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Stable Isotope Constraints on Net Ecosystem Production Under Elevated CO_2

Elise Pendall, Jennifer Y. King, Arvin R. Moser, Jack Morgan, Daniel Milchunas

Introduction: Approaches for Estimating Net Ecosystem Production

Net ecosystem production (NEP) is the total carbon accumulated in an ecosystem over a time interval, usually a year. The C is stored above- and belowground in plants, animals, and soils, and has residence times ranging from less than one to several hundred years or more. NEP varies in response to disturbance and during plant succession, and human-induced perturbations to climate and atmospheric constituents also affect NEP at local to global scales. Because NEP determines how much carbon dioxide is taken up from the atmosphere by terrestrial ecosystems, it is critical to evaluate what forcing factors might be regulating variations in NEP at various temporal and spatial scales. In particular, the effects of elevated atmospheric CO_2 may be altering NEP in many ecosystems, and techniques such as stable isotope analysis are improving our ability to evaluate the magnitude of these effects (Pendall, 2002).

NEP has often been defined operationally, depending on the approach used to conduct measurements and the temporal and spatial scales of interest, but this has led to ambiguity and a lack of comparability among studies (Randerson *et al.*, 2002). NEP was first estimated from changes in ecosystem pool sizes, after accounting for lateral fluxes (Olsen, 1963; Bormann *et al.*, 1974; Aber and Melillo, 1991; Chapin *et al.*, 2002):

$$\mathrm{NEP} = (\Delta\mathrm{plant} + \Delta\mathrm{animal} + \Delta\mathrm{SOM})/\Delta t \pm F_{\mathrm{lateral}} \qquad (11.1)$$

It has been recognized that generally the changes in these inventories are extremely small relative to the entire pool size (particularly for SOM), making accurate measurement difficult unless long time intervals

(10 years or more) are allowed between sampling events. This has led to the development of flux-based methods, which allow NEP estimates to be made at more frequent intervals. A complete accounting can be formulated as the sum of fluxes into and out of the ecosystem (Olsen, 1963; Randerson *et al.*, 2002):

$$\begin{aligned} NEP &= F_{in} + F_{out} \\ &\approx F_{GPP} + F_{R_e} + F_{fire} + F_{leaching} + F_{erosion} + F_{hydrocarbons} \\ &\quad + F_{herbivory} + F_{harvest} \end{aligned} \tag{11.2}$$

where GPP is gross primary production and R_e is ecosystem respiration. Net ecosystem exchange (NEE) has been defined as the balance of GPP and R_e, and in the absence of fire or other disturbance, NEE provides an approximation of NEP at limited temporal and spatial scales (Chapin *et al.*, 2002). NEE is measured at the ecosystem scale by eddy covariance techniques (e.g., Wofsy *et al.*, 1993), or at the plot scale in flow-through chambers by monitoring the change in CO_2 concentration going into and coming out of the chamber (Drake *et al.*, 1996). NEP has also been formulated as the difference between net primary production (NPP) and heterotrophic respiration (R_h):

$$NPP = GPP - R_a \tag{11.3}$$

$$R_e = R_h + R_a \tag{11.4}$$

$$NEE = GPP - R_e = NPP - R_h \tag{11.5}$$

GPP and R_a (autotrophic respiration) are not generally measured, but NPP can be measured directly at the plot scale, or estimated from models based on leaf area index at regional to global scales. Empirical algorithms have been developed to estimate R_h; these are generally based on a relationship between microbial respiration and soil temperature (and sometimes moisture; Chapin *et al.*, 2002). This approach also assumes that the smaller fluxes, such as leaching and erosional losses, can be neglected.

All of the methods used for estimating NEP (or NEE) have limitations. It appears that uptake measured by NEE in open-top chambers is often higher than can be accounted for by measurements of above- and belowground biomass (Drake *et al.*, 1996; Niklaus *et al.*, 2000). This has been termed a 'locally missing' carbon sink (Cheng *et al.*, 2000; Niklaus *et al.*, 2000), and may in part be caused by over-pressurization of chambers suppressing respiration, or by leaching or volatilization of C compounds. There are known issues with eddy covariance technology, particularly with respect to estimation of the respiration flux. Nighttime fluxes can be underrepresented if wind conditions are stable, or if air drainage patterns cause CO_2 to be lost laterally from the system. Inventories miss a portion of the biomass to herbivory, senescence during the growing season, and incomplete sampling, especially

belowground. Improvements in NEE methodology and in belowground inventory methods are narrowing the gap between inventory and exchange techniques.

It is inherently difficult to track small changes in NEP if C is being stored belowground, in part because many experiments are often not long enough (2–5 years) to produce changes in soil organic carbon (SOC) detectable using standard techniques. In certain situations, stable C isotopes improve the resolution with which C storage belowground can be detected. Recently-fixed C entering the soil pool has an isotopic signature distinct from pre-existing SOC in many experiments on elevated CO_2, as well as in cropping situations where a C_4 crop has been planted into a C_3 soil, or vice-versa (Balesdent *et al.*, 1988; Leavitt *et al.*, 1994). In these cases, 'new' inputs from rhizodeposition (root exudates and turnover of fine roots and mycorrhizal hyphae) can be quantified (Hungate *et al.*, 1997; Van Kessel *et al.*, 2000; Leavitt *et al.*, 2001). Rhizodeposition is often too small to measure without isotopic techniques, and may thus constitute some portion of the 'missing' C accumulating in terrestrial ecosystems (Pendall *et al.*, 2004 in review).

Carbon dioxide derived from decomposition of SOC will have a $\delta^{13}C$ value different from that of rhizosphere respiration in the same situations where there is an isotopic disequilibrium between new inputs and pre-existing SOC (Rochette and Flanagan, 1997; Pendall *et al.*, 2001b). This has allowed partitioning of the total soil respiration flux into its components of root/rhizosphere respiration and decomposition. No method exists to fully separate heterotrophic and autotrophic respiration fluxes in the field; decomposition by rhizosphere microbes is generally included with the root respiration component (Hanson, 2000). We suggest that in the case of estimating NEP, C loss by rapid turnover of recent (current year) photosynthate in the rhizosphere is functionally equivalent to autotrophic respiration, and the main C decomposition loss of interest is that which has a residence time of 1 year or longer.

Here we demonstrate a method that relies on biomass measurements and stable C isotopes for evaluating NEP in an experiment on elevated CO_2. This approach is a modification of Eq. 11.5 in that we explicitly include measurement of rhizodeposition to the SOM pool, which is otherwise not included in the inventory:

$$NEP = AG + BG + NSC - R_h \tag{11.6}$$

where AG is annual aboveground C increment, or NPP, BG is annual belowground NPP, NSC is annual 'new' soil C input or rhizodeposition, and R_h is annual C loss by decomposition. We maintain that inventory estimates of NEP should include rigorous estimates of both decomposition and rhizodeposition. This approach would be suitable in any situation where

an isotopic disequilibrium exists between recently fixed C and pre-existing soil organic matter, such as C_3–C_4 land-use or land-cover change. Stable C isotopes allow a more complete accounting of small increases in the soil carbon pool that are contributed by rhizodeposition, and also allow us to estimate decomposition losses by partitioning rhizosphere respiration and SOM decomposition.

Experimental Approach

An elevated CO_2 experiment was conducted in the shortgrass steppe region of northeastern Colorado, at the USDA-ARS Central Plains Experimental Range (CPER; latitude 40°40′ N, longitude 104°45′ W), ~55 km northeast of Fort Collins. The most abundant species at the study site were the C_4 grass, *Bouteloua gracilis* (H.B.K) Lag. (blue grama), and the C_3 grasses *Stipa comata* Trin and Rupr. (needle-and-thread grass) and *Pascopyrum smithii* (Rydb.) A. Love (western wheatgrass). Root biomass (including crowns) is responsible for ~70% of net primary production (NPP) in this ecosystem (Milchunas and Lauenroth, 2001). Beginning in 1997, open-top chambers (OTC; 4.5 m diameter) were used to evaluate the effects of CO_2 on the shortgrass steppe (SGS) ecosystem, with three replicate chambers at ambient (360 ± 20 μmol mol^{-1}) and elevated (720 ± 20 μmol mol^{-1}) CO_2. Three unchambered plots of the same area allowed evaluation of any chamber effects. Chambers were placed on the plots before growth started in late March or early April, and removed at the end of the growing season in late October. Blowers with ambient or elevated CO_2 ran continuously. The experimental and chamber design has been described in detail by Morgan *et al.* (2001).

Figure 11.1 shows the $\delta^{13}C$ values of ecosystem components in elevated and ambient CO_2 chambers at the SGS OTC experiment in 1999. Non-chambered plots had values that were not significantly different from those with the AC treatment. The tank gas used to double the atmospheric CO_2 concentration had an approximate $\delta^{13}C$ value of −40‰ during 1999, which produced air in the elevated chambers of -24.7 ± 1.4‰, which compared with background air $\delta^{13}C$ values of -8.1 ± 0.2‰ (Pendall *et al.*, 2003). The ^{13}C-depleted air in the EC treatment produced depleted plant material, and by 1999 (after 3 years of elevated CO_2), the tracer was evident in soil organic matter. In the AC and NC plots, an isotopic disequilibrium between currently growing plants and SOM of ~4‰ was attributed to a reduction in grazing c. 20 years prior to the start of our experiment (grazing was eliminated completely 2 years prior). Reduction of grazing pressure in the SGS tends to favor C_3 over C_4 grasses (Lauenroth and Milchunas, 1988).

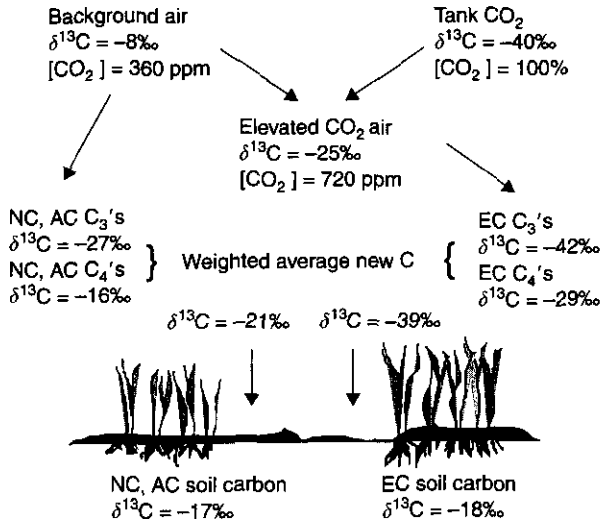

Figure 11.1 *Stable C isotope composition of ecosystem components* in the shortgrass steppe open-top chamber experiment for 1999.

Biomass Inventory Methods

In grassland ecosystems, aboveground biomass harvests are relatively straightforward. At the end of the growing season in October, aboveground biomass was clipped at the root crown from 1.5 m^2 subplots, dried, and weighed. Because plots were clipped to the ground the prior fall, this harvest represents the annual aboveground NPP. We estimate that herbivory losses were less than 5% in all treatments; very occasional pesticide use was needed to reduce aphids. Different subplots were harvested in July and separated by species but this was not practical for plants collected in the fall. Tissue C content was determined by elemental analysis.

Belowground biomass inventories are typically labor-intensive and associated with a wide margin of error. The standing crop of roots in perennial grasslands may have a turnover time of 4–6 years (Milchunas and Lauenroth, 2001), and thus root ingrowth cylinders were used to assess the annual increment of root growth. In the fall prior to the growing season, two 20 cm diameter soil cores were removed from each plot, and replaced with 15 cm diameter PVC pipes to a depth of 40 cm. The outer diameter of each core was lined with plastic mesh (1 mm square openings) and the 5 cm band gap was filled with sieved soil from near the experimental site. At the end of the following growing season, the PVC liners were carefully removed, roots were cut from the mesh liner, and all soil and roots were returned to the laboratory. Roots were separated from soil by flotation and washing over a 1 mm sieve, dried, ground, and analyzed for C content on an

elemental analyzer. Subsamples of roots were ashed at 450°C to determine the ash-free C content.

Both above- and belowground NPP inventory methods tend to underestimate NPP, owing to losses such as herbivory and tissue senescence (Chapin *et al.*, 2002). In the case of belowground NPP, our method of accounting for rhizodeposition should at least partly remedy this situation.

Rhizodeposition or 'New' Soil Carbon

Rhizodeposition is soil C derived from turnover of fine roots, secretion of soluble root exudates, and turnover of rhizosphere-associated microbial biomass. This C increment is generally too small to measure on an annual basis using total C analysis because it is within the measurement error, but by using an isotopic tracer, small changes may be detected in 1–2 years (Leavitt *et al.*, 2001). The fraction of C that has been contributed by rhizodeposition (F_{new}) is determined using the two-part mixing model described by Balesdent *et al.* (1988):

$$\delta^{13}C_{SOC} = F_{new}(\delta^{13}C_{new}) + (1 - F_{new})(\delta^{13}C_{old}) \qquad (11.7)$$

which can be rearranged to:

$$F_{new} = (\delta^{13}C_{SOC} - \delta^{13}C_{old})/(\delta^{13}C_{new} - \delta^{13}C_{old}) \qquad (11.8)$$

In order to use stable isotopes to estimate 'new' C inputs from rhizodeposition to the soil system, it is necessary to define the isotopic 'end-members,' i.e., the $\delta^{13}C$ value of pre-existing SOM and that of the new C being added. Determination of the end-member isotopic values is not a trivial task in many ecosystems, because $\delta^{13}C$ of SOM often increases with depth; therefore, depth-dependent 'old' end-member values must be used. In some ecosystems, the $\delta^{13}C$ of 'new' input C is also challenging to determine. Mixed C_3–C_4 systems require assumptions of the proportion of C allocated belowground as well as the different isotopic values of the different functional groups.

In the case of the SGS OTC experiment, we assumed that the soils on the non-chambered (NC) plots remained at steady-state over the course of the experiment, and that the NC soils represented pre-existing C across the site. (This was confirmed by non-significant changes in $\delta^{13}C$ values in NC soils over time.) Our NC soils therefore provided the old C end-member values. An alternative approach is to use samples collected across the site prior to establishing an experiment (Leavitt *et al.*, 1994). Collecting and analyzing steady-state soils together with treated soils provides analytical control over multi-year experiments. We collected two soil cores (40 cm deep, 15 cm diameter) from each plot at the end of each growing season. Cores were collected volumetrically and divided into five depth increments (0–5, 5–10, 10–20, 20–30, and 30–40 cm). Gravel >2 mm and most roots

were removed immediately after sampling, with additional root picking done by hand on dried samples. Soils were then ground to a fine powder for analysis by elemental analyzer-mass spectrometry (EA-MS) for C content and $\delta^{13}C$ value, with precision of 0.1% and 0.2‰, respectively. Soils were carbonate-free to a depth of 40 cm, and we therefore did not acidify the samples.

In the mixed C_3–C_4 shortgrass steppe, the isotopic composition of new inputs can potentially vary spatially and temporally. Aboveground biomass clipping of 1.5 m^2 subplots was done in July 1999, at peak green biomass. Leaves and stems were separated by species, dried, ground, and analyzed for C content and $\delta^{13}C$ value by elemental analyzer-IRMS. Roots were harvested by species at the end of the experiment in 2001, and averaged 1.4‰ heavier than leaves (Pendall *et al.*, 2004). We used the 1999 leaf $\delta^{13}C$ values, weighted them by the 1999 aboveground biomass amounts, and then added 1.4‰ for the 'new' C end-member signatures ($\delta^{13}C_{new}$; Table 11.1). July harvest samples were assumed to represent the proportion of C_3 and C_4 species for the entire growing season; samples harvested at the end of the season (peak total biomass) were not easily separated by species. The three dominant species maintain similar shoot : root ratios at ambient and elevated CO_2, and therefore we assumed that the C_3 : C_4 ratio of rhizodeposition was proportional to aboveground biomass (Morgan *et al.*, 1994).

F_{new} was multiplied by the total mass of C in each horizon or depth increment to calculate rhizodeposition going into each horizon on a mass basis. This required measurement of bulk density, which can be a particularly challenging measurement in gravelly or organic-rich soils, but was straightforward in our sandy loam soils. Whole-profile rhizodeposition was calculated by summing the depth increments.

Uncertainties in estimating rhizodeposition were determined by accounting for variability in all of the components, including $\delta^{13}C_{SOC}$, $\delta^{13}C_{old}$, and $\delta^{13}C_{new}$, following a first-order Taylor series approach (Phillips and Gregg,

Table 11.1 $\delta^{13}C$ Values of C_3 and C_4 Plants, Proportion of C_3 Biomass, and $\delta^{13}C$ Values of New C Inputs (After Adding 1.4‰ to Account for Average Root ^{13}C Enrichment) During the Growing Seasons of 1999 at the Shortgrass Steppe OTC Experiment

	$\delta^{13}C$, C_4 (‰)	$\delta^{13}C$, C_3 (‰)	C_3 biomass	$\delta^{13}C$ new (‰)
AC	−15.4 (0.21)	−25.2 (0.99)	0.72 (0.03)	−21.1 (1.01)
EC	−33.2 (0.81)	−42.4 (1.92)	0.81 (0.04)	−39.3 (2.09)
NC	−15.4 (0.27)	−26.5 (0.49)	0.67 (0.07)	−21.4 (0.56)

[a] Standard errors in parentheses include error on the concentration and isotopic composition of C in end-members.

2001). Uncertainty estimates for $\delta^{13}C_{new}$ included standard deviations of % biomass and $\delta^{13}C$ of the dominant grass species. We further corrected for covariance of C content and $\delta^{13}C$ values among soil depth intervals when calculating the standard error for the whole profile (Donald L. Phillips, personal communication, 2002).

Decomposition (R_h)

Determination of the in-situ decomposition flux requires first assessment of the total soil respiration flux. We measured soil respiration in this grassland ecosystem by measuring the CO_2 gradient with depth in the soil, and multiplying that by soil diffusivity to obtain the flux (Pendall *et al.*, 2003). This allowed us to obtain a measurement of belowground respiration without disturbing the aboveground vegetation by clipping. In the fall before the growing season, stainless steel tubes (1/8″ OD) were inserted horizontally 15 cm into a pit face at five depths (3, 5, 10, 15, and 25 cm). The pit was then backfilled. Beginning in May, glass syringes, greased with Apiezon M and fitted with gas-tight valves, were used to collect and store the soil gas samples. Volumes of 10 mL were collected at all depths, except for the 3 cm depth, where 6 mL were collected, to ensure that no atmospheric air was pulled into the syringe. A small amount of magnesium perchlorate was used in-line to absorb moisture; this allowed us to analyze the oxygen isotopes as well as the carbon isotopes (Ferretti *et al.*, 2003). Atmosphere samples were also collected on each sampling date, from each chamber or plot into 0.5 L flasks at ~1.5 m above the ground, after flushing ~10 flask volumes. Diffusivity was calculated from soil temperature and moisture using an approach that accounts for inter- and intra-aggregate differences in diffusion rates (Potter *et al.*, 1996).

Soil gas samples were analyzed within 24 hours of collection for CO_2 concentration using an infra-red gas analyzer (Model LI-6251, LICOR, Inc., Lincoln, NE), with a precision of $\pm 3\,\mu mol\,mol^{-1}$ over the concentration range 360–8000 $\mu mol\,mol^{-1}$; most soil gas samples were about 1000 $\mu mol\,mol^{-1}$. Soil gas was analyzed for stable isotopes of CO_2 using gas chromatography-isotope ratio mass spectrometry (GC-IRMS; Isoprime model, Micromass, UK) (Miller *et al.*, 1999). Tests indicated that standards stored in these greased syringes kept for up to one week without significant leakage or isotopic exchange. To ensure a linear response of the mass spectrometer, sample sizes varying from ~7 to ~ 250 µL of soil gas were injected into a carrier gas stream, which was further split before being introduced into the mass spectrometer. This allowed the peak height of the sample to be within ~10% of the peak height of the standard, and precision was better than ±0.1‰. Flasks of atmospheric air were analyzed for CO_2 mixing ratio

by infra-red absorption (Conway *et al.*, 1994) and for $\delta^{13}C$ by dual-inlet mass spectrometry (Trolier *et al.*, 1996).

We used the 'Keeling' plot approach to estimate the $\delta^{13}C$ value of soil respiration ($\delta^{13}C_{SR}$) (Keeling, 1958, 1961; Pendall *et al.*, 2001b, 2003), in order to eliminate the influence of variable amounts of atmospheric CO_2 that diffuse down into the soil profile. When $\delta^{13}C$ is plotted against the inverse of the CO_2 concentration, the y-intercept reflects the flux-weighted average $\delta^{13}C$ value of the biological source of CO_2, resulting from root/rhizosphere respiration and microbial decomposition. The intercepts were calculated using geometric means to account for variability in both independent (CO_2) and dependent (^{13}C) variables (Sokal and Rohlf, 1995). We subtracted 4.4‰ from the y-intercept values to account for kinetic fractionation during diffusion because the soil CO_2 was sampled from within the soil rather than from the soil surface (Cerling, 1984; Amundson *et al.*, 1998). This approach assumes that the biological end-member $\delta^{13}C$ value is constant with depth, reflecting a constant proportion of rhizosphere ('new') and microbial ('old') components.

In order to estimate R_h and ultimately NEP from Eq. 11.6, the soil respiration flux (SR) was partitioned into new and old components using a two-component mixing model similar to Eq. 11.8 (Pendall *et al.*, 2001b, 2003):

$$F_{R_h} = (\delta^{13}C_{SR} - \delta^{13}C_{new})/(\delta^{13}C_{R_h} - \delta^{13}C_{new}) \qquad (11.9)$$

where F_{R_h} is the proportion of CO_2 generated by microbial decomposition of soil C. The main difference from Eq. 11.8 is that old end-member values ($\delta^{13}C_{R_h}$) were not taken from bulk soil C analyses, because a portion of bulk C is 'passive,' or unavailable to microbial decomposition. We instead used a biophysical fractionation approach to reflect decomposition of pre-existing SOC that was available to microbes. Soil samples collected at the end of the 1999 growing season were subjected to long-term (300 days) laboratory incubations (Paul *et al.*, 2001). During the 'slow-pool' phase, after day 100, an approximate steady-state $\delta^{13}C$ value was achieved. Average $\delta^{13}C$ values ($n = 4$ for each of two depths) of CO_2 evolved during decomposition of slow-pool C were used as the old C end-member signatures ($\delta^{13}C_{R_h}$). For AC, $\delta^{13}C_{R_h}$ was -17.0 ± 0.47; for EC it was -21.9 ± 1.52; for NC it was -17.8 ± 0.48 (Pendall *et al.*, 2001a).

The new C end-member signatures ($\delta^{13}C_{new}$) were determined as described above for rhizodeposition (Table 11.1). An offset of at least 4‰ between old and new end-members allowed partitioning on all treatments. In pure C_3 ecosystems, it is possible that photosynthate delivered to roots varies at daily to weekly timescales, depending on atmospheric vapor pressure deficit, leading to variability in the isotopic composition of soil-respired CO_2 (Ekblad and Högberg, 2001).

The decomposition flux was estimated by multiplying F_{R_h} by the total soil respiration flux. Uncertainties in estimating the proportion of soil respiration from decomposition were determined by accounting for variability in all of the components, including $\delta^{13}C_{SR}$, $\delta^{13}C_{R_h}$, and $\delta^{13}C_{new}$, following a first-order Taylor series approach (Phillips and Gregg, 2001). Uncertainty in $\delta^{13}C_{SR}$ was based on least squares standard errors of the Keeling intercepts (which were determined by geometric means; Pataki *et al.*, 2003). Uncertainty for $\delta^{13}C_{R_h}$ was estimated as the standard error of the average of 4 incubation subsamples over 7 sampling dates between 100 and 250 days after the start of the incubation experiment.

Constructing the Ecosystem Carbon Balance: NEP

We combined our isotopic measurements with the more standard inventory measurements of above- and belowground net primary production to estimate NEP; rhizodeposition was added to NPP, and decomposition was subtracted (Eq. 11.6). Bulk soil carbon content was not significantly different among treatments in 1999, but $\delta^{13}C$ values were (Fig. 11.2). By assuming that NC SOC was at steady state, we assessed changes in AC and EC SOC that resulted from the experimental perturbation (chambers as well as elevated CO_2). The new C increment (F_{new} from Eq. 11.8) in individual horizons in AC soils was 2–3%, or 20–35 g m^{-2}, compared with 3–7%, or 30–45 g m^{-2} in EC soils (Table 11.2). At some depths in the AC treatment, F_{new} was a negative value, because $\delta^{13}C$ values of SOC were more enriched than the NC soil 'old' end-member value. Natural variability sometimes causes the isotopic composition of a mixture to fall outside the range of the end-members, in which case the mixing model is invalidated.

Rhizodeposition was similar in magnitude to belowground NPP, and was significantly higher in the EC treatment than in the AC treatment (Table 11.2). When summed over the top 40 cm of soil, and the 3 years of the experiment, the EC soils had accumulated 197 g C m^{-2} compared with 78 g C m^{-2} in AC soils. We simply divided these values by 3 to obtain the amount accumulated in 1999 (Table 11.2). When propagating the errors through the individual horizons to the whole profile value, we accounted for the variability in isotopic end-members and the mixture (Phillips and Gregg, 2001), and also for covariance among soil depths. This correction had the effect of increasing the standard error on the whole-profile value from that which would be calculated from the square root of the sum of the variances.

Soil respiration rates were significantly higher under elevated CO_2 during dry periods (Table 11.3; Pendall *et al.*, 2003). Soil-respired CO_2 was depleted in ^{13}C in EC plots relative to AC and NC plots, reflecting metabolism

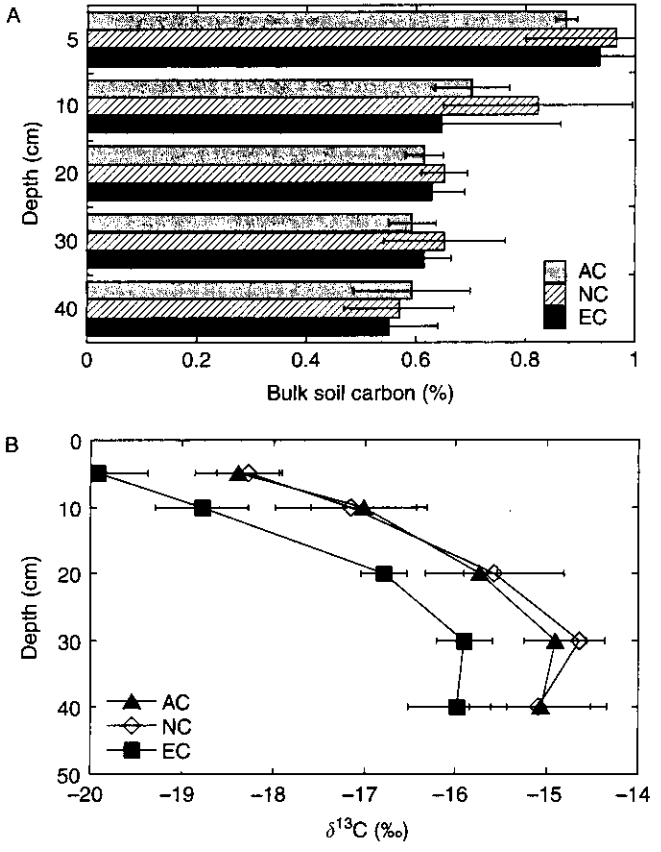

Figure 11.2 (A) Soil C content and (B) $\delta^{13}C$ values in bulk soil collected in October, 1999, after three experimental growing seasons. Ambient CO_2 (AC) and elevated CO_2 (EC) chambers, and non-chambered control plots (NC). Standard errors (bars) for n = 3.

of recently assimilated C (Table 11.3). $\delta^{13}C_{SR}$ values in NC and AC plots were not significantly different from one another. The components of soil respiration, rhizosphere respiration, and decomposition had distinct $\delta^{13}C$ end-member values in all three treatments, which was attributed to reduction of grazing favoring growth and reproduction of C_3 grasses (Pendall *et al.*, 2003). Decomposable organic matter reflected a greater proportion of C_4 carbon, as shown by the relatively enriched microbial incubation $\delta^{13}C$ values.

Elevated CO_2 clearly enhanced decomposition rates throughout the growing season compared with ambient chambers (Fig. 11.3). Annual decomposition losses were estimated by integrating the areas under the curves in Fig. 11.3, assuming the winter fluxes to be negligible (Table 11.4).

Table 11.2 *Rhizodeposition (New C) in Ambient CO_2 and Elevated CO_2 Chambers as Estimated From $\delta^{13}C$ Values*

Treatment	Depth (cm)	Fraction new C	New C ($g\,m^{-2}$)
AC	0–5	0.02 (.25)	18 (4)
	5–10	−0.03 (.22)	—
	10–20	0.02 (.16)	25 (3)
	20–30	0.03 (.13)	35 (3)
	30–40	−0.004 (.15)	—
EC	0–5	0.07 (.09)	41 (4)
	5–10	0.07 (.09)	35 (3)
	10–20	0.05 (.09)	46 (4)
	20–30	0.05 (.08)	46 (4)
	30–40	0.03 (.08)	29 (3)
	Whole Profile New C ($g\,m^{-2}$)		
	3 years	*1999*	
AC	78 (9)	26 (5)	
EC	197 (9)	66 (5)	

AC, ambient CO_2; EC, elevated CO_2.

Standard errors, calculated following Phillips and Gregg (2001), are in parentheses.

Standard error on Profile New C accumulation corrects for autocorrelation among depth increments in addition to including errors on stable isotopic composition of end-members.

Table 11.3 Soil Respiration Rates and $\delta^{13}C_{SR}$ Values for 1999

		Respiration rate ($mg\,C\,m^{-2}\,h^{-1}$)			$\delta^{13}C_{SR}$ (‰)		
Date	DOY	AC (SE)	NC (SE)	EC (SE)	AC (SE)	NC (SE)	EC (SE)
5/26/99	146	103 (8.6)	126 (15.5)	94 (4.4)	−21.2 (.58)	−21.3 (.54)	−30.7 (.33)
6/16/99	166	90 (32.4)	52 (22.9)	126 (36.3)	−19.8 (.58)	−20.4 (.61)	−32.2 (.98)
6/30/99	181	57 (8.4)	83 (13.8)	76 (21.0)	−20.3 (.68)	−19.5 (.29)	−30.7 (.44)
7/15/99	196	51 (1.5)	76 (7.2)	74 (9.7)	−18.7 (.64)	−18.9 (.26)	−30.6 (.48)
8/3/99	215	90 (11.0)	200 (36.1)	122 (13.3)	−20.3 (.4)	−20.3 (.41)	−33.0 (.61)
9/1/99	244	79 (11.5)	182 (22.3)	93 (17.8)	−20.0 (.55)	−21.5 (.5)	−31.8 (.62)
10/13/99	286	40 (4.7)	47 (5.9)	48 (10.4)	−20.2 (.64)	−20.4 (.64)	−30.7 (.93)
11/17/99	321	17 (2.2)	19 (3.6)	21 (1.4)	−20.8 (.49)	−20.5 (.31)	−29.0 (.84)

Respiration rate was estimated by a flux-gradient approach; $\delta^{13}C_{SR}$ estimated from Keeling plot end-members; standard errors in parentheses. DOY, day of year; AC, ambient chambers; NC, non-chambered plots; EC, elevated CO_2 chambers.

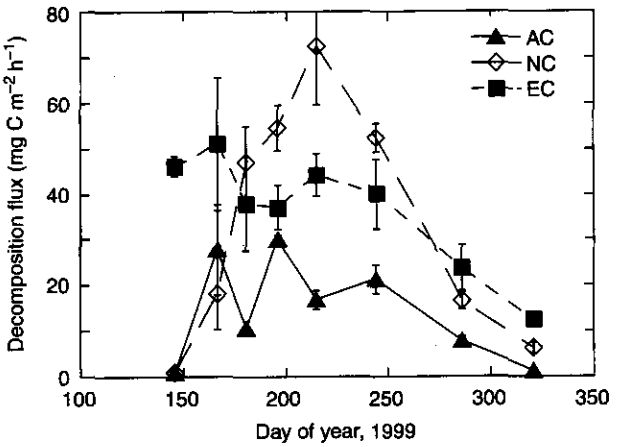

Figure 11.3 Decomposition flux from ambient (AC) and elevated CO_2 (EC) chambers and non-chambered control plots (NC) during 1999. Total soil respiration was multiplied by the fraction from decomposition of old C as estimated from Eq. 11.1. Standard error (bars) included all sources of error: variability of $\delta^{13}C$ values in end-members and mixtures, as well as variability of C_3 and C_4 biomass.

Table 11.4 Components of Net Ecosystem Production (NEP) at the Shortgrass Steppe Elevated CO_2 Experiment in 1999

	EC	AC
AG NPP	74 (9.0)	49 (5.5)
BG NPP	55 (7.3)	40 (6.1)
New soil C	66 (5.2)	26 (4.9)
Decomposition	151 (22.4)	64 (10.2)
NEP	49 (25.7)	51 (14.0)

Values given are g C m^{-2} y^{-1}. EC, elevated CO_2 chambers; AC, ambient CO_2 chambers; AG NPP, aboveground net primary production; BG, belowground; New soil C, also referred to as rhizodeposition.

Errors (in parentheses) were propagated for NEP by taking the square root of the sum of the variance terms for each component (aboveground and belowground biomass, decomposition, and whole-profile rhizodeposition).

The non-chambered plots showed the highest soil respiration and decomposition fluxes, possibly because the soils were slightly drier inside the chambers (Table 11.3; Fig. 11.3). Low decomposition rates early and late in the growing season reflect higher rates of rhizosphere respiration during

these moister parts of the growing season. Rhizosphere respiration was positively correlated with soil moisture for all treatments (Pendall *et al.*, 2003).

Elevated CO_2 also altered the temperature sensitivity of decomposition. Significant exponential relationships between soil T and decomposition rate were found for all treatments (Pendall *et al.*, 2003). Interestingly, Q_{10} values for EC soils were lower than for AC soils, with NC soils intermediate. This suggests that decomposition processes under elevated CO_2 are being altered qualitatively in addition to quantitatively, which may relate to an increasing importance of fungal decomposers. Soil xylanase analysis suggests that EC soils have greater fungal activity (E. Kandeler, personal communication), and fungi are known to be generally less temperature sensitive than bacteria (E. Paul, personal communication).

We estimated NEP by summing above- and belowground biomass and rhizodeposition, and subtracting the annual decomposition loss (Fig. 11.4; Table 11.4). During the 1999 growing season, which had 50% greater than average precipitation, NEP was the same on EC and AC treatments, roughly $50\,g\,C\,m^{-2}\,y^{-1}$. Apparently, stimulation of decomposition under elevated CO_2 during a moist year can negate the increased production of biomass in the shortgrass steppe. Carbon cycling rates on this semi-arid grassland were stimulated by elevated CO_2, but no net C accumulated during 1999, as shown by Hungate *et al.* (1997).

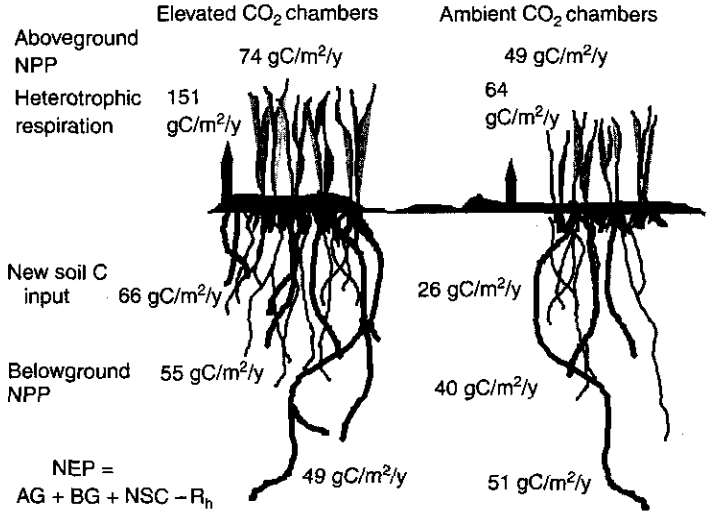

Figure 11.4 Net ecosystem production (NEP) for 1999 (all units are $g\,C\,m^{-2}\,y^{-1}$). Above- and belowground NPP were determined by standard methods. Heterotrophic respiration and new soil C input were determined using stable C isotope tracers.

We defined our experimental plots as the ecosystem boundaries, including soil down to a depth of 40 cm. We have not accounted for leaching losses, but in our semi-arid region this is not likely to be a significant portion of the C balance. Volatilization of organic compounds is also expected to be small; methane is always taken up by the shortgrass steppe ecosystem (Mosier *et al.*, 1991). These factors contribute to unquantified errors in our NEP estimate (Randerson *et al.*, 2002).

Conclusions

Stable C isotopes provided a unique method of tracing C movement through individual components of this shortgrass steppe ecosystem. These analyses enabled us to assign estimates for C pools and fluxes which were otherwise difficult to measure directly, and therefore allowed us to constrain estimates of NEP. Based on the measurements made in our study on the shortgrass steppe, we found that elevated CO_2 stimulated not only plant productivity but rhizodeposition and decomposition fluxes, thus resulting in no overall difference in NEP between ambient and elevated CO_2 during a moist growing season.

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