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Net ecosystem carbon exchange in two experimental grassland ecosystems

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Abstract

Increases in net primary production (NPP) may not necessarily result in increased C sequestration since an increase in uptake can be negated by concurrent increases in ecosystem C losses via respiratory processes. Continuous measurements of net ecosystem C exchange between the atmosphere and two experimental cheatgrass (*Bromus tectorum* L.) ecosystems in large dynamic flux chambers (EcoCELLs) showed net ecosystem C losses to the atmosphere in excess of 300 g C m^{-2} over two growing cycles. Even a doubling of net ecosystem production (NEP) after N fertilization in the second growing season did not compensate for soil C losses incurred during the fallow period. Fertilization not only increased C uptake in biomass but also enhanced C losses through soil respiration from 287 to 469 g C m^{-2}, mainly through an increase in rhizosphere respiration. Fertilization decreased dissolved inorganic C losses through leaching of from 45 to 10 g C m^{-2}.

Unfertilized cheatgrass added 215 g C m^{-2} as root-derived organic matter but the contribution of these inputs to long-term C sequestration was limited as these deposits rapidly decomposed. Fertilization increased NEP but did not increase belowground C inputs most likely due to a concurrent increase in the production and decomposition of rhizodeposits. Decomposition of soil organic matter (SOM) was reduced by fertilizer additions. The results from our study show that, although annual grassland ecosystems can add considerable amounts of C to soils during the growing season, it is unlikely that they sequester large amounts of C because of high respiratory losses during dormancy periods. Although fertilization could increase NEP, fertilization might reduce soil C inputs as heterotrophic organisms favor root-derived organic matter over native SOM.

Keywords: Bromus tectorum, carbon sequestration, grasslands, net ecosystem productivity

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Introduction

It has been hypothesized that the buildup of atmospheric CO_2 can be reduced by enhancing C sequestration in terrestrial ecosystems, for instance by increasing net primary production (NPP, equal to the total C uptake by plant photosynthesis minus the C loss via plant respiration; Prentice *et al*., 2000; Ciais *et al*., 2001). Increases in NPP will not necessarily result in increases in C storage in terrestrial pools, however, because most of the CO_2 taken up from the atmosphere by plant photosynthesis and converted to NPP eventually returns to the atmosphere via heterotrophic respiration (R_h). Thus, actual ecosystem C sequestration is determined by the balance between NPP and R_h, i.e. net ecosystem production (NEP; Schulze *et al*., 2000; IGBP, 1998).

Accurate determination of NEP by tracking changes in C inventories over short time periods may be difficult because of uncertainties associated with

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sampling errors of highly variable soil and vegetation C pools and difficulties in detecting relatively small changes in ecosystem C pools against large background levels (IGBP, 1998; Lal *et al*., 2001; Smith, 2002). Calculation of NEP from estimates of NPP and R_h is complicated because of difficulties in including root litter production in NPP estimates, and separating root respiration from microbial respiration when estimating R_h. Direct measurement of net ecosystem CO_2 exchange (NEE) permits the most accurate calculation of NEP (i.e. integrated NEE) and thus ecosystem C sequestration. Although NEE is being measured in the field in a wide variety of ecosystems using eddy covariance techniques (e.g. Baldocchi *et al*., 1988; Valentini *et al*., 1996; Suyker *et al*., 2003), problems with measurements under stable atmospheric conditions, inclement weather, and spatial variability as well as inability to partition NEE into various ecosystem fluxes limit the potential for obtaining a rigorous mechanistic understanding of observed patterns in NEE.

In this paper, we present results from a 2-year study in which we directly measured NEE in two experimental grassland ecosystems using the Desert Research Institute's Ecologically Controlled Enclosed Lysimeter Laboratories (EcoCELLs). For this study, we used cheatgrass (*Bromus tectorum* L.), a species native to Eurasia but now present in large parts of the United States (Mack, 1981; D'Antonio & Vitousek, 1992). The success of this species has been related to several factors, including livestock grazing, fire, and use of fertilizers, with the competitive ability of cheatgrass being greatly enhanced by increased nutrient availability (Kay, 1966). We constructed grassland ecosystems by growing cheatgrass in soils originating from the Konza prairie. These soils were chosen since isotopic composition of Konza prairie soil is different from that of cheatgrass allowing us to measure directly the contribution of plants to belowground C flows. In addition, organic matter content of the Konza prairie soil used in our study is comparable to cheatgrass-dominated ecosystems (e.g. Acker, 1992). While cheatgrass is not as prominent at the Konza prairie as it is in semi-arid areas of the western United States (Smith & Knapp, 1999), increased N availability, for instance through grazing (Collins, 1987; McNaughton *et al*., 1997), may lead to increased invasion of tallgrass prairie by exotic species (Wedin & Tilman, 1996; Stohlgren *et al*., 1999) including cheatgrass.

The main objectives of this paper were to (1) test whether N-enhanced increases in cheatgrass productivity increases NEP and thus C sequestration, (2) determine the contribution of soil C fluxes including soil respiration and leaching to overall ecosystem fluxes, and (3) compare flux-based NEP and NPP estimates as measured in the EcoCELLs with pool inventories.

Materials and methods

Experimental system

The EcoCELLs are open flow mass balance systems using the same principles as leaf-level gas exchange measurements but at a much larger scale. The total volume of each chamber is 183.5 m^3 of which 20.1 m^3 is occupied by three lysimeters that can be filled up with soil. Each $2.85 \times 1.3 \times 1.8\,m^3$ ($L \times W \times D$) lysimeter is mounted on four truck scales each capable of measuring a weight of 5 000 kg with a combined precision of 1 kg. The environmental control includes temperature, CO_2 concentration, and relative humidity. The chambers receive natural light and light attenuation by the chambers is 22%. A detailed description of the EcoCELL facility is given by Griffin *et al*. (1996). For this study we used two EcoCELLs.

Grassland ecosystems were constructed by sowing cheatgrass in three adjacent 3.7 m^2 lysimeters in each of the two EcoCELLs. In July 1998, each lysimeter was filled with a 1 m layer of washed pea gravel as a space holder, and the gravel was covered with a root-impermeable landscape fabric. A 40 cm layer of washed noncalcareous coarse sand followed by a 40 cm layer consisting of a 1 : 2 mixture of soil from the Konza Prairie Long-Term Ecological Research site near Manhattan, Kansas, USA (39°05′N, 96°35′W) and sand were layered on top of the fabric. Roots were removed from the soil by handpicking before mixing with the sand. Soil water content was maintained at field capacity throughout the study and water was applied daily using polyethylene drip-irrigation lines put on top of the soil with a spacing of 15 cm. Seeding did not occur until 8 months after the soils were put in the lysimeters to minimize the transient disturbance effects on microbial activity (R_h and N mineralization) from soil handling before the start of our study. Day and nighttime temperatures in the EcoCELLs were maintained at 28 °C and 22 °C, respectively, with daytime temperatures starting at 05:00 PST and ending at 19:00 PST.

The grass was seeded on February 23, 1999 (70 seeds m^{-2}). Above- and belowground biomass was harvested after 108 days (June 10, 1999) when the grass started to senesce, and resprouted biomass was harvested on July 30, 1999. Following this harvest, the soils were left fallow for 6 months. The grass was reseeded on January 31, 2000. Two weeks after the second seeding, we applied nitrogen (N) fertilizer as $(NH_4)_2SO_4$ to each grassland: the equivalent of 88 kg N ha^{-1} in one application to ecosystem 1 and the

same amount to ecosystem 2 in 15 weekly additions of 5.87 (totaling 88) kg N ha^{-1}. At the start of senescence, above- and belowground biomass of the second crop was harvested 128 days after seeding (June 8, 2000).

Measurements

Net ecosystem C exchange was measured continuously using the EcoCELL gas exchange system by monitoring CO_2 concentrations in the air entering and leaving the EcoCELL chambers using LI-COR 6262 infrared gas analyzers (IRGAs) while holding airflow through the chambers constant. The accuracy of the NEE measurements was routinely verified by injecting known amounts of CO_2 into each EcoCELL at night when photosynthetic CO_2 uptake was absent. The NEE data were corrected for IRGA drift occurring between instrument spans and for variation in airflow meter performance. Fluxes and environmental parameters were measured every 10 s and stored as 15 min averages. Data points affected by the presence of people inside the chambers (6.7% of the 49 880 15 min data points) were removed and replaced by values calculated using light response curves for each day (daytime during planting phases) or by linear interpolation (night-time during planting phases and day- and night-time during fallow periods). Only 1.3% of all the data were lost due to computer failures. The environmental setpoints inside the EcoCELLs were maintained without interruption (0% failure). Cumulative ecosystem C storage, or NEP, was obtained by integrating NEE over time.

Soil respiration was measured continuously using an automated open-flow gas exchange system (Cheng *et al.*, 2000). We placed two open-flow chambers in each lysimeter giving a total of six chambers per EcoCELL. The contribution of rhizosphere respiration to the total soil respiration C flux was determined biweekly using a closed circulation trapping system followed by ^{13}C analysis of the trapped CO_2 over a 24 h period (Harris *et al.*, 1997; Cheng *et al.*, 2000). Vegetation on the Konza prairie was dominated by C_4 plants resulting in a $\delta^{13}C$ value of the soil of -17.9 ± 0.1‰ ($n = 10$), while the $\delta^{13}C$ of the cheatgrass roots (C_3) was -29.2‰. Plant-derived CO_2 was calculated as (Cerri *et al.*, 1985; Cheng, 1996):

$$C_3 = C_t(\delta_t - \delta_4)/(\delta_3 - \delta_4), \quad (1)$$

where $C_t = C_3 + C_4$, the total C from belowground CO_2; C_3 the amount of C derived from C_3 cheatgrass; C_4 the amount of C derived from C_4 soil; δ_t the $\delta^{13}C$ value of C_t; δ_3 the $\delta^{13}C$ value of C_3; and δ_4 the $\delta^{13}C$ value of C_4. Cumulative root-derived CO_2 was calculated by fitting a curve to the $\delta^{13}C$ values using linear regression.

During the first growing period, the lysimeters were drained biweekly and leachate was collected for chemical analyses. During the fallow period, leachate was sampled less frequently. During the second growing period, lysimeters were allowed to drain continuously and samples were analyzed every 2 weeks. To measure C losses via leaching, leachate samples were analyzed for total and inorganic C. At two dates during the fertilized planting phase, we measured $\delta^{13}C$ of dissolved inorganic C (DIC). Total and DIC were analyzed using a Shimadzu 5050 TC analyzer (Shimadzu, Kyoto, Japan). Total organic C (TOC) was calculated by subtracting DIC from total C. The $\delta^{13}C$ of the DIC was measured after precipitating DIC with $SrCO_3$. Samples were analyzed for ^{13}C at the University of California, Davis Stable Isotope Facility using a Europa Scientific Integra mass spectrometer (PDZ Europa, Northwich, UK). To assess the potential disturbance effects of soil handling, we measured NH_4 and NO_3 concentrations in leachate. Ammonium and NO_3 were analyzed at the Desert Research Institute using a Technicon Automated Colorimetric Analyzer (Technicon Instruments Corporation, Tarrytown, NY, USA).

At the end of the first growing period, aboveground biomass was harvested by clipping at ground level. Initially, root crowns were not removed. Resprouted root crowns and shoots were removed during a second harvest but unfortunately this biomass was not measured. Biomass data at the end of the first growing period excluded root crowns and regrown shoots. At the end of the second growing period, both shoots and root crowns were removed to prevent regrowth. At each harvest and at the start of the second growing period, root biomass was measured by washing roots out of six replicate soil columns (two per lysimeter; 25 cm diameter and 80 cm depth) over a sieve (63 μm mesh size) to avoid loss of roots during washing. Mineral material adhering to roots after washing was removed with tweezers. Soil cores were separated into soil (0–40 cm) and sand (40–80 cm) layers. Shoots, roots, and soil were oven-dried (70 °C) and subsamples were ground up for total C and ^{13}C analysis (soil and roots only). Total C was analyzed at the Desert Research Institute using a PerkinElmer CHN analyzer (PerkinElmer, Wellesley, MA, USA). Samples were analyzed for ^{13}C at the University of California, Davis Stable Isotope Facility using a Europa Scientific Integra mass spectrometer. Carbonate C was measured at the beginning of the study by treating samples with 6 N HCl and trapping evolved CO_2 in 2 N NaOH traps. The NaOH solutions were analyzed for DIC using a Shimadzu 5050 TC analyzer. Because carbonate C was almost undetectable (0.01%), we only measured carbonate C at the start of the study.

We compared estimates of NPP and NEP based on flux and pool measurements for the fertilized growing period. The flux-based NPP (NPP_{flux}) was calculated as the sum of NEP and heterotrophic respiration originating from soil organic matter (SOM) decomposition, while the pool-based NPP (NPP_{pool}) was calculated as the sum of shoots, roots, and rhizodeposits (Cheng *et al.*, 2000). In addition, we compared flux-based NEP estimates (NEP_{flux}) as measured directly by the EcoCELLs with pool-based NEP estimates (NEP_{pool}) calculated as the sum of biomass and change in soil C at the time of harvest. The errors associated with each of the NPP and NEP estimates were estimated by propagation of the errors associated with the individual measurements.

Effects of fertilizer application method on various ecosystem variables were analyzed using analysis of covariance with prefertilization data as the co-variable. Since the N application method did not affect any of the measured variables, we lumped the two fertilizer treatments and used each EcoCELL as a replicate. All monolith-level data were used to create an EcoCELL-level average, so $n = 2$ for each measurement.

Results

Net ecosystem exchange

At the beginning of the first planting period, ecosystem respiration exceeded canopy photosynthetic uptake for 44 days (Fig. 1) causing NEE to remain below zero for this period and resulting in an initial loss of C from both ecosystems to the atmosphere. As plants developed, NEE started to increase and peaked at $6\,g\,C\,m^{-2}\,day^{-1}$. Initial ecosystem C losses were not compensated until 62 ± 2 days (standard error) after seeding (more than half the growing period; Fig. 1). Ecosystem C sequestration peaked at $132 \pm 18\,g\,C\,m^{-2}$ at the time of the first harvest. Net ecosystem C exchange dropped below zero again immediately after the first harvest, causing a rapid loss of ecosystem C. This loss rate was slowed by a temporary increase in NEE as a result of resprouting of leaves, but increased again after resprouted shoots were harvested. Net ecosystem C loss during the 6-month fallow period totaled $262 \pm 27\,g\,C\,m^{-2}$. The pattern of NEE observed after the second seeding was nearly identical to that observed after the first seeding. Because both ecosystems lost large amounts of C during the 6-month fallow period, almost the entire second growing period (106 ± 12 days of 128 days) was required for ecosystem C stocks to reach levels present at the time of the first seeding. This occurred despite a fertilizer-induced doubling of ecosystem C gains in the second growing period ($287 \pm 6\,g\,C\,m^{-2}$). Although the net ecosystem C balance was positive at final harvest, ecosystem C stocks returned to starting levels in 29 ± 22 days after the final harvest and continued to decline as the experiment was terminated (Fig. 1).

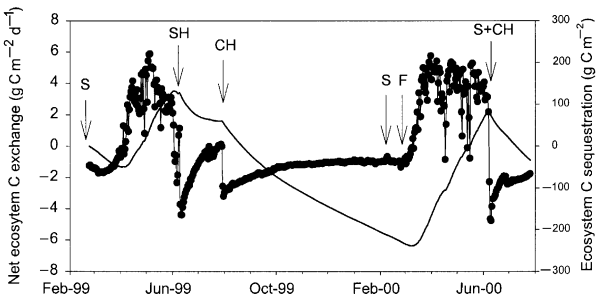

Fig. 1 Mean net ecosystem C exchange (line and symbols) and net ecosystem C sequestration (solid line) in two grassland ecosystems. The first growing period is unfertilized while during the second period $88\,kg\,N\,ha^{-1}$ was applied. The arrows point at times of seeding (S), shoot harvest (SH), root crown harvest (CH), fertilizer application (F), and shoot and root crown harvest (S + CH).

Soil fluxes

Soil respiration rates increased throughout the growth period in both ecosystems (Fig. 2). The N fertilization resulted in a more rapid increase as well as higher soil

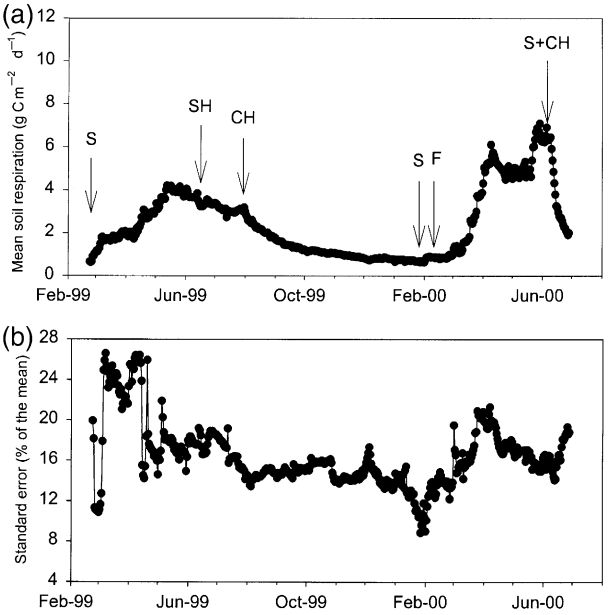

Fig. 2 Mean soil respiration (a) and standard errors (b) in two grassland ecosystems. The arrows point at times of seeding (S), shoot harvest (SH), root crown harvest (CH), fertilizer application (F), and shoot and root crown harvest (S + CH).

respiration rates. The total amount of C lost through soil respiration during the unfertilized planting period (including the period of resprouting of the root crowns) was $287 \pm 16\,g\,C\,m^{-2}$. Fertilization increased C losses through soil respiration to $469 \pm 22\,g\,C\,m^{-2}$. The C loss through soil respiration for the entire experimental period was $1139 \pm 6\,g\,C\,m^{-2}$.

The contribution of root-derived C to the total C flux from soil respiration increased in both growing periods as evidenced by a decrease in $\delta^{13}C$ values of the soil-respired CO_2 (Fig. 3). Prior to planting, $\delta^{13}C$ values of the respiration (-13.2 ± 0.7‰) were higher than the soil (-17.9 ± 0.1‰), even though theoretically the isotopic value of respired CO_2 should be equal to its source (Amundson *et al.*, 1998). The discrepancy may have been caused by introduction of atmospheric air ($\delta^{13}C \approx -8$‰) or by drawing air enriched in ^{13}C from deeper soil layers (Cerling *et al.*, 1991; Amundson *et al.*, 1998). Blanks showed no adsorption of atmospheric CO_2 to the NaOH traps during the preparation and installation of the traps and precipitation and cleaning of the $SrCO_3$. Soil CO_2 is enriched by 4.4‰ compared with respired CO_2 as a result of diffusional fractionation (e.g. Cerling *et al.*, 1991), which is very close to the observed discrepancy between source and respired CO_2. Therefore, we suspect that the relatively high flow rates combined with rapid removal of CO_2 in the NaOH traps in our study may have caused ^{13}C-enriched CO_2 to be drawn out of the soil. Since flow rates of the trapping system were constant, we corrected all $\delta^{13}C$ of respired CO_2 by the difference between the $\delta^{13}C$ value of soil-respired CO_2 and soil organic C measured prior to planting. In both growing periods, the maximum contribution of rhizosphere respiration to the total respiration flux was 75% (Fig. 3). Fertilization caused the cumulative contribution of rhizosphere respiration to the soil respiration C flux to increase from 40% to 58%. Respiration from SOM decomposition (calculated as total soil respiration minus rhizosphere respiration) increased in both growing periods, but fertilization initially decreased SOM decomposition compared with unfertilized conditions (Fig. 4).

After the soil was put into the lysimeters, TOC concentrations in the leachate decreased from 40 to $3\,mg\,L^{-1}$ prior to planting in both ecosystems, while the decrease in DIC was less pronounced (Fig. 5). Concentrations of DIC in leachate increased as plants developed, while TOC concentrations stayed below $10\,mg\,L^{-1}$. The cumulative C loss through leaching was $44.6 \pm 0.6\,g\,C\,m^{-2}$. During the second, fertilized growing phase C leaching losses decreased to $10.4 \pm 1.4\,g\,C\,m^{-2}$. Leaching of DIC was underestimated during the first 2 months of the second planting phase because the lysimeters were allowed to drain continuously. Initially, the leachate was collected in large buckets exposed to the air causing samples to equilibrate with atmospheric CO_2 levels. After the first 2 months, leaching samples were taken directly from the drainage hoses instead of the buckets minimizing equilibration with atmospheric CO_2. To estimate the potential error in DIC losses, we calculated the soil P_{CO_2} based on observed pH and DIC concentrations for samples equilibrated with soil CO_2 concentrations. We used these soil P_{CO_2} and pH values for nondegassed samples to calculate equilibrium DIC concentrations for the degassed samples. The calculations showed that we underestimated DIC concentrations by approximately 50% during this period. Increasing the concentrations

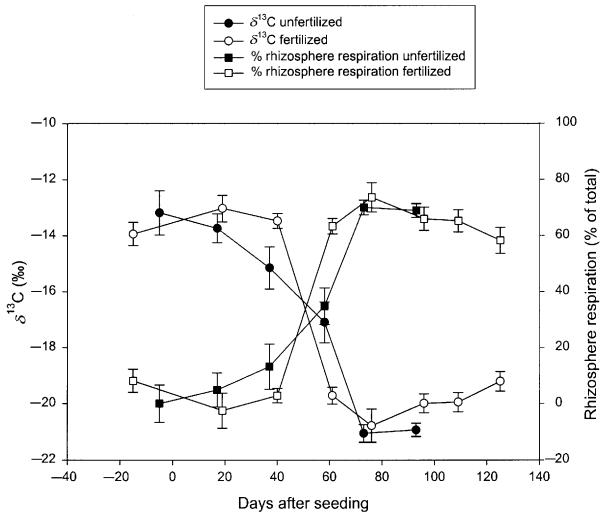

Fig. 3 $\delta^{13}C$ values of soil respiration and contribution of rhizosphere respiration (RR) to the total soil respiration C flux in unfertilized and fertilized growing phases in two grassland ecosystems.

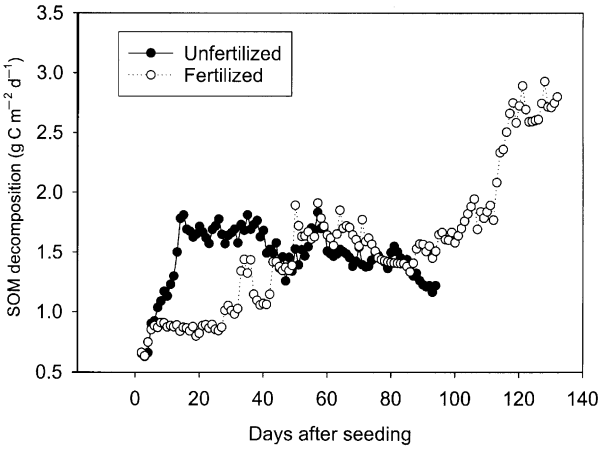

Fig. 4 C losses from soil organic matter decomposition before and after fertilizer applications.

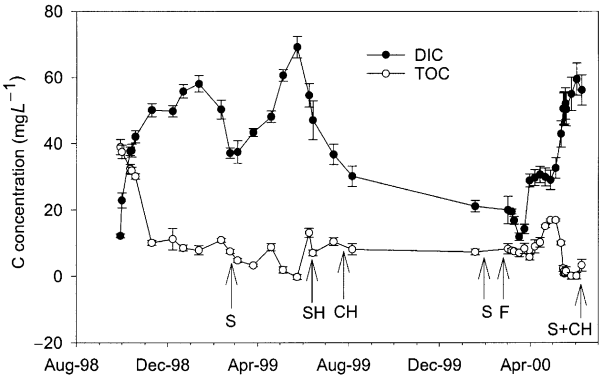

Fig. 5 Total organic C (TOC) and dissolved inorganic C (DIC) concentration in leaching water from two grassland ecosystems during unfertilized and fertilized growing periods. The arrows point at times of seeding (S), shoot harvest (SH), root crown harvest (CH), fertilizer application (F), and shoot and root crown harvest (S + CH).

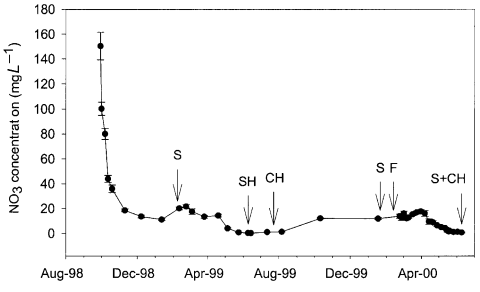

Fig. 6 Nitrate concentrations in leaching water from grassland ecosystems before and after fertilizer applications. The arrows point to times of seeding (S), shoot harvest (SH), root crown harvest (CH), fertilizer application (F), and shoot and root crown harvest (S + CH).

by 50% until March 31, 2000 resulted in an increase in cumulative C loss by 20%.

The $\delta^{13}C$ of the DIC did not change between sampling times (-16.4 ± 0.4‰ on May 5, 2000 and -16.9 ± 0.3‰ on May 26, 2000). Ammonium concentrations were below detection limits throughout the experiment. Nitrate concentrations rapidly decreased after the soil was put into the lysimeters, and concentrations stabilized prior to seeding (Fig. 6). Nitrate concentrations slightly increased during vegetation development.

Ecosystem C pools

The fertilizer application almost doubled total biomass C (Table 1). Fertilization caused C shoot/root ratio to increase significantly. The additional amount of C gained in root crowns in the fertilized growing period was 16 g C m^{-2}. Since shoot biomass was lower in the unfertilized phase we assume root crown biomass was lower as well. We, therefore, underestimated total biomass C at the first harvest by less than 10% by not harvesting root crowns. Temporal changes in soil C concentrations were not significant (Table 2). The $\delta^{13}C$ value of the soil decreased by less than 1‰ from preplanting levels after the first and second planting phase. Prior to the second planting phase, the $\delta^{13}C$ returned to preplanting levels. Even though $\delta^{13}C$ of the roots was -29.2‰, the $\delta^{13}C$ value of the sand increased from -23.5‰ to -21.9‰ during the first planting phase, indicating that native SOM had leached into the sand layer. We estimated root-derived inputs into the sand layer in two steps. First, we assumed that the increase in sand C by 160 g m^{-2} (Table 2) originated from a mixture of SOM and root-derived organic matter. Using Eqn (1), we calculated that the average $\delta^{13}C$ of these additional C inputs had to be -20.8‰ to explain the observed change in $\delta^{13}C$ of the sand. Second, using Eqn (1) we then calculated that the fraction of root-derived inputs was 25% or 40 g C m^{-2}. For the second, fertilized, growing period, we did not observe a significant change in sand C or $\delta^{13}C$ so the inputs of rhizodeposits and/or SOM were assumed to be zero.

Table 1 Biomass C content (g m^{-2}) in grassland ecosystems

	Unfertilized	Fertilized	*P*-value (unfertilized vs. fertilized)
Shoot	84.1 (1.9)	166.3 (6.1)	<0.001
Root	71.3 (4.1)	115.9 (3.7)	<0.001
Total	155.4 (7.5)	282.2 (13.1)	<0.001
Shoot/root	1.20 (0.004)	1.44 (0.09)	0.04

Standard errors are in parentheses.

NPP and NEP estimates

The pool-based NPP (NPP_{pool}) was not significantly different from the flux-based NPP (NPP_{flux}) estimate (Table 3). The largest source of variation in the NPP_{pool} estimates was associated with the measurements of root-derived organic matter as a result of relatively small changes in $\delta^{13}C$ of the soil (less than 1‰) combined with a large uncertainty in total soil C numbers (Eqn (1)). Both NEP estimates were not significantly different due to the large uncertainty associated with the soil C pool measurements.

Discussion

Ecosystem C sequestration

The EcoCELL facility allowed us to measure accurately NEE for a period of 2 years without interruption over

Table 2 Soil C concentrations and contents and contribution of root-derived organic matter to the total soil C pool

	Pre-planting	After first planting	After fallow	After second planting
Soil C (%)	0.67 (0.01)	0.64 (0.01)	0.61 (0.02)	0.62 (0.02)
Sand C (%)	0.04 (0.004)	0.08 (0.01)	0.06 (0.004)	0.05 (0.004)
Soil C ($g\,m^{-2}$)	3489 (32)	3342 (101)	3101 (121)	3150 (103)
Sand C ($g\,m^{-2}$)	220 (4)	380 (30)	275 (3)	270 (8)
Soil $\delta^{13}C$ (‰)	−17.9 (0.1)	−18.7 (0.2)	−17.9 (0.1)	−18.6 (0.1)
Sand $\delta^{13}C$ (‰)	−23.5 (0.1)	−21.9 (0.1)	−22.7 (0.2)	−22.2 (0.3)
Root-derived C ($g\,m^{-2}$)	0	216 (30)	0 (17)	166 (19)

Standard errors are in parentheses.

Table 3 Flux- and pool-based NPP and NEP estimates for fertilized grassland ecosystems

Components	$g\,C\,m^{-2}$	Standard error
NEP	286	21
SOM decomposition	204	50
NPP_{flux}	*490*	54
Shoot mass	166	12
Root mass	116	1
Rhizodeposition	166	19
NPP_{pool}	*448*	15
$NPP_{flux}-NPP_{pool}$	42	
NEP_{flux}	*286*	21
ΔBiomass	282	13
ΔSoil	49	107
NEP_{pool}	*332*	109
$NEP_{flux}-NEP_{pool}$	−46	

NPP, net primary production; NEP, net ecosystem production; SOM, soil organic matter.

two planting cycles. Despite the rapid development of the vegetation, it took 62 days from seeding for the C balance to become positive. After the harvest, the ecosystem C balance rapidly approached zero, indicating that the grass did not substantially contribute to overall ecosystem C sequestration. Maximum NEE values were similar for both unfertilized and fertilized growth periods, indicating that an increase in C uptake was balanced by an increase in respiratory C losses. Still, NEP was larger for the fertilized than for the unfertilized period as a result of a longer period during which maximum NEE was attained. The increase in NEP after fertilization did not compensate for the C losses incurred during the fallow period causing the total C balance to be negative at the end of the study.

In our NEP calculations, we initially assumed that all harvested aboveground biomass was retained. If, on the other hand, we assumed that harvested biomass will eventually decompose and release CO_2 back to the atmosphere, for instance through fire, then net ecosystem C losses would have been $280 \pm 30\,g\,C\,m^{-2}$ at the end of the study. Had harvested biomass entered the soil as litter, the release of CO_2 to the atmosphere from residue decomposition would have been delayed and net ecosystem C losses would have been between 33 and $280\,g\,C\,m^{-2}$. Suyker *et al.* (2003) observed that tallgrass prairie switched from a sink to a source for C when C losses from fires were included in NEP calculations.

Carbon losses would have been smaller if the unplanted period had been shorter but even if we had reseeded the soils immediately after the first growing period ecosystem C stocks would have fallen below starting levels, assuming that the harvested aboveground biomass eventually returns to the atmosphere. Had we fertilized the first crop and observed the same fertilizer response as we did with the second crop, the unplanted period could have lasted up to 6 weeks before ecosystem C dropped below starting levels.

Soil disturbance at the start of the study may have stimulated decomposition of SOM and thus ecosystem C losses. Leachate C and N concentrations and soil respiration were stable prior to the seeding, however, indicating that short-term disturbance effects from soil handling had disappeared (Figs 5 and 6). Furthermore, after all rhizodeposits had decomposed during the fallow period, leaching concentrations and soil respiration rates were the same as prior to the first seeding (Fig. 2). Soil respiration rates before seeding were comparable to rates measured at the Konza prairie during winter (Bremer *et al.*, 1998; Knapp *et al.*, 1998) when temperatures were lower but plants were present. It is therefore most likely that soil handling caused labile C pools to be decomposed and thus caused heterotrophic respiration to be under- rather than overestimated during this study. We cannot discount the possibility that soil were not in equilibrium on a long term but this will also be the case in the field when native plants are being replaced by exotic species.

Soil C fluxes

Decomposition of SOM was stimulated by the presence of plants but fertilization lowered SOM decomposition,

especially during the first 3 months after planting (Fig. 4). The effect of plants on SOM decomposition remains a subject of controversy with studies finding increases (Helal & Sauerbeck, 1984; Cheng & Coleman, 1990) and decreases (Cheng, 1996) in SOM decomposition when plants are present. Cheng & Johnson (1998) found that elevated CO_2 reduced SOM decomposition by 18% without N fertilization, but increased it by 22% with N fertilization. Kuikman *et al*. (1990) and Lekkerkerk *et al*. (1990) hypothesized that, under sufficient N supply, soil microorganisms prefer labile, root-derived organic matter over SOM. Our data support this last hypothesis since the stimulatory effect of plants on SOM decomposition was lower after fertilization. The increase in rhizosphere respiration was most likely caused by an increase in root biomass but we cannot determine whether this was due to an increase in root respiration, decomposition of root-derived organic matter, or a combination of both. Van Ginkel & Gorissen (1998) observed that the production of rhizodeposits was highly correlated with root biomass so C inputs from root-derived organic matter were likely to be higher during the fertilized period. Since the amount of root-derived organic matter present was the same for both growing periods, decomposition of rhizodeposits must have increased in response to fertilization. Root-derived organic matter did not contribute to long-term soil C storage since all plant-derived belowground C inputs had disappeared during the fallow period, demonstrating the labile nature of these rhizodeposits (e.g. Cheng *et al*., 1993, 1994; Verburg *et al*., 1998).

During the unfertilized growing period, C leaching losses equaled 16% of the losses through soil respiration. Potentially, DIC in leachate could originate from four C sources: (1) the atmosphere ($\delta^{13}C = -8‰$), (2) SOM ($\delta^{13}C = -18‰$), (3) roots and rhizodeposits ($\delta^{13}C = -29.2‰$), and (4) $CaCO_3$ ($\delta^{13}C = -2‰$ to 4‰). If we assume an enrichment in $\delta^{13}C$ upon dissolution of CO_2 (g) to HCO_3^- (the dominant form in the leachate at the measured pH) between 8‰ and 10‰ (Mook *et al*., 1974; Amiotte-Suchet *et al*., 1999), then the original CO_2 had a $\delta^{13}C$ of approximately −25‰ to −28‰, indicating a substantial contribution of plant-derived CO_2 (Atekwana & Krishnamurthy, 1998). The exact contribution of each source cannot be calculated because too many sources were present. Even though DIC appeared to be plant derived, the fertilizer-induced increase in (root)biomass did not cause an increase in DIC leaching. During the fertilized phase drainage hoses were open, so atmospheric CO_2 may have entered the soil from the bottom of the lysimeter causing DIC to equilibrate with lower soil CO_2 concentrations than in the first planting phase. Data from the first planting phase show that C losses through DIC leaching below the rooting zone can be significant in alkaline soils when precipitation exceeds evapo-transpiration.

Comparison of NPP and NEP estimates

The EcoCELL facility allowed us to compare directly NPP and NEP estimates using flux measurements and pool inventories. Both pool- and flux-based NPP estimates agreed well considering potential errors in both estimates. For example, Cheng *et al*. (2000) compared flux- with pool-based NPP estimates in sunflower ecosystems under ambient and elevated CO_2 levels and found approximately $80\,g\,C\,m^{-2}$ missing in the NPP_{pool} estimates under elevated CO_2 concentrations. They speculated that part of this 'missing C' could be ascribed to emission of volatile organic compounds (VOC) from the plant canopy, a flux that is currently not measured in the EcoCELL facility. VOC emissions were not likely to influence C budgets in our study as emissions from grasses are very small ($<1\,mg\,C\,m^{-2}\,day^{-1}$; König *et al*., 1995). In our study, NPP_{flux} was overestimated since C losses from leaching were not included in the NEP flux measurements. This would have caused NEP to decrease by about $10–12\,g\,C\,m^{-2}$ assuming that most of the DIC was plant derived. Changes in DIC in soil solution could have resulted in an, albeit small, underestimation in NPP_{pool}. Assuming a volumetric soil moisture content of 30% in the soil layer and 15% in the sand layer yields a total amount of 180 L of water present per m^2 at the time of harvest. Soil solution total C concentrations were around $70\,mg\,L^{-1}$ prior to harvest giving a total of almost $13\,g\,C\,m^{-2}$ present in the soil solution most of which may have been derived from the vegetation. The gravel layer contained little moisture compared with the soil and sand layers as the lysimeters were drained continuously during the fertilized growth period. Including dissolved C fluxes and pools would have resulted in a closer agreement between NPP_{pool} and NPP_{flux}. Additional uncertainty in both NPP calculations was caused by the inability to differentiate between autotrophic root respiration and heterotrophic respiration due to decomposition of root-derived organic matter. This problem most likely caused an overestimation in NPP_{flux} since we implicitly assumed that R_h was equal to SOM decomposition. NPP_{pool} was most likely underestimated as some of the root-derived organic matter produced may have decomposed during the growth period.

Both NEP estimates agreed relatively well but the errors were very large for the NEP_{pool} mainly due to uncertainties in soil C pools. The direct NEE measurements showed that ecosystem C losses during the

fallow period were $260 \pm 26\,g\,C\,m^{-2}$ due to a loss of soil C. The pool inventories showed a loss of $350\,g\,C\,m^{-2}$ from the soil and sand but this change was not significant (Table 2). Still, these soil C losses were similar to the total biomass production in the fertilized planting phase. The large background in soil C made these changes (statistically) undetectable, despite having homogenized soils and a sampling density of one sample per m^2. In this study, NEP_{flux} was almost equal to NEP_{pool} calculated as the change in biomass alone ignoring any changes in soil C pools. Over longer time periods, changes in soil C pools may become significant but our data show that these changes have to be considerable to be accounted for in pool inventories.

Implications for natural systems

Our study was designed to measure the effects of changes in NPP on ecosystem C sequestration. By keeping temperature and soil moisture constant, we eliminated the potential confounding effects of changes in environmental conditions. Cheatgrass occurs both as a winter and spring annual (Mack & Pyke, 1984) so temperature and soil moisture during the growing season and dormancy periods can vary widely depending on the location. It is unclear as to how these differences in growth patterns impact NEE/NEP making it difficult to extrapolate our results to field conditions. Obrist *et al*. (2003) observed daily NEP values around $0\,g\,C\,m^{-2}\,day^{-1}$ with maximum values of $2\,g\,C\,m^{-2}\,day^{-1}$ throughout a very dry year (precipitation $< 150\,mm\,yr^{-1}$) in postfire mixed grass communities in the Great Basin in the western United States. To our knowledge, this is the only study, however, that has measured NEE in a cheatgrass-dominated grassland. Leaching losses of C are likely to be smaller under field conditions than in our study, but data from Cline *et al*. (1977) indicated that leaching below the root zone of cheatgrass occurs even in semi-arid environments. Overall, it appears that in our study both growing season C uptake and dormant season C losses were higher than in the field due to the relatively warm and humid conditions employed in our study. It is not clear if these seasonal differences in NEE between our study and the field would result in differences in annual NEP (integrated NEE). Given that the environmental conditions employed in this study resulted in higher NPP and heterotrophic respiration, it may be that our results are more representative for warmer, more humid grasslands. Indeed, both rates and temporal patterns in NEE compared well with those observed in native tallgrass prairie in Oklahoma (Suyker *et al*., 2003) even though species composition was very different between studies.

Our study showed that the presence of cheatgrass is not likely to result in large increases in ecosystem C sequestration even though cheatgrass can potentially add considerable amounts of C to the soil. The long dormancy period during summer relative to the short growing period will allow most of these labile belowground C inputs to be decomposed. These C losses will be especially important in areas receiving summer rains, which are predicted to occur more frequently as a result of climate change (Baldwin *et al*., 1999). If N availability increases, for instance due to increased grazing, belowground C inputs could be reduced even further despite an increase in biomass as heterotrophic organisms will favor root-derived organic matter over native SOM as their primary energy source. Our results support modeling studies showing that conversions of native rangeland communities including pinyon-juniper woodlands, sagebrush scrublands, and bunchgrasslands to cheatgrass as occurs in the western United States will result in C losses (Sobecki *et al*., 2001) since large C stocks accumulated over long time periods are replaced by a small pool of regrowth, while C inputs into stable SOM pools are reduced (Schulze *et al*., 2000).

Our study demonstrated the potential effects of increases in NPP on ecosystem C sequestration in a species that is becoming increasingly important on local and global scales. The controlled environment facility allowed us to study mechanisms that explained observed patterns in ecosystem C exchange. Despite the increasing significance of these ecosystems, there is a clear lack of field studies that measure NEE (Angell *et al*., 2001; Obrist *et al*., 2003) that will allow us to include the effects of natural temperature and moisture variability on ecosystem C exchange.

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