly placed polyvinylchloride (PVC) collars were taken every 7–10 days during the growing season (April–November) and occasionally during the rest of the year. To avoid overestimating  $\text{CO}_2$  fluxes from loose PVC collars, recently disturbed soil, or cut roots, we beveled the collars at the ground edge to minimize disturbance upon

installation, moved the collars approximately every six weeks to ensure measurement of representative soil conditions (Wagai et al. 1998), and waited at least 24 hours to take measurements following collar movement (Norman et al. 1997).

We developed site-specific regression models to estimate continuous soil respiration for each treatment using soil-surface temperature (0–10 cm depth) and moisture (0–6 cm depth) measured adjacent to  $\text{CO}_2$  collars. Simple and multiple linear regressions were performed using log-transformed response data ( $R_S$ ) to meet the assumptions of linear regression. Models were selected based on significance of parameters and  $r^2$  and mean square error values. We then used the continuous mean daily soil temperature and moisture measured at our data logger station to estimate daily soil  $\text{CO}_2$  flux, and summed these from 1 June 2002 to 31 May 2003 to get an annual estimate. We took 2 d of diurnal measurements and found midday measurements varied <10% from measurements taken at other times of day, and concluded that they were adequately representative to scale from the measured intervals to an annual basis (Knapp et al. 1998).

There is no consensus on methods for partitioning between heterotrophic and autotrophic components of soil respiration (Hanson et al. 2000, Rustad et al. 2000, Franzluebbers et al. 2002; see Kuzyakov [2006] for a review of techniques highlighting current shortfalls and the need for method standardization). Most studies use annual estimates of the proportions of  $R_H$  (annual heterotrophic respiration) and  $R_R$  (annual autotrophic root respiration) for an ecosystem, although these allocations between the two components may change throughout the year and depend on growing conditions (Hanson et al. 2000), land management, and plant phenology. We estimated  $R_H$  for the grasses using three values representing a reasonable range for tallgrass prairie systems: 60%, 70%, and 80% of total respiration (see comparison with grassland  $R_H$  fraction reported in Table 2). This proportion remained fixed throughout the year, as no data were available to describe changing partitioning of  $R_H$  and  $R_R$  in these systems.

#### Estimating net ecosystem production (NEP)

We estimated the annual NEP by applying the biometric approach to our field data using the following equation, in units of  $\text{g}\cdot\text{C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ :

$$\text{NEP} = \text{NPP}_T - R_H$$

where

$$\text{NPP}_T = \text{ANPP} \times \%C_{\text{ANPP}} + \text{BNPP} \times \%C_{\text{BNPP}}$$

and

$$R_H = R_S - R_R.$$

Here  $\text{NPP}_T$  is total annual carbon partitioned above and belowground, ANPP and BNPP are above- and belowground net primary production, and  $R_S$ ,  $R_H$ , and  $R_R$  are annual total soil respiration, heterotrophic respiration, and autotrophic root respiration, respectively. The carbon content of ANPP was measured, and BNPP carbon concentration was taken from tallgrass prairie root-carbon concentrations reported by Rice (1999); both were near 40% C. Both ANPP and BNPP were estimated for the 2002 growing season, while annual  $R_S$  was based on the year from 1 June 2002 to 31 May 2003 when soil respiration observations were available. Note that above- and belowground NPP are reported in units of g biomass/m<sup>2</sup> for consistency with other published reports; we multiplied each fraction of monthly biomass by its relative carbon content to convert dry masses of above- and belowground biomass to carbon content.

We combined our three methods for estimating ANPP, five methods for estimating BNPP, and three methods for estimating the fraction of  $R_H$  for a total of 45 estimates of NEP for the prairie treatments (Fig. 2). For partitioning the  $R_S$  in the crop treatment, we took two approaches: a constant proportion throughout the year of 65%  $R_R$  reported for soybeans (Wagner and Buyanovsky 1989), and an estimate where the allocation varied over the growing season for an annual crop because fine-root growth and decay is completed in one growing season (Rochette et al. 1999). Because the planting dates of this study and that of Rochette et al. (1999) were similar, we assumed the same partitioning of  $R_R$  here. Our simplified assumptions about above- and belowground NPP for the crop treatment yielded a single  $\text{NPP}_T$  estimate and two estimates of NEP from the two approaches of partitioning  $R_S$ .

#### *Statistical analysis*

We used the SAS General Linear Model (version 8.2, SAS Institute, Cary, North Carolina, USA) for one-way analysis of variance (ANOVA), using a randomized experimental design, to compare all characteristics other than CO<sub>2</sub> flux among treatments. Where an ANOVA *F* statistic showed that not all means for a given characteristic were equal, Fisher's protected least significant difference (LSD) test was used to determine differences among means at  $\alpha = 0.05$ .

To compare soil surface CO<sub>2</sub> fluxes among treatments, we used a mixed-model ANOVA to account for the repeated measures on the same collars over time, and a spatial power approach to account for the varying

amounts of time between flux measurements. This model assumed that treatment and date were fixed effects, while effects from individual collars were random to allow generalizing inferences from the collar to the whole-plot level.

To find standard errors for our calculations of NEP, we converted ANPP, BNPP, and  $R_S$  to a common unit (g C·m<sup>-2</sup>·yr<sup>-1</sup>) and combined the variances of each NEP term. We assumed that the three component estimates were independent of each other, so that the estimated variance for NEP was simply the sum of the variance estimates of the components. For each different method of calculating NEP, we assumed that the selected method was "correct" and ignored model error. We report the standard error of the estimate of NEP as the square root of the sum of the three variance terms.

We acknowledge the shortcomings of reporting measurements from a relatively short time span and from only one site, which could not be experimentally replicated because of the unique pairing of agricultural land and prairies, shared land-use history, and the two diverse species mixes used by the landowner during restoration. The paired-plot approach used here is useful for inferring the effects of different land-use practices on soil and vegetation characteristics and carbon sequestration (Wagai et al. 1998, Brye et al. 2002, Kucharik et al. 2003, 2006). However, inferences about ecosystem structure should be drawn for this study only. Nonetheless, we believe these data are a valuable contribution because of the richness of attributes measured in one site, especially root biomass, and the opportunity this provides to compare carbon budget components that are not frequently measured at one site. The novelty of bringing together a range of NEP estimates from the published literature is also a valuable contribution, using our data as a case study to illustrate the range of uncertainties. Finally, these data are a valuable point of comparison with other sites. They are likely to be different than restored grasslands reported elsewhere because state management protocols for CRP lands in Wisconsin promote higher species diversity than other regions of the central United States.

## RESULTS

### *Vegetation*

Although the C<sub>4</sub>-dominated treatment had different species composition and greater total plant cover (95%) than the C<sub>3</sub>-dominated treatment (81%), functional group proportions were similar for both prairie treatments (~77% of total plant cover was grasses and ~23% forbs). All treatments had substantial quantities of non-seeded species (e.g., patchy bunches of switchgrass in the C<sub>3</sub> treatment), and some species that were seeded had failed to establish (e.g., big bluestem and Indiangrass in the C<sub>4</sub>-dominated treatment and timothy in the C<sub>3</sub>-dominated treatment). Two C<sub>4</sub> grasses, big bluestem and Indiangrass, were seeded into the C<sub>3</sub>-dominated treatment by the landowner 8 years after establishment with

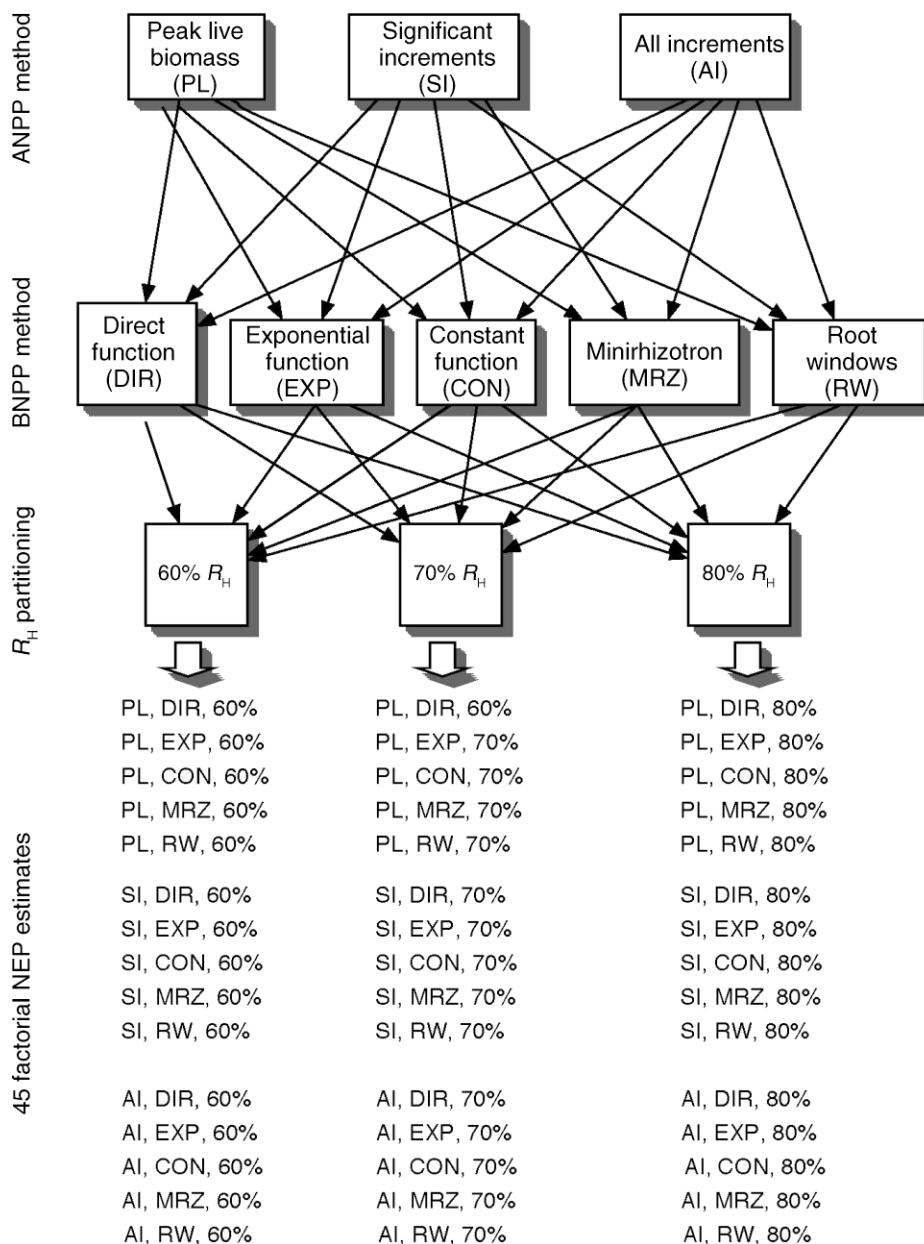


FIG. 2. Flow chart of the calculations used for the 45 factorial estimations of NEP in our study of carbon cycling and ecosystem characteristics among two prairie restoration treatments and adjacent cropland in the Conservation Reserve Program in southwestern Wisconsin, USA.

the intention of “filling out” the vegetative cover, but comprised <5% of the grasses in that treatment. The most abundant species in the C<sub>4</sub> treatment was switchgrass; in the C<sub>3</sub> treatment, smooth brome. Mean species richness was significantly different among all three treatments (4.4 ± 0.2 species per 0.20 × 0.50 cm quadrat for the C<sub>3</sub> treatment, 3.9 ± 0.3 for the C<sub>4</sub> treatment, and 3.0 ± 0.2 for the crop treatment; mean ± SE). There were 12 species total observed across all quadrats in the C<sub>4</sub> treatment and 23 for the C<sub>3</sub> treatment.

Leaf area index in both the C<sub>3</sub>- and C<sub>4</sub>-dominated treatments was significantly higher than in the crop treatment throughout the 2002 growing season (Table 3). There was a significantly higher C:N ratio in litter, the pool from which aboveground litter is potentially incorporated into the soil for sequestration, from the C<sub>4</sub>-dominated than the C<sub>3</sub>-dominated treatment (P = 0.02 for the season; data not shown).

Considered over the growing season, mean aboveground live biomass did not differ significantly among

TABLE 3. Observed growing-season leaf area index (LAI,  $n = 10$  per treatment) and aboveground vegetation ( $n = 4$ ) and root biomass ( $n = 8$ ) pools.

Measurement, by period	Treatment		
	C <sub>3</sub> dominated	C <sub>4</sub> dominated	Crop
May–October			
Mean LAI*	2.4 <sup>a</sup> ± 0.1	2.7 <sup>a</sup> ± 0.2	0.9 <sup>b</sup> ± 0.2
Aboveground			
Live biomass	254 <sup>a</sup> ± 56	228 <sup>a</sup> ± 86	175 <sup>a</sup> ± 52
Standing dead biomass*	115 <sup>a</sup> ± 53	225 <sup>b</sup> ± 79	24 <sup>c</sup> ± 28
Litter layer*	235 <sup>a</sup> ± 50	346 <sup>b</sup> ± 81	164 <sup>a</sup> ± 62
Total aboveground biomass*	593 <sup>a</sup> ± 88	786 <sup>b</sup> ± 167	364 <sup>c</sup> ± 67
Peak aboveground biomass†	351 ± 48	428 ± 60	312 ± 23
8 August 2002			
Belowground			
Live root biomass*	315 <sup>a</sup> ± 24	525 <sup>b</sup> ± 116	32 <sup>c</sup> ± 5
Dead root biomass*	165 <sup>a</sup> ± 37	139 <sup>a</sup> ± 19	40 <sup>b</sup> ± 11
Total root biomass*	480 <sup>a</sup> ± 42	664 <sup>a</sup> ± 114	71 <sup>b</sup> ± 10
Root biomass range	314–611	367–1188	23–114
Total live roots in top 10 cm (%)	61 <sup>a</sup>	65 <sup>a</sup>	59 <sup>a</sup>
Total root biomass in top 30 cm (%)	85 <sup>a</sup>	78 <sup>a</sup>	80 <sup>a</sup>

Notes: All biomass quantities are in units of g/m<sup>2</sup>; values are given as mean ± SE. Quantities not connected by the same letter across rows are significantly different at the 95% confidence level and are marked with an asterisk (\*) in the first column.

† Peak aboveground biomass was observed in June for the C<sub>3</sub> site, July for C<sub>4</sub>, and September for the crop site. Because it was measured at different times in the different plots, the comparisons were not direct and not appropriate for statistical comparison.

any of the treatments ( $P = 0.159$ ), but standing dead biomass and total biomass were significantly different among all three treatments (Table 3), implying that the C<sub>4</sub>-dominated treatment is either more productive or its residues are decaying more slowly. The C<sub>4</sub>-dominated treatment had significantly ( $P = 0.02$ ) more live fine-root biomass than the C<sub>3</sub>-dominated treatment, and both had significantly ( $P < 0.01$ ) more live fine-root biomass than the crop treatment (Table 3).

#### Estimates of net primary production (NPP)

Aboveground NPP (ANPP) was estimated at 351 g/m<sup>2</sup> for the C<sub>3</sub> treatment, 428 g/m<sup>2</sup> for the C<sub>4</sub> treatment, and 312 g/m<sup>2</sup> for the crop treatment using the peak live (PL) approach (Fig. 3, Table 4). The significant increment (SI) estimates of ANPP were >30% higher (Table 4). Calculating all positive increments (AI) in the standing dead and litter pools regardless of significance resulted in an ANPP estimate ~2.4 times greater than the PL estimate, and ~1.7 times greater than the SI estimate (Table 4). All three ANPP estimation algorithms agreed that productivity in the C<sub>4</sub>-dominated treatment was between 25% and 35% greater than that of the C<sub>3</sub>-dominated treatment (Table 4).

Belowground NPP (BNPP) estimates range from 135 to 1199 g/m<sup>2</sup> for the C<sub>3</sub>-dominated treatment, and 225 to 1660 g/m<sup>2</sup> for the C<sub>4</sub>-dominated treatment (Table 4). The two lowest BNPP estimates were derived with the root turnover values from the exponential function of mean annual temperature and the 1987 root-window study. The BNPP estimated using the Brye et al. (2002)

minirhizotron-derived turnover coefficient was an order of magnitude higher than all other approaches.

We estimated total NPP by adding estimates of above- and belowground biomass from our three ANPP estimates and five BNPP estimates. These total NPP estimates varied by a factor of four. In the C<sub>3</sub>-dominated treatment, total estimated NPP ranged from 485 to 2033 g/m<sup>2</sup>; in the C<sub>4</sub>-dominated treatment, the range was from 645 to 2731 g/m<sup>2</sup> (Table 4). For both prairie treatments, the BNPP methodology had greater influence on total NPP. The mean of the 15 individual estimates for total NPP was 937 g/m<sup>2</sup> for the C<sub>3</sub>-dominated treatment and 1293 g/m<sup>2</sup> for the C<sub>4</sub>-dominated treatment.

#### Soil respiration

Total soil respiration within the C<sub>3</sub>-dominated plot was significantly higher than the C<sub>4</sub>-dominated and crop treatments ( $P = 0.035$ ; Fig. 4). Soil respiration was not significantly different between the C<sub>4</sub>-dominated and the crop treatments overall, although the crop treatment experienced a peak flux in May that was not observed in either of the prairie treatments (Fig. 4). For all three treatments, the selected model to estimate soil respiration used soil temperature only; additional terms did not improve model fit. The selected models were:

$$1) \text{ C}_3\text{-dominated treatment: } R_S = \exp(-0.238 + 0.0439T_{S,10}), r^2 = 0.71;$$

$$2) \text{ C}_4\text{-dominated treatment: } R_S = \exp(-0.344 + 0.0469T_{S,10}), r^2 = 0.73;$$

$$3) \text{ Crop treatment: } R_S = \exp(-0.111 + 0.0298T_{S,10}), r^2 = 0.43$$

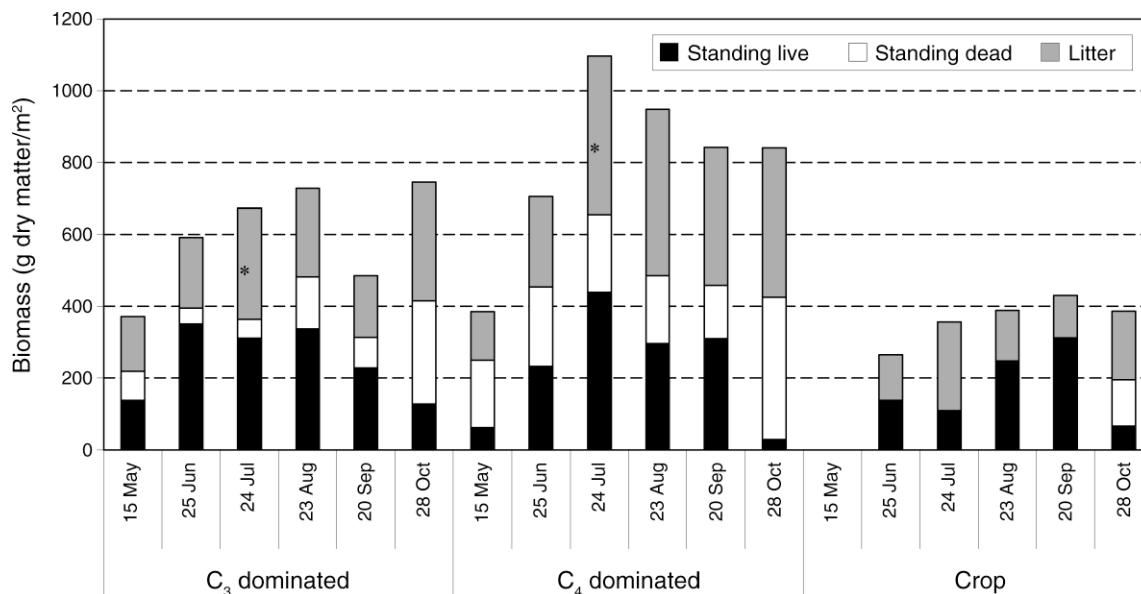


FIG. 3. Aboveground biomass of standing live, standing dead, and litter from the three land cover treatments: C<sub>3</sub>, C<sub>4</sub>, and crop plants (*n* = 4 per treatment per sampling date). These data were used to calculate aboveground net primary productivity (ANPP) using the three methods reported in Table 4. Soybeans had not yet been planted in May. Nonliving biomass pools marked with an asterisk were significantly different ( $0.02 < P < 0.06$ ) from the same pool at the previous sampling date and were used to calculate the significant increments (SI) estimate of ANPP.

where  $R_S$  is soil respiration and  $T_{S,10}$  is 0–10 cm soil-surface temperature in °C. (The range of  $T_{S,10}$  measured over the year was between  $-4^{\circ}\text{C}$  and  $+28.4^{\circ}\text{C}$ .)

Using the daily mean temperature measured for each treatment and the corresponding regression equation,

we estimated an annual soil carbon flux from the C<sub>3</sub>-dominated treatment of  $964 \pm 23 \text{ g C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$  (mean  $\pm$  SE), which was significantly higher than that of the C<sub>4</sub>-dominated treatment,  $816 \pm 14 \text{ g C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$  (mean  $\pm$  SE), and the crop treatment,  $798 \pm 1 \text{ g C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$

TABLE 4. Estimates for above-, belowground, and total net primary productivity, in  $\text{g biomass}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ , for the grassland treatments using different methods.

ANPP method	BNPP method	C <sub>3</sub> dominated			C <sub>4</sub> dominated		
		BNPP	TNPP	BNPP:TNPP	BNPP	TNPP	BNPP:TNPP
Peak live biomass, PL (ANPP = 351 for C <sub>3</sub> and 428 for C <sub>4</sub> )							
	DF	178	529	34%	334	762	44%
	CF	204	555	37%	341	770	44%
	EXP	135	485	28%	225	654	34%
	MRH	1199	1550	77%	1660	2088	79%
	RW	169	519	32%	246	674	37%
Significant increments, SI (ANPP = 464 for C <sub>3</sub> and 618 for C <sub>4</sub> )							
	DF	210	674	31%	424	1042	41%
	CF	204	668	31%	341	960	36%
	EXP	135	599	23%	225	844	27%
	MRH	1199	1663	72%	1660	2278	73%
	RW	169	632	27%	246	864	37%
All increments, AI (ANPP = 834 for C <sub>3</sub> and 1071 for C <sub>4</sub> )							
	DF	314	1148	27%	334	1708	37%
	CF	204	1038	20%	341	1412	24%
	EXP	135	969	14%	225	1296	17%
	MRH	1199	2033	59%	1660	2731	61%
	RW	169	1002	17%	246	1317	19%

Notes: ANPP is aboveground net primary productivity; PL, peak live ANPP method; SI, significant increments ANPP method; AI, all increments ANPP method; BNPP, belowground net primary productivity; DF, direct function BNPP method; CF, constant function BNPP method; EXP, exponential BNPP method; MRH, minirhizotron BNPP method; RW, root window BNPP method; TNPP, total (above + belowground) NPP. Percentages are the fraction of TNPP represented by BNPP. DF uses the PL in its calculation, while the other BNPP terms do not; TNPP changes as a function of the ANPP.

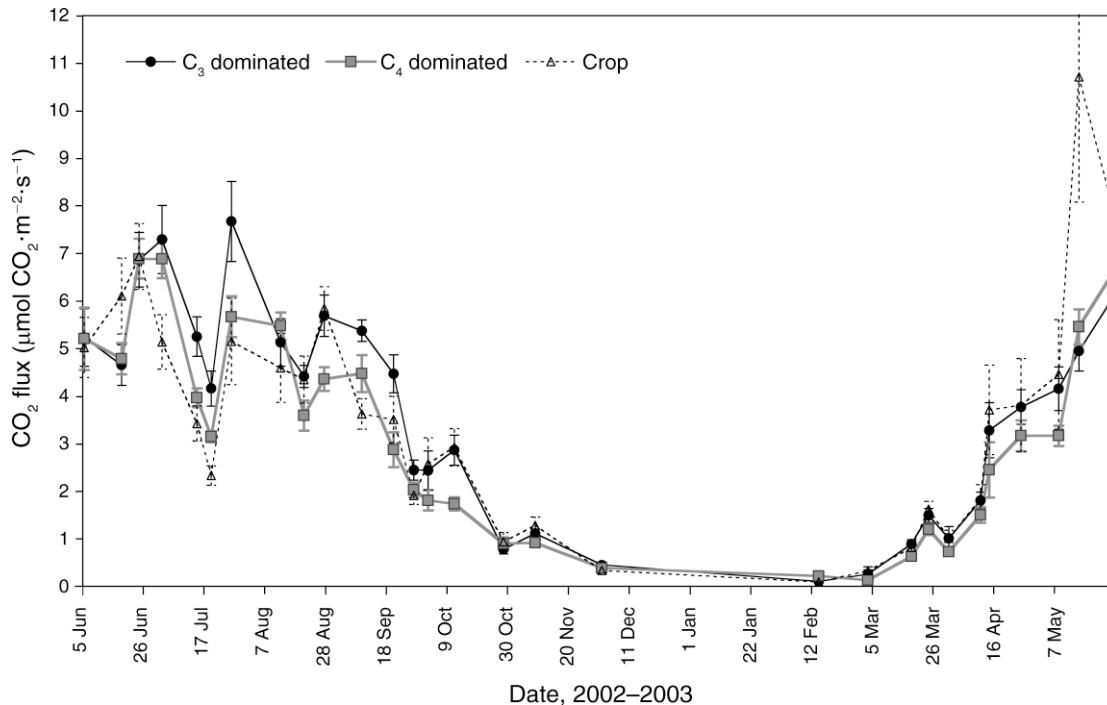


FIG. 4. Mean soil-surface  $\text{CO}_2$  flux measured from collars ( $n = 10$  per treatment). Over the entire sampling period,  $\text{C}_3$  fluxes were significantly higher than the other two treatments, which were not significantly different from each other. The error bars represent  $\pm\text{SE}$ .

(mean  $\pm$  SE). These errors were estimated from the standard error of the annual respiration estimates produced by each independent soil temperature replicate ( $n = 4$ ).

#### Estimates of net ecosystem productivity (NEP)

Forty out of 45 of our factorial estimates found the  $\text{C}_3$ -dominated treatment to be a net annual source of carbon to the atmosphere, with estimates ranging from  $-10$  to  $-564 \text{ g C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ . Fifteen out of 45 estimates

found the  $\text{C}_4$ -dominated treatment to be a carbon sink, including all estimates using the minirhizotron BNPP coefficient and all estimates for the all increments (AI) ANPP at the lowest  $R_H$  (Table 5, Fig. 5). We estimate the crop treatment to be a carbon source under both methods of partitioning  $R_H$ . At a constant  $R_H:R_S$  of 65%, crop NEP is  $-147 \text{ g C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ . When the  $R_H:R_S$  was allowed to vary during the course of the growing season, the NEP is  $-515 \text{ g C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ .

TABLE 5. Total net ecosystem production using three different methods to estimate ANPP, BNPP, and  $R_H$ , (mean  $\pm$  SE).

	Peak live			Significant increments			All increments		
	$R_H = 60\%$	$R_H = 70\%$	$R_H = 80\%$	$R_H = 60\%$	$R_H = 70\%$	$R_H = 80\%$	$R_H = 60\%$	$R_H = 70\%$	$R_H = 80\%$
<b><math>\text{C}_3</math>-dominated grassland</b>									
DF	$-367 \pm 25$	$-464 \pm 26$	$-560 \pm 28$	$-309 \pm 34$	$-406 \pm 35$	$-502 \pm 36$	$-119 \pm 47$	$-216 \pm 48$	$-312 \pm 49$
CF	$-357 \pm 25$	$-453 \pm 27$	$-549 \pm 28$	$-311 \pm 34$	$-408 \pm 35$	$-504 \pm 36$	$-163 \pm 47$	$-260 \pm 48$	$-356 \pm 49$
EXP	$-384 \pm 25$	$-481 \pm 26$	$-577 \pm 28$	$-339 \pm 34$	$-435 \pm 35$	$-532 \pm 36$	$-191 \pm 47$	$-288 \pm 48$	$-384 \pm 49$
MRH	<b><math>41 \pm 49</math></b>	$-55 \pm 49$	$-152 \pm 50$	<b><math>87 \pm 53</math></b>	$-10 \pm 54$	$-106 \pm 55$	<b><math>235 \pm 63</math></b>	<b><math>138 \pm 63</math></b>	<b><math>42 \pm 64</math></b>
RW	$-371 \pm 26$	$-467 \pm 28$	$-564 \pm 29$	$-326 \pm 35$	$-422 \pm 36$	$-518 \pm 37$	$-178 \pm 48$	$-274 \pm 49$	$-370 \pm 50$
<b><math>\text{C}_4</math>-dominated grassland</b>									
DF	$-185 \pm 37$	$-266 \pm 37$	$-348 \pm 37$	$-73 \pm 50$	$-154 \pm 50$	$-236 \pm 50$	<b><math>194 \pm 99</math></b>	<b><math>112 \pm 99</math></b>	<b><math>31 \pm 99</math></b>
CF	$-182 \pm 40$	$-263 \pm 40$	$-345 \pm 40$	$-106 \pm 52$	$-187 \pm 52$	$-269 \pm 52$	<b><math>75 \pm 100</math></b>	$-6 \pm 100$	$-88 \pm 100$
EXP	$-228 \pm 33$	$-309 \pm 33$	$-391 \pm 33$	$-152 \pm 47$	$-233 \pm 47$	$-315 \pm 47$	<b><math>29 \pm 98</math></b>	$-52 \pm 97$	$-134 \pm 97$
MRH	<b><math>346 \pm 117</math></b>	<b><math>264 \pm 117</math></b>	<b><math>183 \pm 117</math></b>	<b><math>422 \pm 122</math></b>	<b><math>340 \pm 122</math></b>	<b><math>259 \pm 122</math></b>	<b><math>603 \pm 149</math></b>	<b><math>521 \pm 149</math></b>	<b><math>440 \pm 149</math></b>
RW	$-220 \pm 40$	$-301 \pm 40$	$-383 \pm 39$	$-144 \pm 52$	$-225 \pm 52$	$-307 \pm 52$	<b><math>37 \pm 100</math></b>	$-44 \pm 100$	$-126 \pm 100$

Notes: Units are in  $\text{g C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ . Positive numbers (in bold) denote a net carbon sink from the atmosphere; negative numbers denote a carbon source to the atmosphere.  $R_H$  = heterotrophic respiration, percentage of total soil respiration attributed to non-plant sources; other abbreviations are as in Table 4.

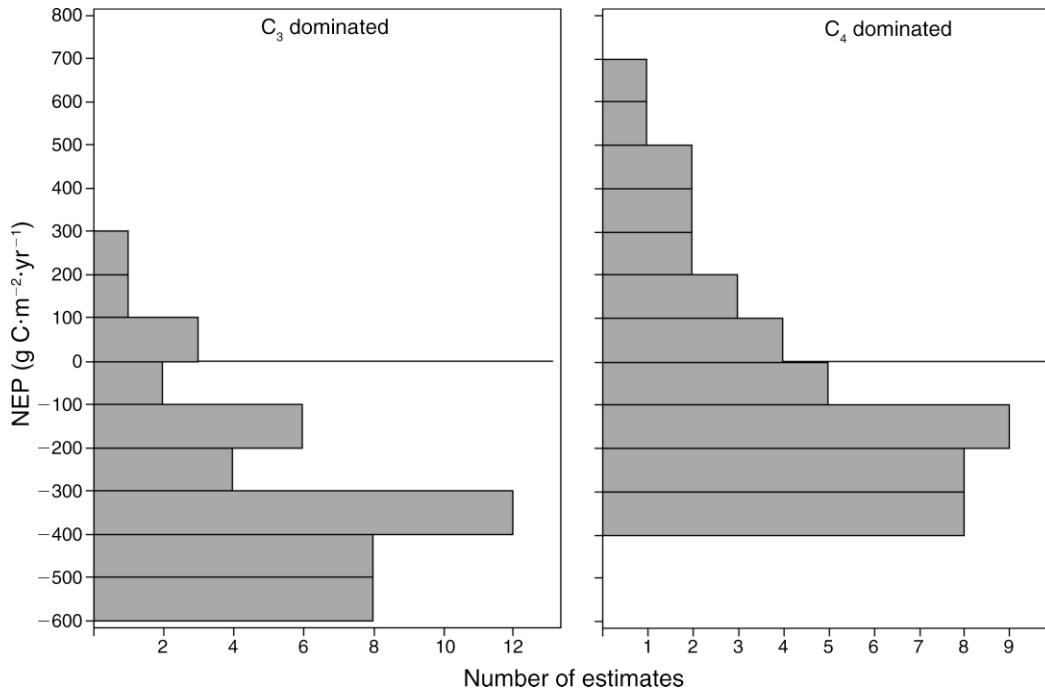


FIG. 5. Distribution of the 45 net ecosystem production (NEP) estimates for both grassland treatments resulting from five BNPP estimates  $\times$  three ANPP estimates  $\times$  three  $R_H$  estimates. Positive values denote a carbon sink and negative values denote a carbon source.

#### *Soil composition and structure*

The prairie treatments had similar soil characteristics, with carbon and total organic matter significantly higher than the crop treatment in the surface 5 cm, and significantly lower bulk densities and pH (Table 1). The  $C_3$ -dominated treatment with its N-fixing species had significantly more N than the crop treatment in the surface 5 cm of soil, while the  $C_4$ -dominated treatment was intermediate (Table 1). Mean monthly soil temperatures at 10 cm in the two prairie treatments were not significantly different from each other, but were significantly less variable (cooler in three months of summer and warmer in two months of winter) compared with the crop treatment. Surface soil moisture was significantly higher in the  $C_4$ -dominated treatment than in the  $C_3$ -dominated treatment, and both were significantly wetter than the crop treatment (Table 1).

#### *Soil carbon sequestration*

Both the  $C_3$ - and  $C_4$ -dominated restored prairies had significantly higher levels of soil carbon in the surface 5 cm than the crop treatment ( $1477 \pm 73$  g C/m<sup>2</sup> for the  $C_3$ -dominated treatment,  $1413 \pm 137$  g C/m<sup>2</sup> for the  $C_4$ -dominated treatment, and  $1216 \pm 68$  g C/m<sup>2</sup> for the crop treatment; mean  $\pm$  SE). Thus we found that prairie restoration increases soil carbon accumulation vs. an agricultural system, but we did not find that varied grassland restoration protocols led to different rates of sequestered soil carbon in these systems, although the intermingling of functional groups between the two

treatments made it difficult to determine the importance of functional group composition in driving outcomes for ecosystem properties.

To estimate annual mean rates of soil carbon sequestration in the two prairie treatments, we assumed the soil carbon content of the crop treatment has been constant since establishment, and further assumed a constant rate of accumulation for the two prairie treatments over the 16 years since restoration. The mean annual carbon accumulation rate in the top 5 cm using this approach was 16.3 g C/m<sup>2</sup> for the  $C_3$ -dominated treatment and 12.3 g C/m<sup>2</sup> for the  $C_4$ -dominated treatment.

#### DISCUSSION

Key differences between the two prairie treatments occurred in plant-related rather than soil-related variables. Although our field observations suggested greater quantities of soil residue inputs in the  $C_4$ -dominated treatment due to higher amounts of standing dead biomass, litter, total aboveground biomass production, and greater live-root biomass, but correspondingly lower soil respiratory losses, we measured slightly higher (though nonsignificant) carbon content in the soil of the  $C_3$ -dominated treatment.

#### *Vegetation*

The prairies studied here have a much higher abundance of forbs than reported in previous studies of the Conservation Reserve Program (CRP) (e.g.,

negligible in Baer et al. 2002) and native tallgrass prairies (e.g., 3% reported by Suyker and Verma 2001). A common goal of the CRP is reducing soil erosion, and diffuse-rooted forbs might not stabilize highly erodible soils as well as sod-forming grasses.

The ratios of live and dead roots (65% live roots in the C<sub>3</sub> treatment and 79% in the C<sub>4</sub> treatment) are in excellent agreement with other values published in the literature (Rice et al. 1998, Jackson et al. 2000, Gill et al. 2002).

#### *Estimates of net primary production (NPP)*

Our estimates for ANPP using the peak live (PL) and significant increments (SI) methods agreed well with several other studies, while the estimate from the all increments (AI) method was fairly high relative to other studies. However, the AI estimates may still be reasonable given the high interannual variability of ANPP (Rice et al. 1998) and the fact that ANPP is commonly estimated using peak live biomass in grasslands, which may underestimate actual ANPP (Scurlock et al. 2002). Estimates of ANPP at other restored tallgrass prairie sites in Wisconsin range from 120 to 901 g/m<sup>2</sup> (Brye et al. 2002, Kucharik et al. 2006). The high temporal variability of ANPP is illustrated by long-term estimates from Konza Prairie between 143 and 501 g/m<sup>2</sup> (Rice et al. 1998).

The most widely reported estimates for root turnover in tallgrass prairies range from 25% to 40% of all roots dying per year (Kucera et al. 1967, Sims and Singh 1978, Hayes and Seastedt 1987, Rice et al. 1998). The root-turnover coefficients we derive here for the three models of Gill et al. (2002) all suggest that root turnover is occurring more rapidly than these previous estimates, and the Brye et al. (2002) minirhizotron suggests an order of magnitude more rapid turnover, with complete cycling estimated to occur every ~146 days. The BNPP calculation using the Brye et al. (2002) turnover estimate might appear to be the outlier, but could represent the best estimate of prairie root dynamics in these sites since it is observational data from a similar site. Scurlock and Hall (1998) support the idea that grassland root biomass may turn over in a year or less. We note that only the direct function of Gill et al. (2002) allows root turnover coefficients to vary between our prairie treatments (based on ANPP), which is important because differences in ANPP were observed and evidence suggests that C<sub>3</sub> and C<sub>4</sub> grasses allocate resources differently belowground.

Unfortunately, there is no perfect method for quantifying root production (Nadelhoffer and Raich 1992). Milchunas et al. (2005) point out that there are different ways to calculate root turnover even within one method, which they illustrate using a minirhizotron in a shortgrass steppe. Thus, the uncertainty introduced by the choice of method for selecting root turnover is not only a problem among different methods of measuring

or estimating belowground productivity; it can be a problem within one method as well.

Ratios of belowground to total productivity (BNPP:TNPP) range from 60% to 90% for grasslands in general and are estimated at 48–64% for tallgrass prairies (Stanton 1988). We report our ratios of BNPP to total NPP in Table 4. Only those estimates using the fine-root turnover constant of 2.5 from Brye et al. (2002) found BNPP to be more than half of the total, as is usually reported. Using the two observation-based estimates from Hayes and Seastedt (1987) and Brye et al. (2002), our results were similar to findings reported from those two studies, but quite different from each other. For example, using the fine-root turnover constant derived using minirhizotrons reported by Brye et al. (2002), BNPP represents between 59% and 77% of TNPP for the C<sub>3</sub>-dominated and 61–79% for the C<sub>4</sub>-dominated treatment, depending on calculated ANPP (Table 4). This agreed well with the value of 73% reported by Brye et al. (2002). Similarly, using the constants suggested by Hayes and Seastedt (1987), we closely approximated their BNPP of 208 g/m<sup>2</sup> in our study (169 g/m<sup>2</sup> for C<sub>3</sub> and 246 g/m<sup>2</sup> for C<sub>4</sub>; Table 4). However, the Hayes and Seastedt (1987) approach suggested a much different ratio of belowground total NPP, ranging from 17% to 37% (Table 4). This suggests that methodology strongly influences estimates of BNPP and therefore NEP. Uncertainty in the quantification of fine-root turnover may be the largest single source of error in estimating grassland NPP (Scurlock et al. 2002).

#### *Soil respiration*

Our finding that soil surface respiration in the prairie and crop treatments was highly correlated with soil temperature, and only weakly related to soil moisture, suggests that soil moisture may be of secondary importance to soil respiration in these generally non-water-limited systems. However, significantly higher summer temperatures in the crop treatment did not lead to significantly higher soil respiration. Possible mechanisms to explain the lower respiration observed in the agricultural soil include surface “crusting” as it dries, which may seal pores at the soil–atmosphere interface, or lower belowground carbon allocation in cropping systems. The high spring peak flux observed in the crop treatment (Fig. 4) may have been due to warmer soil conditions or greater root respiration vs. the prairie treatments, which were burned the previous month and may have shifted more carbon allocation aboveground to increase leaf area.

Vegetation influences soil respiration by impacting soil structure through root growth and microclimate, the quantity and quality of litter inputs, and root respiration rates (Raich and Tufekcioglu 2000). It has been assumed that species differences within similar vegetation forms (i.e., grasses) are not important to soil respiration (Raich and Tufekcioglu 2000), but our data showing significantly more C respired from the C<sub>3</sub>-dominated

TABLE 6. Reported NEP in other recent grassland ecosystem studies.

Authors	Years	Ecosystem type	NEP (g C·m <sup>-2</sup> ·yr <sup>-1</sup> )	Biosphere source/sink
Zhao et al. (2006)	2003	high-altitude grassland	+59	sink
	2004	high-altitude grassland	+76	sink
Byrne et al. (2005)	2004	new, intensively managed temperate grasslands	+150	sink
	2004	permanently managed temperate grassland	+380	sink
Li et al. (2005)	2003–2004 (one year)	grazed steppe	–23 to +20†	near balanced
Aeschlimann et al. (2005)	2001	managed grassland (with N and elevated CO <sub>2</sub> manipulations)	+210 to +631‡	sink
Li et al. (2004)	1998	semiarid grassland	+59 to +109§	sink
	1999	semiarid grassland	+5 to +21§	sink
	2000	semiarid grassland	–33 to –18§	source
Lohila et al. (2004)	2002	perennial forage grass	–79	source
Welker et al. (2004)	1998–2000	alpine grassland (grazed)	–170 (2-year mean)	source
	1998–2000	alpine grassland (ungrazed)	+83 (2-year mean)	sink
Xu and Baldocchi (2004)	2000–2001	mediterranean grassland	+132	sink
	2001–2002	mediterranean grassland	–29	source
Verburg et al. (2004)	1998–1999	cheatgrass	–286	source
	1999–2000	cheatgrass	–332	source

Notes: NEP is positive when carbon gains to the biosphere exceed losses to the atmosphere (a biosphere sink of carbon). All values are for one year of NEP unless otherwise noted.

† Range depended on estimates of C removed by sheep grazing.

‡ Range depended on species and manipulation treatment.

§ Range depended on whether estimate was modeled (first number) or calculated (second number).

prairie than the C<sub>4</sub> prairie suggests that this may not always hold true.

#### Estimates of net ecosystem productivity (NEP)

Our range of estimates for NEP for the C<sub>3</sub>- and C<sub>4</sub>-dominated prairie treatments is somewhat disturbing, as we find that not only the magnitude but also the sign can change depending on the methodology used to estimate above- and belowground productivity and for partitioning soil respiration into  $R_H$  and  $R_R$ . Undoubtedly some of the combinations of parameters we used are more appropriate for this system than others. However, all represent reasonable and common approaches. When only one estimate for ANPP, BNPP, or  $R_H$  is reported or used to calculate ecosystem carbon balance, it may misrepresent the certainty that can reasonably be assigned to the result, particularly when the method selected for estimation is not reported. Although many of the NEP values from various methods seem to converge in one direction, that does not necessarily mean that those are the true values. Precision in estimating NEP using the biometric method as applied here is more likely a sign of methodological bias than a reflection of the accuracy of the estimate.

A comparison of these findings with other studies is illustrative (Table 6). The mean of all ecosystem carbon balance estimates reported in Table 6 is a sink of +54 g C·m<sup>-2</sup>·yr<sup>-1</sup>, with a range from a sink of +380 g C·m<sup>-2</sup>·yr<sup>-1</sup> for an unmanipulated grassland (+631 g C·m<sup>-2</sup>·yr<sup>-1</sup> for a grassland under manipulated N and CO<sub>2</sub>) to a source of –332 g C·m<sup>-2</sup>·yr<sup>-1</sup>. In a compilation of 38 annual carbon balance estimates from 14 sites, the highest grassland sink was +772 g C·m<sup>-2</sup>·yr<sup>-1</sup> in a tallgrass prairie, and the largest source was –521 g

C·m<sup>-2</sup>·yr<sup>-1</sup> in a mixed-grass rangeland, with a mean over all sites and years of +52 g C·m<sup>-2</sup>·yr<sup>-1</sup> (our calculations are from Novick et al. 2004: Table 1).

#### Soil carbon sequestration

The cessation of intensive soil disturbance from agriculture and the establishment of grassland species were likely the key drivers of the greater surface soil carbon storage and decreased bulk density observed in the prairie restoration treatments. The significantly higher root biomass in the two prairie restoration treatments also may be important to the higher soil carbon accumulation from belowground carbon inputs, although Schlesinger and Andrews (2000) note that soil carbon accumulation is typically driven by limits on decomposers (e.g., litter quality, temperature, and moisture), rather than large carbon inputs.

The assumption that soil carbon is constant in the cropped site may not be entirely correct, but given the continued tillage and soil erosion in the crop plot, it is unlikely that soil C increased in the crop system. Therefore, estimates using this approach are more likely to overstate carbon accumulation rather than understate it, and the annual rates we report are low relative to other findings (e.g., 24.7 g C·m<sup>-2</sup>·yr<sup>-1</sup> for 8–12-year-old CRP sites in the same county [Kucharik et al. 2003]). Carbon sequestration rates for CRP sites in southern Wisconsin, USA have been reported to decrease over time from 72 g C·m<sup>-2</sup>·yr<sup>-1</sup> for the top 5 cm of soil for 4–5 year old prairies, to a mean of 13 g C·m<sup>-2</sup>·yr<sup>-1</sup> for 17 study sites >10 years old (Kucharik 2007). Ideally this estimate could be checked with a sequential measurement of soil carbon in the future, although it might not confirm anything; Brye et al. (2002) found soil carbon

content in a restored tallgrass prairie in Wisconsin did not change significantly over 5 years, beginning 19 years after restoration from agriculture.

The higher quality litter in the C<sub>3</sub>-dominated treatment (C:N mean 44.4 vs. 51.8 for the C<sub>4</sub>-dominated treatment) potentially reflects differences in both photosynthetic physiology and in nutrient retention and retraction strategies. The litter quality input is consistent with the significantly higher soil surface CO<sub>2</sub> flux, presumably from microbial decomposition, but is not consistent with the observed (although not statistically significant) levels of higher soil carbon storage in the C<sub>3</sub>-dominated treatment if the carbon inputs are more labile. There does not appear to be a direct relationship in this system between plant-litter input and actual carbon storage based on aboveground litter quantity or litter quality, as represented by C:N ratio.

There is serious disagreement among our estimates of ecosystem carbon accumulation based on vegetation carbon inputs and soil respiratory losses (fluxes) and observed levels of carbon storage. Following conversion from intensive agriculture to the grassland treatments upon enrollment in the CRP, we would expect to see accumulation of soil carbon relative to the low-input crop treatment. The soil carbon stocks imply that these prairies are weak carbon sinks relative to the crop treatment (mean sequestration rates since establishment of 16.3 and 12.3 g C·m<sup>-2</sup>·yr<sup>-1</sup> for the C<sub>3</sub>-dominated and C<sub>4</sub>-dominated treatments, respectively), but most of our biometric based NEP estimates show the prairies to be a net carbon source. This leads to doubts about obtaining reliable estimates of carbon sources and sinks using these methodologies, particularly over a short time scale (~5–15 yr). However, even longer term studies that take into account interannual variability in climate and other relevant ecosystem variables may not be able to improve biometric estimates of NEP to a desirable level of precision, particularly to assign credits (i.e., Brye et al. 2002, Kucharik et al. 2006).

#### CONCLUSION

While the Conservation Reserve Program (CRP) program favors planting diverse mixes of native species, ongoing management is not currently a required part of the program. Our observations suggest that without subsequent follow-up, species composition and abundance may shift significantly over time, potentially countering some management goals (Kucharik 2007). These goals may include native plant species diversity, wildlife habitat, erosion control, biomass production for biofuels, and aesthetics. These are clearly important functions, but some involve a tradeoff with other potential management goals such as carbon sequestration (e.g., through planting high numbers of forbs). Ideally, monitoring and management would be given more resources by the CRP program; realistically, managers should at least be aware that restored systems

may differ in composition and possibly in function from those intended with the initial species planted.

Carbon sequestration in established grassland ecosystems is only belowground in the soil carbon pool, which greatly affects the magnitude and sign of net ecosystem production (NEP) estimates, but the belowground components are often ignored or estimated from the literature. Improved and easy to implement methods are needed to quantify fine-root biomass, to partition live from dead biomass, and estimate the fraction of annual root turnover if estimates of ecosystem carbon sources and/or sinks are to be more credible.

The large uncertainties we found in estimating NEP using the biometric approach likely exist in similar grassland carbon balance studies, although they have not generally been reported previously. This cautions against using estimates of carbon sequestration based on this approach, particularly for policy applications interested in assigning carbon credits. While the Kyoto Protocol only allows credits for “verifiable changes in stocks,” other emerging carbon credit markets may be prone to inaccuracies that might undermine the credibility of the trading process if component measurements, as used here, are allowed for verification of carbon sequestration. Biometric methods to estimate NEP clearly need further refinement before they are employed to estimate grassland carbon balance with a desired level of precision. Greater standardization of methodologies and transparent reporting of the methods used are also important to provide consistency among studies and allow closer constraints on carbon balance estimates.

This study highlights the inherent difficulties in correlating short-term observations of carbon cycle components with longer term studies of carbon stock changes to quantify soil carbon sequestration. These findings also illustrate the complexities of predicting soil carbon storage based on known ecosystem properties without direct measurements, and caution against using broadly developed models for site-specific estimates of carbon storage, such as may be increasingly demanded for quantification of greenhouse gas offset credits. The high variability and complexity of factors influencing ecosystem properties controlling carbon sequestration mean that rigorous field measurements of carbon stocks are essential to obtain reliable estimates, and can be used to constrain biometric estimates.

Further refinement of methodologies using both stocks and biometric approaches are necessary for accurate estimates of ecosystem carbon balances, and to validate models developed to estimate carbon balances at a regional scale. At the present time, our methods lag behind the policy-driven agenda to reduce greenhouse gas emissions and accumulation in the atmosphere. Improvements and standardization in scientific methodologies to quantify carbon sequestration using both biometric and stocks approaches are essential to support policy efforts along these lines.

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