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Belowground Primary Production by Carbon Isotope Decay and Long-term Root Biomass Dynamics

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Abstract

The isotope decay method of estimating belowground net primary production (BNPP) has the potential to overcome the assumptions and biases associated with traditional methods. Isotope loss through in situ decomposition after pulse-labeling is considered the inverse of production, and turnover times are estimated by regression to time of zero remaining isotope. Method development and estimates of production were previously published using 4 years of data, which showed a clear linear loss rate over time. A slow, distinctly different phase in isotope loss developed 5–10 years postlabeling. We assess reasons for the two-phase loss functions and the implications for estimates of BNPP and compare the isotope decay method with standard coring methods over a 13-year period. Reasons for the two-phase dynamics of carbon 14 (^{14}C) loss could include various biological and/or methodological factors. Results suggest that ^{14}C in soil embedded in roots as they grow, a small proportion of roots that live for a much longer time than the majority of roots, and method of separating roots from soil organic matter may influence estimates of BNPP by isotope methods. Remobilization of label in structural tissue or reuptake of label from the soil did not appear to be responsible for the slow, second phase of loss dynamics. Isotope decay produced more reliable estimates than standard coring methods. Estimates using harvest sum of increments were zero in 6 of 13 years. Thirteen years of root biomass data showed no predictable trend over winter or consistent seasonal pattern, although longer-term cycles were evident. Aboveground:belowground ratios were generally smaller during dry periods, but root biomass was not as responsive as aboveground biomass to annual precipitation.

Key words: belowground turnover; carbon dynamics, crowns; decomposition; isotope methods; net primary production, root production; shortgrass steppe; soil carbon.

Introduction

Belowground net primary production (BNPP) is still very difficult to estimate accurately, even though it is a basic and important ecological variable (Lauenroth 2000). New technology such as minirhizotrons, developed to overcome the large problems and biases of traditional coring methods, is also flawed by inherent biases (Pages and Bengough 1997). In 1992, we published a paper that focused on the testing of methods using carbon-14 (^{14}C) decay (Dahlman and Kucera 1965) and ^{14}C dilution (Caldwell and Camp 1974) to estimate root, crown, and aboveground net primary production (ANPP), based on an experiment initiated in 1985 (Milchunas and Lauenroth 1992). The ^{14}C decay method provided values for the more accurately estimated ANPP that were consistent with known biases of the traditional methods it was compared to, thereby providing some confidence in estimates of BNPP for which actual values are very poorly approximated

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by traditional methods. However, new methods always require critical, ongoing evaluation. Major disadvantages of the carbon isotope decay method include the time necessary to obtain an estimate of BNPP and the fact that the estimate is an average over several years. Our initial estimates were based on data collected from 1985 through the spring of 1988. Turnover coefficients for ^{14}C in various biomass pools were obtained by extrapolating straight-line regressions of isotope mass remaining over time to the time of complete turnover (zero mass remaining) (Dahlman and Kucera 1965). A few years after the publication of the first report on our labeling experiment, we noted that the rate of loss of ^{14}C in all plant components had decreased substantially. Our objective here is to report results collected for the 10 years following labeling of the original experimental plots in the shortgrass steppe of the North American Great Plains. First we show evidence for the two-phase dynamics; then we examine some of the potential causes for the decreased, nonlinear loss rates of label and discuss the implications of this change in isotope decay for estimating production of root, crown, and aboveground plant components.

The isotope decay method of estimating net primary production is conducted in situ, without artificial surfaces or conditions (Milchunas and Lauenroth 1992). The method consists of pulse-labeling an area of vegetation by exposing the plants to labeled CO_2 under a clear tent. We used ^{14}C, but conceptually it should be possible to use ^{13}C. After all isotope in a labile form (soluble compounds that can be translocated or respired) has been either stabilized in structural tissue or respired (after the first growing season in this system), temporal loss of the isotope via decomposition plus herbivory is estimated by evaluating the isotope content of plant parts by coring and clipping. Production estimates are obtained by dividing the time for complete turnover (years) by the annual average biomass (both estimates have an associated error). Loss is considered to be equal to production when biomass does not change. Non–steady-state conditions are accounted for by adding or subtracting the increase or decrease in biomass from the beginning to the end of the study.

Assumptions for the carbon isotope decay method of estimating net primary production are relatively minor, especially compared to those involved in traditional methods. Assumptions are (a) that at the time of labeling, roots have the same potential for growth throughout the profile (maximized by watering prior to labeling), and (b) that the time or season of labeling does not influence the relative proportion of label in tissue fiber fractions (celluloses and lignin). Time or season of labeling effects on aboveground vs belowground allocation of isotope or the proportion of isotope in labile vs fiber fractions would not influence estimates because it is sufficient to know starting quantities whatever the specific value may be, and starting quantities are based on fiber fractions alone, after all labile isotope has been incorporated into structural material. It is not an assumption that label be distributed uniformly throughout all roots, which is a major problem with the isotope dilution method (Milchunas and Lauenroth 1992). Label is preferentially translocated to actively growing sites and is not translocated to roots that have recently died; therefore, it is not possible to obtain a uniform label. However, because the isotope decay method encompasses the time necessary for plant organs to age, die, and decompose, it avoids the necessity of a uniform label. Although herbivory and decomposition are accounted for as parts of the loss process, these processes may not vary from year to year in parallel with production. However, loss rate of the label is an integration over many years. Therefore, annual estimates of production are biased by any degree of nonparallel dynamics between production and herbivory plus decomposition, whereas average annual production is not.

The decreased rate of loss of label we observed many years after pulse labeling suggests that additional assumptions may need to be considered. A slowing of loss rate would result in an increase in turnover time, which would alter the estimate of production if the reduced loss rate were directly associated with the decomposition of plant tissue and not mediated through some indirect mechanism or artifact of sampling. We will take a chronological approach in this paper, showing how questions arose and were tested as results from the long-term dynamics unfolded.

Methods

The study was conducted at the Central Plains Experimental Range (latitude 40°49′N, longitude 104°46′W) in north central Colorado. Mean annual precipitation is 321 mm, with a standard deviation of 98 mm (Lauenroth and Sala 1992). Approximately 71% of the precipitation occurs during the growing seasons from May to September. Mean monthly air temperatures ranged from 22°C in July to below 0°C in January. The vegetation is dominated by *Bouteloua gracilis* (H.B.K.) Lag., and total basal cover is typically 25%–35% (Milchunas and others 1989).

In the summer of 1985, eight 3 m × 3 m plots were pulse-labeled with ^{14}C; each plot was exposed to a total of 2.22×10^8 Bq ^{14}C (6 mCi). Details of the labeling procedures are given in Milchunas and Lauenroth (1992). Each plot was divided into a grid of 64 squares, with five squares in each plot randomly chosen for each sample date. Within each square, two 66.5-mm inside diameter soil cores were removed, one to a depth of 20 cm and the other to 40 cm. Cores were not divided into depth increments, because the small amount of roots at deep depths would not suffice for the number of analyses necessary to address the original objectives. Differences in carbon dynamics with depth are inferred from differences between the 0–20-cm and 0–40-cm samples. Sampling of the long-term plots began in the spring of 1986, after all labile carbon had been incorporated into structural material or respired/exuded (samples from an additional eight plots were used to assess short-term dynamics reported in the 1992 paper). Sample dates reported here were from March and October 1986 and 1987 and then once each year in early spring through 1995, except for 1994.

An unlabeled paired plot approximately 10 m × 10 m was located north of each labeled plot for the purpose of obtaining seasonal root biomass dynamics and estimates of BNPP by conventional biomass calculations (Lauenroth 2000). Five cores to a 20-cm depth (66.5 mm inside diameter) were removed from each of the eight plots once each month through the growing season from 1985 through 1997. These frequent samples were restricted to a 20-cm depth due to regular breakage of expensive large corers when sampled to a 40-cm depth. Estimates of ANPP were obtained by clipping current-years growth from 15 0.25-m^2 quadrats at the time of peak standing crop (late August) each year in the same ungrazed fenced level upland as the labeled and unlabeled cored plots, although these plots were approximately 200 m from the labeled plots.

Core samples were separated into aboveground, crown, and root plant material. Prior to driving the soil cores, each location was scored with the core bit, and aboveground leaf and stem material was clipped. Litter was picked from the area and combined with the other aboveground material. Isotope loss from this combined leaf-plus-litter sample represents the same process as that for crowns and roots—the length of time for transfer of plant organ label to soil organic matter (SOM). Crowns were removed from the scored area by clipping beneath the soil at the crown–root interface. Roots were separated from the soil by the flotation method of Lauenroth and Whitman (1971) using a 0.5-mm sieve, after removing a small subsample of soil for analysis. Roots from the soil subsample were removed by hand-picking larger roots and vacuum-lifting small roots onto veil material; they were then combined with the bulk of roots retrieved through flotation. Subsampling of soil below 20 cm was abandoned after the 1st year because ^{14}C levels were close to background. Samples were oxidized and counted as described in Milchunas and Lauenroth (1992). Root and crown biomass is expressed on an organic matter basis, after correcting for whole-sample ash. Soil samples represent bulk soil. Statistical analyses are described in the Results and Discussion section.

Results and Discussion

Two-phase Dynamics of Isotope Loss

It is clear that different estimates of turnover can be obtained by regressions through different numbers of sampling dates (Figure 1A–D). For plant tissues, it is also clear that a distinct leveling off in ^{14}C mass occurred, with the possible exception of crowns. Soil ^{14}C mass was generally level from year 2 through year 4; then it declined to a second, generally level state from year 5 through year 10 (Figure 2). Whether or not the leveling off in ^{14}C mass of the plant samples can be related directly to tissue label loss or indirectly to tissue contamination or other confounding factors is critical in establishing an appropriate estimator for turnover. We examined several potential confounding factors.

We first describe the method for determining whether one or two distinct processes or phases may statistically be considered to have occurred. We tested for a leveling-off point in the ^{14}C time series by performing two-phase regression tests. This method assesses whether two regression models better explain the variance in a data set than a single model; it also examines which points are included in the first and second phase of the dynamics (the breakpoint defining the two separate dynamics) if two models are appropriate. This is determined by finding the largest F value resulting from performing one regression using the full data set and then comparing it with the F value from a series of two-model regressions over different sequential groupings of points (that is, the left three points and the right remainder, the left four points and the right remainder, and so on). The single best-fitting two-phase model of the several possible two-phase models is then compared to the single model. We found that two-phase models were sig-

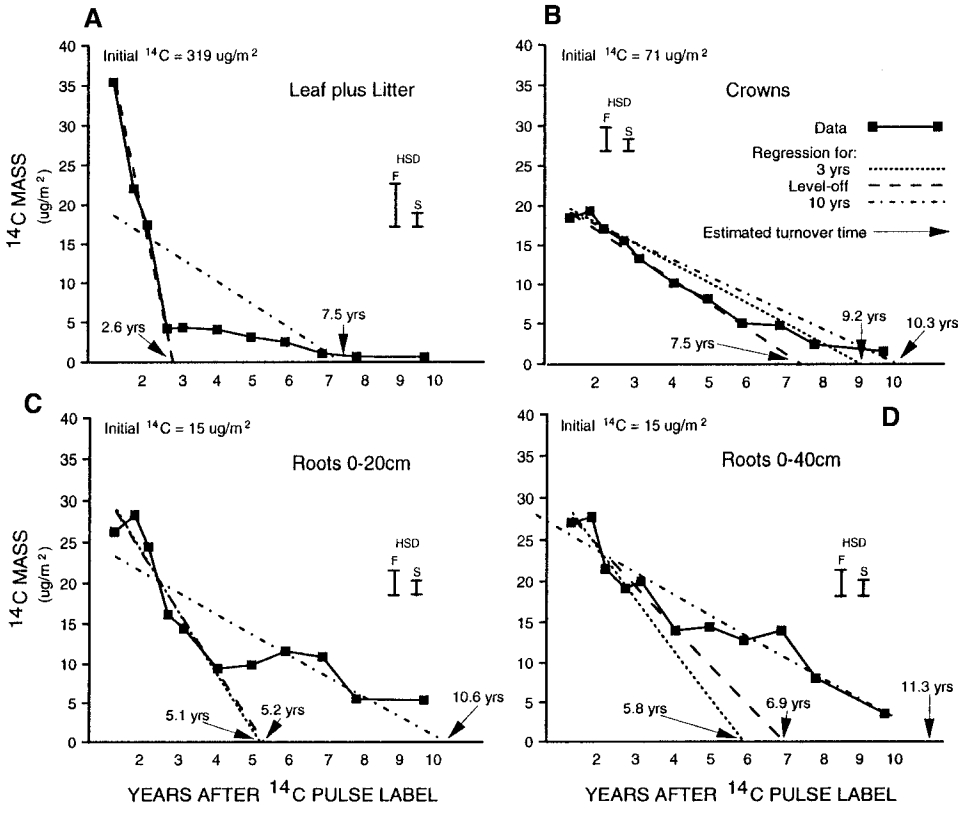

Figure 1. ^{14}C mass loss ($\mu g^{14}C/m^2$) over 10 years post–pulse-labeling and turnover regressions and turnover times for the first 3 years of data, for data up to the time of leveling off in the two-phase dynamics (the point separating initial rapid loss period from a later, slower loss period) and for all 10 years of data. **A** leaf plus litter, **B** crowns, **C** roots from 0 to 20 cm, **D** roots from 0 to 40 cm. Initial ^{14}C is the amount in that tissue component 0.2 day after pulse-labeling. HSD are $P = 0.05$ confidence intervals for the first-phase (F) and second-phase (S) dynamic portions of the data. First-phase dynamics do not include 1st-year data prior to the stabilization of labile ^{14}C.

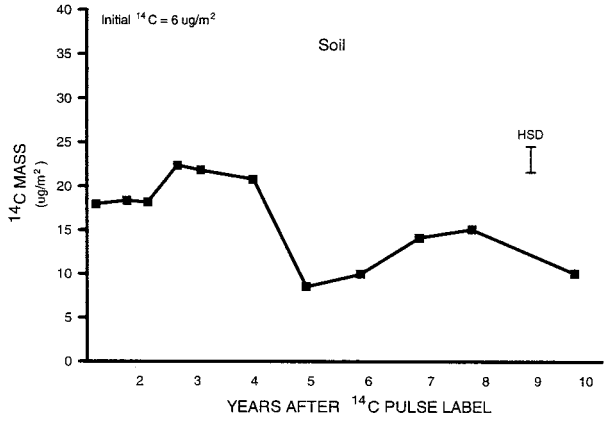

Figure 2. Soil ^{14}C mass ($\mu g^{14}C/m^2$) over 10 years post–pulse-labeling. Initial ^{14}C is the amount 0.2 day after pulse-labeling. HSD is $P = 0.05$ confidence interval.

nificantly better at describing the dynamics of all plant tissue components than the single models. The left-hand portion of the two-phase models is represented in Figure 1 as the "leveling-off" regression (the point separating initial rapid loss period from a later, slower loss period). The number of points comprising the first-phase dynamics is therefore defined statistically and can vary with tissue type, depending on the sampling time when leveling off (second-phase dynamics) began. A single exponential model would not be appropriate in this situation, because the tail may never reach the x-axis (turnover time).

Potential Reasons for the Two-phase Dynamics

Reasons for the two-phase dynamics of ^{14}C loss could include (a) remobilization of structural ^{14}C or retranslocation of labile ^{14}C, (b) inclusion of SOM in plant tissue samples due to recovery through sieving methods, (c) reuptake by plants of ^{14}C containing compounds from the soil (Amiro and Ewing 1992; Jones and Darrah 1992), (d) contamination by soil embedded in roots as they grow, and (e) the possibility that some roots live for a long time, relatively much longer than the bulk of the roots (Eissenstat and Yanai 1997). The following is an assessment of the potential for each of the above to have contributed to the two-phase dynamics.

Retranslocation/remobilization. Retranslocation of labile ^{14}C or remobilization of ^{14}C in fiber fractions could prolong the presence of label when incorporated into new growth fiber fractions some time after pulse-labeling. We attempted to control for retranslocation of labile ^{14}C as a bias in turnover estimates (Milchunas and others 1985) by using data after which cell wall ^{14}C as a percentage of

total ^{14}C had leveled off. To a large extent, this occurred at the end of the 1st year postlabeling (Milchunas and Lauenroth 1992). At this time, from 60% to 80% of ^{14}C was in the residue following neutral detergent fiber (NDF) extraction of solubles (Van Soest 1967). The NDF, acid detergent fiber (ADF), lignin procedure is a widely used method that improves on previous "proximate analysis." However, NDF removes some plant constituents, such as pectins (Van Soest 1975), in addition to cell contents or labile, respirable, and translocatable carbon. Therefore, 100% of the total ^{14}C in NDF residue would not be expected, and we based our turnover start date on leveling-off criteria. Although it is possible that some stored labile ^{14}C was incorporated into structural material after the 1st year, it would be difficult to believe that this could result in the relatively flat long-term second-phase dynamics observed 5–7 years postlabeling. Further, we are not aware of any reports in the literature suggesting that structural material can be remobilized into labile material and then reincorporated into new structural material (similar to the continual breakdown–rebuilding of muscle protein in animals), with two possible minor exceptions. Cellulases could be involved in cellulose or hemicellulose degradation in abscission zones of deciduous leaves (Osborne 1973), and cleavage and resynthesis of linkages in cell walls may be involved in changes in cell wall plasticity (Lamport and Miller 1971). Although we cannot totally disregard this as a possibility, it is unlikely that these mechanisms could remobilize sufficient label to account for the two-phase dynamics.

Inclusion of SOM. There is a problem associated with all methods that entail physical retrieval of roots through sieves: sieve mesh size defines the separation between what is considered root biomass or SOM (Hook and others 1994). Flotation/wet sieving generally retrieves more roots than dry sieving/hand-picking (Bohm 1979) but these samples may also contain more detrital SOM, which can have a long turnover time and contribute to the slow second-phase dynamics we observed. Sieve sizes finer than 0.5 mm capture a large percentage of fine-root biomass, but they also trap a large amount of SOM in the form of detritus (Bohm 1979; Amato and Pardo 1994). In soil studies, common methods of SOM fractionation include a portion of root material in the coarse particulate organic matter fraction of the soil (Cambardella and Elliott 1994). There is overlap between root and SOM in both root and soil studies, with a tradeoff in root studies between capturing a larger proportion of the more rapidly turning over, very fine root biomass and the bias of including a SOM component. Material collected on fine sieves may represent a portion of SOM that does not correlate with current rooting distributions of live plants (Hook and others 1994). This could influence the long-term dynamics of material recovered from labeled plants simply because samples include a portion of ^{14}C in SOM with a different loss rate.

We tested the possibility that detrital SOM contamination was responsible for the slow second-phase dynamics. Root samples collected in year 8 were floated out from the bulk soil as usual but then hand-separated into either "obvious root segments" (most probably live) or "other" material that included unidentifiable detritus, dead or decayed small root fragments, and material too fine to separate. There were no significant differences (by one-way analysis of variance [ANOVA]) between the ^{14}C in the two types of root materials for either 0–20- or 0–40-cm sample increments, although the mean ^{14}C mass for 0–20-cm detrital material was higher than for obvious roots (Table 1). We had expected much lower or no ^{14}C in what was mostly live roots 8 years after labeling. The similar values for obvious "live root" vs "other" suggests that inclusion of detrital SOM in tissue samples due to recovery through sieving methods was not the single factor in the second-phase dynamics, because obvious root segments without detrital SOM also contained label.

Detrital SOM could be eliminated as a potential confounding factor in labeling studies by separating live roots or all roots from detrital material. However, this may not be feasible in some systems. Prior to initiation of this experiment, we tested the Congo red dyeing method of live–dead root discrimination. This dye has previously been reported to be successful in aiding separation, because it results in a darker coloration of dead roots (Ward and others 1978; van der Maarel and Titlyanova 1989). An area of approximately 1 m^2 was scraped and kept clear of vegetation for 2 months; then both this area and an adjacent live one were cored. Roots from both areas were treated with the dye and examined under a dissecting scope. Although a minor portion of new root growth and a minor portion of unknown material were respectively lighter and darker than the majority of roots in the live treatment, no clear differences in color between the live vs dead area samples could be discerned for the majority of root mass. Roots of the dominant *B. gracilis* in this semiarid environment are all of a very small diameter (Lee and Lauenroth 1994) and drought-resistant in nature (Lauenroth and others 1987), which may make them less amenable to

Table 1. ^{14}C Mass of Root Tissue Sampled 8 Years after Pulse-labeling and Hand-separation into Either Obvious Live Root Segments or Other Material and of Dead Leaves plus Litter and Live Leaves Sampled 10 Years after Pulse-labeling

Plant Material	^{14}C Mass ($\mu g^{14}C/m^2$)	(Standard Deviation)
Belowground 1993		
Obvious large live roots 0–20 cm	2.30	1.18
Detritus, fine or dead roots 0–20 cm	3.24	1.32
Obvious large live roots 0–40 cm	4.05	1.00
Detritus, fine or dead roots 0–40 cm	4.02	1.22
Aboveground 1995		
Dead leaves plus litter	0.63	0.39
Live leaves	0.001	0.0005

No significant difference was observed between root tissue types, and no significant difference was observed between live leaves and background activity.

dyeing methods. Further, the relatively very fine diameter of all roots makes root/detrital separation impractical in this particular plant community. In any plant community, distinguishing live from dead roots involves a degree of over- or underestimation (Eissenstat and Yanai 1997), and visually judging when a root becomes SOM will always be very qualitative.

Reuptake from soil. We tested the possibility of reuptake by plants of compounds containing ^{14}C from the soil. In year 10, samples of obviously new live leaves were collected from the plots in early spring, in addition to the usual aboveground sampling. Live leaves have minimal soil contamination compared to roots and are easily distinguished from detrital material. Dead leaves plus litter (containing soil contamination) had higher activities than live leaves. ^{14}C in live leaves could not be distinguished from levels found in live leaves outside the labeled plots (Table 1). This eliminated the possibility of reuptake of ^{14}C-containing compounds from the soil, unless carbon sink strength of roots exceeded that of new leaves. We would then have to assume that labile carbon in roots does not "circulate" within the plant, but translocation is continually unidirectional from leaves down to roots.

Soil contamination. Soil is inevitably embedded in roots as they grow and press through dense soil. Some SOM pools have long turnover times, and new unlabeled roots growing into soil containing ^{14}C years after all labeled roots had decomposed could become contaminated. Ash contents in this experiment typically were 30% to 40% of total weights, even though roots were floated (washed) from bulk soil. Obvious "live roots" did not differ from "other root/detrital material" in ^{14}C content (Table 1), indicating that live roots, and not just older detrital SOM, contributed to the slow second-phase dynamics.

We attempted to adjust for ^{14}C in root ash to test for contamination. We multiplied the ash content of the roots by the mass of ^{14}C/g of soil and subtracted this from root ^{14}C contents. The assumption in this correction is that embedded soil, as well as other internal elements of roots not volatilized during ashing, has the same ^{14}C-specific activity as bulk soil. This is likely to be a conservative adjustment, because rhizosphere soil may contain more labeled carbon than bulk soil (Milchunas and others 1985), the ash content of soil would weigh less than bulk soil since carbon is volatilized in ashing, and the smaller amount of internal mineral ash would not be as great as the first two factors.

Regressions for turnover times using the soil-adjusted root ^{14}C masses lowered the estimated turnover times of roots (x-intercept) but did not completely bring the temporal leveling-off phase in ^{14}C values to background (Figure 3). Adjusting turnover times for soil ^{14}C embedded in root material compared to unadjusted values resulted in turnover times for 0–20-cm roots of 4.1 (adjusted) and 5.1 years (unadjusted), respectively, and turnovers for 0–40-cm roots of 5.4 (adjusted) and 6.9 years (unadjusted). Crowns and aboveground leaf plus litter were not adjusted for soil contamination, because applying 0–15-cm increment soil values to surface soil did not seem appropriate. Adjusted values for the second, leveling-off phase of aboveground leaf plus litter fell well below zero (figure not shown). The ^{14}C mass in aboveground leaf plus litter during the leveling-off phase was

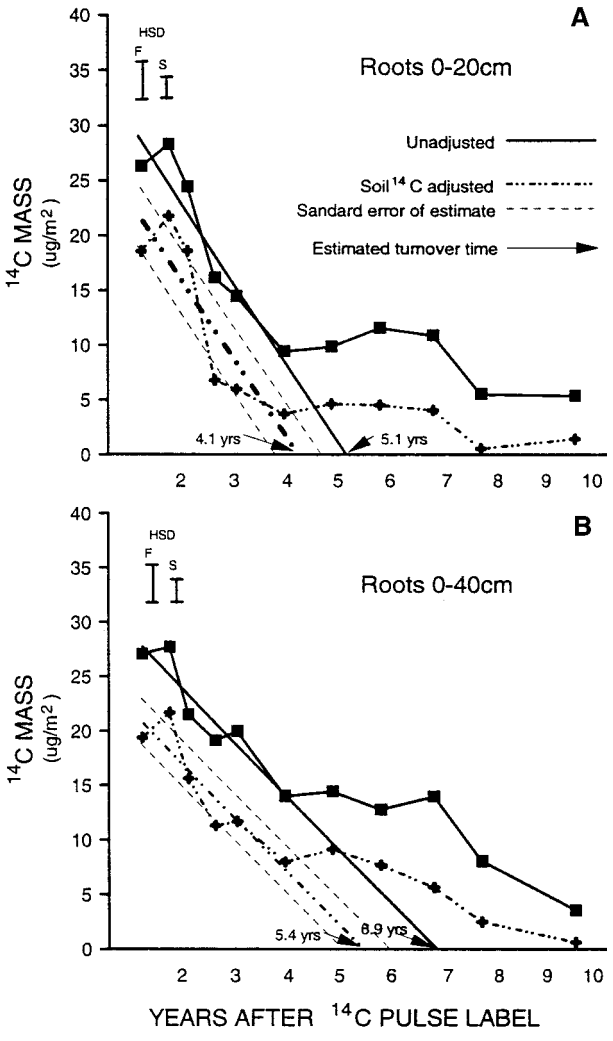

Figure 3. ^{14}C mass loss ($\mu g^{14}C/m^2$) over 10 years post–pulse-labeling for root data unadjusted and adjusted for soil ^{14}C embedded in root tissue (ash), with their associated turnover regressions and turnover times. The standard error of estimate is for the soil ^{14}C adjusted regression with $r^2 = 0.90$ for (**A**) 0–20-cm roots and $r^2 = 0.89$ for (**B**) 0–40-cm roots. HSD are $P = 0.05$ confidence intervals for the first-phase (F) and second-phase (S) dynamic portions of the unadjusted data. First-phase dynamics do not include 1st-year data prior to the stabilization of labile ^{14}C.

relatively small compared to initial values and compared to variance in the data.

Correction of root activities for soil contamination was based on the ^{14}C content of bulk soil. Corrections based on rhizosphere soil could potentially further lower estimates of root ^{14}C contents. Although their estimates were made less than 1 year after labeling, when a rhizosphere effect would be expected to be large, Milchunas and others (1985) found 90% of ^{14}C in soil was associated with the rhizosphere in a greenhouse pot experiment. If we assume that adjustment of root ^{14}C contents for rhizosphere levels of ^{14}C and for potential SOM recovered with roots during sieving would lower the leveling-off phase to background, then turnover times for both 0–20- and 0–40-cm roots would become 3.8 years, as compared to 4.1 and 5.4 years for level-off-point regressions, respectively. This represents the x-value of the lowest point of the first-phase dynamics.

Long life of a few roots. Alternatively, the leveling off could be due to the very long life span of a few major roots. Working within the same exclosure, Liang and others (1989) found that approximately 20% of the roots to a depth of 100 cm were greater than 2 mm in diameter. These may represent the long-lived component of the root system. The presence of similar levels of isotope in "obvious live" and "other" root separations (Table 1) leaves open the possibility that the slow loss of isotope during the second-phase dynamics is due to the long life span of a small proportion of roots. In contrast to the possible rhizosphere–soil contamination effect, which would lower estimates of turnover time, a long life of a few roots would mean that estimates based on the first-phase dynamics would slightly underestimate turnover times. Estimates would need to be based on separate two-phase dynamics rather than an exponential decay curve, because the latter would mean that the turnover time (x = intercept when y = 0) would be greatly extended due to a few roots, while this turnover time would be applied to total root biomass. BNPP estimates based on two separate pools (fast and slow) could be obtained if the two pools were physically distinct, such as fine (less than or equal to 2 mm) and coarse (greater than 2 mm), and were analyzed separately at each sample date.

Estimates of Production by Different Methods

How do adjustments to ^{14}C turnover regressions affect estimates of BNPP, and how do these compare to BNPP estimated by traditional methods? Estimates of BNPP were calculated using the sum-of-increments method with core biomass data, using ^{14}C turnover times from 10 years of data adjusted and unadjusted for soil ^{14}C embedded in root material and using data published after only 4 years and unadjusted for embedded soil ^{14}C (Table 2). In the sum-of-increments method, increases in biomass from one particular time to the next are summed within a year and decrements are ignored. Ignoring decrements gives this method a positive bias (Persson 1978; Singh and others 1984; Lauen-

Table 2. Root Production and Root Biomass Average, Minimum, and Maximum Estimates Based on Annual Averages for 1985–97

	Root Production ($g \cdot m^{-2} \cdot y^{-1}$) Estimated by:						
	Harvest Sum of Increments Using Statistical Constraint			^{14}C Turnover Using Regression			
	HSD	STD	None	Adjusted	Unadjusted	Original	Root Biomass (g/m^2)
Average	178	213	364	223	179	183	915
Minimum	0	0	81	189	152	155	774
Maximum	1003	1159	1376	255	205	209	1046

Harvest sum-of-increments method was calculated using HSD (ANOVA and Tukey's mean separation test), STD (one standard deviation), and no statistical constraints on positive increments in biomass.
^{14}C turnover estimates were calculated from regressions using 10 years of data adjusted and unadjusted for soil ^{14}C embedded in root tissue (ash) and 4 years of unadjusted data as originally presented in Milchunas and Lauenroth (1992) and applied to annual biomass estimates from 1985 through 1997. Respective ^{14}C turnover time estimates were 4.1, 5.1, and 5.0 years.

roth and others 1986; Sala and others 1988). Whether or not an increment is included in the total may be determined by various statistical tests of significance. BNPP estimates by this method depended strongly upon the statistical test used to determine whether or not increments in biomass were added. There were wide ranges in minimum and maximum annual estimates when using the sum-of-increments method, with minimum estimates of BNPP of zero when either conservative or liberal statistical constraints were used. An estimate of zero BNPP was obtained in 8 of 13 years when using the conservative ANOVA-HSD constraint (see Table 2), and in 6 of 13 years when using a standard deviation. This occurred even though each data point represented a very large sampling effort (40 large cores with a 66.5-mm inside diameter) in a system that is relatively lawnlike compared with many others (see Figure 1 in Milchunas and Lauenroth 1992, Milchunas and Lauenroth, 1989).

Estimates of ^{14}C turnover depended on whether the regression used to estimate turnover time was from data adjusted or unadjusted for soil ^{14}C embedded in root material. Little difference in estimates of BNPP was observed based on 4 years of unadjusted data and 10 years of unadjusted data, when the 10-year data regressions were applied through the declining, first-phase dynamics of ^{14}C mass loss (183 and 179 g/m^2, respectively) (Table 2). However, estimates adjusted for soil ^{14}C embedded in root material averaged 25% higher than unadjusted values.

Making the assumption that the second-phase dynamics represent an embedded soil fraction places a lower bound on the estimate of turnover; the upper bound in the case of this study is the bulk soil ^{14}C adjusted regressions. The upper bound could potentially be lowered or eliminated if rhizosphere soil ^{14}C activities were known. BNPP estimates based on the lowest point of the first-phase dynamics (that is, we drop the data straight down to where the lowest point is at $y = 0$) were approximately 8% higher than the estimates based on the bulk soil ^{14}C adjusted regression for 0–20-cm roots. However, the difference between the estimate of turnover time was only 0.3 year, as compared to a 1.6 year difference for 0–40-cm roots.

Estimates of aboveground production by ^{14}C turnover remain the same as the original 4-year estimates. Even a full adjustment to zero isotope mass at the end of the first-phase dynamics would have very little effect on the turnover estimate, because of both the steep slope of first-phase loss for this component and the small quantity of ^{14}C remaining during the second-phase dynamics. The ^{14}C turnover estimate of ANPP averaged 109 $g \cdot m^{-2} \cdot y^{-1}$, as compared to 91 $g \cdot m^{-2} \cdot y^{-1}$ based on harvest of peak standing crop. Harvest of peak standing crop underestimated ANPP by 16% compared to estimates based on ^{14}C turnover, which is reasonable considering that harvest of peak standing crop misses a small amount of production by early, cool-season forbs in this system and there can be a small amount of regrowth after clipping at peak crop. Crowns represent the second largest component of biomass (430 g/m^2 average); they are positioned approximately equally above- and belowground but contribute only 57 $g \cdot m^{-2} \cdot y^{-1}$ to production due to very slow turnover times. Previous estimates for crown production at this short-

grass steppe site, based on summation of statistically significant increases in biomass during two growing seasons, averaged 225 g m^{-2} y^{-1} (Sims and Singh 1978). This especially large discrepancy between estimates based on ^{14}C turnover and the traditional coring method suggest that overestimation errors due to a statistical artifact in summing only increments in growth (Persson 1978; Singh and others 1984; Lauenroth and others 1986; Sala and others 1988) are more likely to occur when production is low compared to the standing stock of biomass, as suggested by Sala and others (1988).

Semiarid grasslands, such as the shortgrass steppe, are considered to be dominated by belowground inputs of organic matter to soil. This perception may be due to the very low aboveground-to-belowground biomass ratios (see Long-term Biomass Dynamics below). Previous aboveground-to-belowground production estimates averaged 0.41, based on summation of increments in biomass by 0–10-cm increments in soil depth to 30 cm for roots (Sims and Singh 1978). Estimates from this study using similar methods of calculation averaged 0.51 using conservative statistical constraints and 0.25 using no statistical constraints. Aboveground-to-belowground production ratios averaged 0.49 based on ^{14}C decay, indicating a contribution of aboveground biomass to total productivity of approximately one-third.

We calculated root turnover for adjusted values according to the method used by Gill and Jackson (2000) (production/maximum standing crop) to compare values from this shortgrass steppe site with other grassland/shrubland sites compiled in their review. Root turnover from our site was 0.213; this figure was similar to other reports based on isotope decay or dilution: 0.231, Missouri tallgrass prairie (Dahlman and Kucera 1965); 0.314, New Zealand *Agrostis/Lolium* (Saggar and others 1997); 0.21, Utah *Atriplex* (Caldwell and others 1977); 0.123, Utah *Ceratoides* (Caldwell and others 1977); and 0.489, Missouri tallgrass prairie (Buyanovsky and others 1987). Root ingrowth estimates of root production ranged from 0.015 for a Poland *Dactylis/Arrhenatherion* community (Szanser 1997) to 0.531 for a *Cholochloa* community in Manitoba, Canada (Neill 1992, 1994). Estimates based on minirhizotrons/rhizotrons were either much higher or lower than isotope methods: 0.673, central Netherlands *Deschampsia-Molinia* (Aerts and others 1992); 0.634, UK *Festuca-Nardus-Juncus* (Fitter and others 1997); 0.135, Alaska *Carex-Eriphorum* (Miller and others 1980); 0.116, Alaska *Carex-Dupontia* (Shaver and Billings 1975). This variation may have been due to large temperature differences among the communities. Preliminary data from minirhizotrons at our shortgrass steppe site also suggest a long turnover time (Figure 4), but not as long as that estimated by isotope decay. The estimate for turnover for this site based on the Gill and Jackson (2000) prediction model for grasslands is 0.43 (Gill personal communication), as compared to our estimate of 0.213. However, their model is heavily based on coring studies. Coring studies can produce estimates of

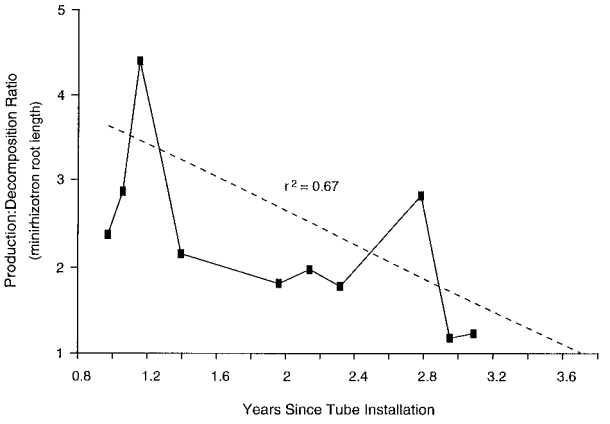

Figure 4. Preliminary data for production:decomposition ratios from minirhizotrons at the Central Plains Experimental Range shortgrass steppe site. Tubes were installed approximately 1 year prior to first sampling. An oscillating equilibrium about production (new root length):decomposition (root length loss) ratio of one has not occurred over 3 years. Regression suggests an equilibrium intercept (turnover time) of 3.7 years, as compared to an isotope decay turnover time of 5.4 years for 0–40-cm roots.

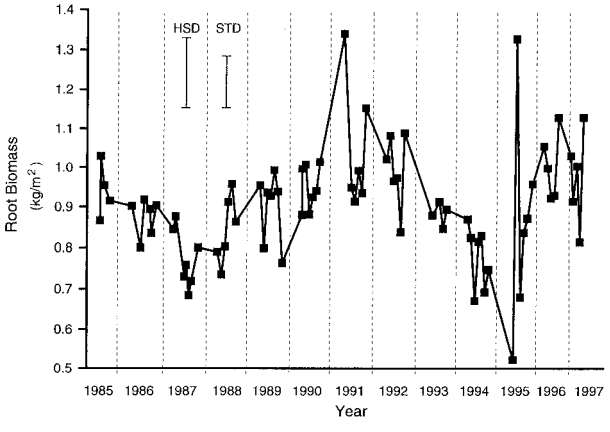

Figure 5. Seasonal dynamics of root biomass (kg/m^2, 0–20-cm depth) from 1985 through 1997. HSD represents a conservative confidence interval; STD is a standard deviation.

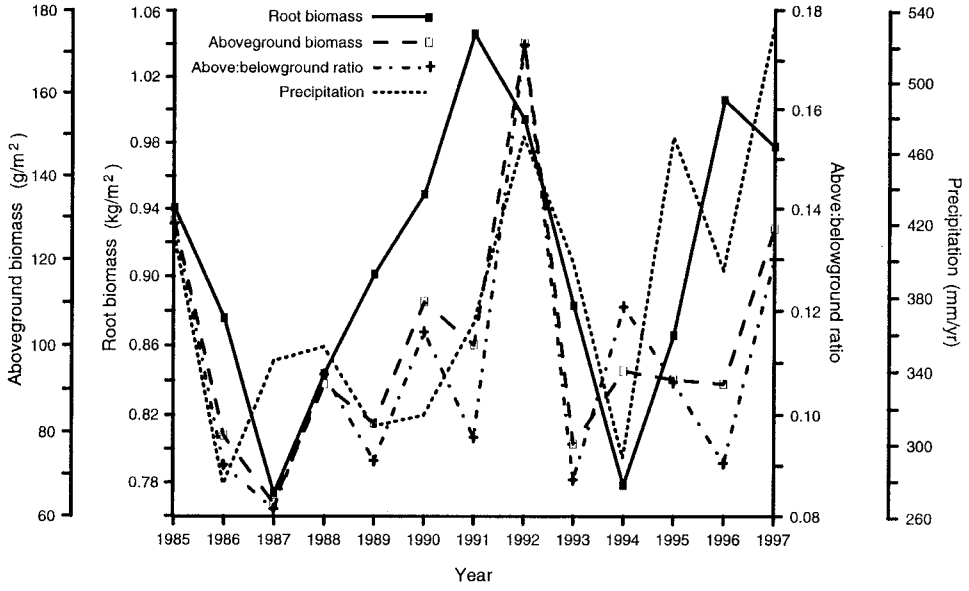

Figure 6. Annual root biomass (kg/m^2, 0–20-cm depth), aboveground biomass (g/m^2, peak standing crop), aboveground-to-belowground ratios, and precipitation (mm/y, October through September for 1985–97. Root biomass data are the annual means from the seasonal data in Figure 5.

zero production (as in 6 of 13 years in this study) to overestimates of 130% (Sala and others 1988). The discrepancy between isotope decay and model turnover is likely to be the result of the model representing an average over many sites. Our preliminary minirhizotron data suggest that turnover estimates may be shorter than those based on isotope decay.

Long-Term Root Biomass Dynamics

The large number of zero estimates of BNPP by harvest sum of increments calculations suggests that periods of root growth are often synchronized with decomposition, yet the periodic high estimates indicate that artificial peaks due to sampling variance may often appear. Temporal dynamics of root biomass within a year were statistically relatively flat, with periodic high peaks and low troughs (Figure 5). There was no consistent within-year seasonal pattern and no consistent increase or decrease over the winter period between years. There were, however, distinct cycles in longer-term root biomass dynamics.

We examined whether the long-term cycles in root biomass were related to aboveground biomass dynamics and how they related to precipitation. Although aboveground peak standing crop tracked annual precipitation to some degree, temporal dynamics of annual root biomass displayed a looser relationship with precipitation than did aboveground biomass (Figure 6). Temporal dynamics of root biomass had a relatively smooth temporal trend, whereas aboveground biomass displayed sharper responses to annual fluctuations in precipitation. Aboveground-to-belowground biomass ratios ranged from 0.08 to 0.17 and were generally higher during periods of greater precipitation. Correlations between biomass and precipitation were 0.57 for aboveground and 0.44 for belowground biomass. The correlation for aboveground:belowground biomass ratios with precipitation was 0.5. Only the correlation between aboveground biomass and precipitation was significant ($P < 0.05$). Root biomass was significantly correlated with aboveground biomass ($r = 0.57$, $P < 0.05$).

The long-term root biomass dynamics in this shortgrass steppe highlight two interesting points. First of all, it is often assumed that spring and autumn are the periods of maximum root growth, as plants grow rapidly in the spring and translocate to belowground organs in fall. Cool temperatures during these periods would not favor rapid decomposition rates, because temperature is a primary rate-regulating factor (Berg and others 1993; Kirshbaum 1995). One would therefore expect to observe seasonal patterns in root biomass dynamics, but no consistencies were observed over the 13 years of this study. Second, it is also sometimes assumed that plants allocate more carbon to roots than to shoots during dry periods, because a greater water limitation necessitates greater exploitation of the soil volume. This was generally, but weakly, confirmed in our study.

Conclusion

In summary, estimates of belowground net primary production from isotope decay regressions of turn-

over times need to be adjusted for soil ^{14}C embedded in plant tissue. Turnover time estimates are variously sensitive to this adjustment, depending on the slope of the decomposition regression and the quantity of ^{14}C in soil material representing a relatively slow second phase of loss dynamics. Adjustment based on rhizosphere soil rather than bulk soil may prove useful to more accurately separate plant tissue from soil–organic matter dynamics. Particulate SOM included in root–tissue samples due to sieving procedures, or a long life span of a small proportion of roots, may additionally account for the observed two-phase dynamics. Both soil factors would tend to cause a positive bias in turnover estimates and lead to an underestimate of BNPP. Basing isotope decay regressions on live roots or roots further sorted from sieved material may additionally reduce bias for systems in which this is possible. Estimates of BNPP using the isotope decay method appear to be more reliable than estimates obtained using traditional harvest methods. In an area of research where absolute values are unknown and bounded by relatively wide confidence intervals, comparison of results from multiple techniques will provide important comparative information for interpreting estimates arrived at by any particular technique. Combining isotope decay and minirhizotron technologies may be fruitful in this regard.

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