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Estimating Root Production: Comparison of 11 Methods in Shortgrass Steppe and Review of Biases

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Abstract

Estimating root production has been difficult due to multiple potential biases associated with both old and new methods. This shortgrass steppe site is the only place we are aware of that can compare most methods including sequential coring, ingrowth cores, and ingrowth donuts, ^{14}C pulse-isotope dilution, ^{14}C pulse-isotope turnover, rhizotron windows, and minirhizotron, and indirect methods including nitrogen budget, carbon flux, simulation carbon flow model, and regression model. We used the studies at this site, other comparisons, a summary of potential directional biases, and different ways of calculating estimates in a logical, comparative approach of evaluating methods. Much of the literature for root production is based on sequential biomass coring, a method resulting in erroneous estimates. Root ingrowth estimates of production are generally conservative compared to minirhizotron and isotope turnover methods. The size of the ingrowth area may be the most important determinant of the underestimation. Estimates based on pulse-isotope dilution are also erroneous due to non-uniform labeling of tissues. Uniform labeling is not an assumption of the pulse-isotope turnover method, and this method has the least severe potential biases. Root production estimates from pulse-isotope turnover were lower than those using minirhizotron when the most common method of calculation was used. This agrees with literature concerning bomb ^{14}C continuous-isotope labeling comparisons with minirhizotron, although some potential biases between isotope methods are different. However, good agreement between pulse-isotope turnover and minirhizotron were obtained when minirhizotron estimates were calculated from regression of decomposition versus production to equilibrium and when pulse-isotope turnover estimates were calculated from two-phase life-span regressions. This minirhizotron method bypasses biases associated with the artificial surface similar to root-cohort methods that may be practical only in mesic systems, and takes into account both short- and long-lived roots and corrects for soil-isotope contamination that the continuous-isotope labeling bomb ^{14}C method is not able to account for. Comparisons of these direct methods are also made with four indirect methods.

Key words: belowground net primary production (BNPP); carbon flux; excavation window; isotope decay; minirhizotron; nitrogen budget; root coring; root ingrowth cores and donuts; root longevity; root turnover; root decomposition.

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Introduction

New methods for estimating root production or belowground net primary production (BNPP) have only recently been developed in response to a general acknowledgment of serious biases and problems associated with traditional sequential coring of biomass methods. The new methods also possess potential biases which may lead to under- and overestimates. Because of the number of new methods and their potential differences, Arnone and others (2000) proposed that general conclusions concerning the effects of elevated CO_2 on root production and aboveground versus belowground allocation could not be made based on a review of field studies. Hendricks and others (2006) indicated that numerous investigators (Hendricks and others 1993; Nadelhoffer 2000; Norby and Jackson 2000) have hypothesized that contrasting theories of soil resource controls on aboveground to belowground allocation may in part be caused by differences in methods used to assess root production.

The problem with evaluating any BNPP method is that the true answer is never known. Two approaches have been taken in our attempts to evaluate root production methods; direct tests of potential errors within a method and comparisons among methods. Tierney and Fahey (2001) evaluated several assumptions of the minirhizotron method and presented an excellent discussion of some of the current issues with this methodology. Steingrobe and others (2000, 2001) examined soil conditions and root disturbance effects of the ingrowth core method of root production, and Milchunas and Lauenroth (1992, 2001) and Swinnen and others (1994a, b, c, 1995) evaluated several factors influencing estimates by isotope-pulse labeling. In the second approach, Hendricks and others (2006) working in forests presented perhaps the only multi-method, multi-site comparison, and reviewed 18 additional sites that compared biomass coring with one other method (see their Table 5). Tierney and Fahey (2001) compared estimates of fine-root production obtained with minirhizotrons with earlier ones from ingrowth cores, and cite Swinnen and others (1994c) as the only study they knew of at that time that compared the minirhizotron with a non-coring method. Similarly, Nadelhoffer and Raich (1992, Table 1) show 18 forest communities where more than one method has been used, but all of these comparisons include one with biomass coring. Comparisons among methods at the same site when absolute values are not known can be a basis for reconciling directional biases among methods and thereby improve ways of obtaining and calculating estimates within a method and in synthesizing literature using different methods. Hendricks and others (2006) state that ''it has been widely suggested that multiple assessment techniques should be employed as a means of corroborating estimates, or, in the absence of corroboration, of evaluating the shortcomings of each method.''

One of the objectives of this article is to compare 11 distinct methods of estimating BNPP that were obtained at the same shortgrass steppe research site. Seven of the methods are direct methods whereby some aspect of roots are measured in the field, and four of the methods are indirect whereby root production is calculated based on various non-root parameters. The methods include traditional biomass coring, isotope-pulse labeling to obtain both isotope turnover and isotope dilution estimates, root ingrowth concentric coring and root ingrowth donuts, minirhizotron, excavation window, indirect field methods of nitrogen budget and carbon flux, and indirect methods based on simulation modeling and regression modeling. Although all method estimates were obtained at the same research site in the same plant community, locations and years sometimes vary, but estimates are in most cases for multiple years. There are obvious weaknesses and strengths in this broad approach. Ways of calculating BNPP estimates from the same method/data are also compared for coring, minirhizotron, and isotope decay methods. Different ways of calculation even within a new method can determine the particular set of assumptions involved in that estimate, and are an important way of improving estimates within a technique (Tierney and Fahey 2001). A second objective of this article is to compare methods for estimating several root growth and biomass parameters in relative terms independent of their absolute root production relationships. Relative rather than absolute values may be acceptable for answering some questions for some research purposes. A third objective of this article is to compile a comprehensive list of potential positive, negative, and unknown directional biases associated with direct methods of estimating root production. Although there are several excellent recent reviews of root methods (Fahey and others 1999; Lauenroth 2000; Hendricks and others 2006) directional biases are addressed to a different extent, and new insights or problems appear as the use of new technology increases. In this area we focus on new methods that are promising and particularly on direct rather than indirect methods. We use hereafter the term root production because in forests and large-shrub communities many methods assess only fine root production, whereas

in grasslands fine root production and BNPP are essentially the same.

Methods

All studies were conducted at the Central Plains Experimental Range (CPER) (40°49′ N, 104°46′ W) in north central Colorado. Mean annual precipitation is 321 mm, with 71% occurring during the May through September growing season (Milchunas and Lauenroth 1992). Mean monthly air temperatures range from 22°C in July to below 0°C in January. All studies of direct methods (excluding models and balance methods) were in a plant community dominated by the warm-season grass *Bouteloua gracilis*, and all studies were on level upland topographic positions. However, studies were at two locations (except for the root ingrowth by concentric coring method) and the years in which the studies were conducted varied. Site one encompasses root production estimates reported in this article for isotope decay, isotope dilution, excavation window, and all traditional coring methods. Site two encompasses root production estimates reported in this article for minirhizotron and root ingrowth donuts, and for all data used for assessing comparisons of relative values. The following data for aboveground net primary production and root biomass are provided to link the two sites and the years that are being compared. Average aboveground net primary production (ANPP) for the period over which root production estimates by direct methods in this article were obtained was 91 g/m^2/y at site one (standard deviation 16, Milchunas and Lauenroth 1992) and 86 g/m^2/y at site two (standard deviation 25, Milchunas and others 2005b). Root ingrowth by concentric coring occurred during only two of the five years of the latter period, and ANPP for those two years averaged 60 g/m^2/y. ANPP at the excavation window root production site was 62 g/m^2/y for the one year of study. Root biomass data to 20 cm depth for 13 years at site one averaged 870 g/m^2 (225 average standard deviation) and for the five years at site two averaged 913 g/m^2 (272 average standard deviation). For the common five years that root biomass data were collected, and in the same month of collection, the average annual value was 917 (min. 744, max. 1148) g/m^2 at site one and 913 (min. 570, max. 1540) g/m^2 at site two. Therefore, sites and periods were considered similar for method comparisons except for ingrowth by concentric coring and the period for excavation window where ANPP was lower (these two methods are later dropped from consideration for other reasons). Further, from 4, 5, 10, to 13 years of data were used for most method comparisons (except for the two listed above, and see Table 1).

Details of all methods can be found in the articles cited below and in tables; we here provide only condensed necessary pertinent information. Some small differences in depth of measurement among studies were normalized to a standard of 40 cm deep using a long-term 10-year biomass mean (Milchunas and Lauenroth 2001) and depth increment percentages based on 5 cm incremental sampling (Leetham and Milchunas 1985); this assumes that production or turnover is constant among depths and follows biomass distributions. Root biomass depth distributions were practically identical for site one and site two.

Direct Methods

Isotope Decay

Eight 3 × 3 m long-term sampling plots were pulse labeled twice (June, July) by covering with tents with fans for 2 h after releasing 1.1 × 10^6 Bq ^{14}C each labeling. Plots were sampled over the subsequent 4 years (Milchunas and Lauenroth 1992) and through 10 years post labeling (Milchunas and Lauenroth 2001), starting the second year after allowing all labile C (soluble C) to be incorporated into structural tissue (a critical assumption of both isotope methods). Dried roots were floated onto a 0.5-mm mesh sieve, oxidized into CO_2 traps, mixed with cocktail, and activity determined by scintillation counting. Biomass was ash corrected to organic matter basis in this and all following studies. Turnover times for roots (as well as crowns and aboveground) were determined by regression of ^{14}C mass remaining over time and using the *x*-intercept as the turnover time estimate. Production was calculated as average biomass for the year divided by the number of years for complete turnover.

Isotope Dilution

This method is based on the reduction of the ratio of $^{14}C/^{12}C$ after ^{14}C-labeling when plants are assimilating primarily ^{12}C. The same raw data as above were used, but the turnover coefficient was calculated as $TC = [(^{14}C_{t1}/^{12}C_{t1})/(^{14}C_{t2}/^{12}C_{t2})] - 1$, where t1 and t2 are different sampling dates (Caldwell and Camp 1974). Production = TC * biomass time 1.

Traditional Biomass Coring

A paired plot for each of the eight replicate ^{14}C plots above was established and cored on average

six times per year over 13 years (five cores each plot each date as above). Root production was calculated by summing positive increments in biomass between dates, using conservative (ANOVA/HSD), more liberal (standard deviations), and no statistical constraints.

Minirhizotron

The minirhizotron method was used, and the root ingrowth donut method was developed, during an open-top chamber CO_2 experiment that used three replicate blocks of non-chambered control and chambered ambient and elevated CO_2 treatments, where two minirhizotron tubes were installed in each at a 23° angle to the surface of the year prior to the start of treatments to allow for equilibration (Milchunas and others 2005b). Imaging was done on four to five dates from April through October each year for five years, with sampling frequency based on the very slow 5.4 year turnover observed in this semiarid system using the ^{14}C decay methodology. Video images were taken using a Bartz BCT-100X camera system with a square indexing handle, and processed using RooTracker software (David Tremmel, Duke University, Durham, NC) for diameter, length growth, or loss between dates, and disappearance recorded for individual roots.

Ingrowth Donut

This method was developed to minimize destructive sampling within the limited space of CO_2 treatments, and to obtain newly grown roots under the treatments for chemical analyses in contrast to multi-age-class roots from coring (Milchunas and others 2005a). Donut refers to the ring, or geometric toroid shape when viewing the concentric circles from above the ground. Two holes of 20.3 cm outside diameter were created in each of the nine treatment-replicates by driving steel cylinders into the ground to a depth of 40 cm with a front-loader tractor and removing them and the soil. The surfaces of the smooth-faced sides of the holes were lined with ''plastic canvas'' fabric as the ingrowth cloth. Steel cylinders of 15.2 cm outside diameter were placed in the middle of the holes and filled with sand bags. This created a donut space of 2.54 cm width between the cylinder and the ingrowth cloth which was filled with root-free sifted soil from an adjacent area, and packed to similar bulk density as outside soil. Roots were sampled by removing the sand bags, lifting out the cylinders, and cutting the root and soil donut away from the mesh surface. The same donuts were refilled with new root-free sieved soil annually each October. Root biomass was processed as described above. This method has several assumptions different from ingrowth concentric cores (see Results and Discussion) and is considered a separate method here. The donut method provides a relatively large surface area exposed to ingrowing roots compared to smaller cores.

Ingrowth Concentric Core

In two years in early May, 7.62 cm internal diameter cores were excavated at two locations in each of four replicate plots, and refilled with root-free sieved soil collected at the site (McCulley and others 2005). Ingrowth samples were collected each year the following October by driving a 4.8 cm internal diameter core in the center of the larger root in-growth hole. Samples were wet-floated onto a 0.147 mm sieve, which is smaller than the 0.5 mm used in all other sieving work reported here, but detrital material was hand picked out. Problems associated with this difference are discussed later.

Excavation Window

Four large square holes were excavated and five glass plates 91 by 102 cm placed against a smoothed surface at a 10 degree incline from the vertical whereby the glass slanted in at the top (Ares 1976). The glass was braced against the soil surface and covered with insulating foam sheets and black plastic when not being measured, and the hole covered with plywood. The length, growth, and loss of roots were recorded by obtaining photographs during dark hours at a distance of 0.4 m from the glass surface using a wide-angle lens, and projecting images onto a white board for drawing and measurement. Installation of windows was completed by February 1973, and samples were obtained at 7–14 day intervals on 11 dates between April 22 and August 14 of 1973. Therefore, there was essentially no equilibration period before starting measurements. Estimates of turnover were based on the reported range of 30–60% root-loss/y, normalized to standard depth and biomass as described above.

Indirect Methods

Nitrogen Budget

This method was developed by Aber and others (1985), Nadelhoffer and others (1985), and Nadelhoffer and Raich (1992) as an alternative to biomass coring methods that were known to have serious errors and biases. The method is based on

calculating the annual allocation of N to roots based on other N fluxes in the system, and dividing this by the root-N pool to obtain an estimate of root-N turnover that is assumed to equal root biomass turnover. The equation using annual allocation estimates in $g/m^2/y$ is: root turnover = ([net N mineralization + N input in precipitation − N lost to leaching] − [leaf litter N + non-leaf-litter N + perennial tissue N])/(total standing root N pool). Two root production estimates were calculated: the low estimate uses plant and litter N data from Schimel and others (1985) for the four latter variables in the above equation, with roots to 20 cm depth separated from soil by flotation through 1 mm sieve. The high estimate is for plant and litter N data from King and others (2004), with roots to 60 cm depth separated from soil by hand picking. Roots were normalized to 40 cm depth as described above. N-mineralization is from Schimel and others (1985). N in precipitation is from the National Atmospheric Deposition Program web site for total N for 2004 for data collected at this site. Leaching and allocation to perennial wood was assumed to be zero in this arid grassland. The problem of perennial crowns in grasslands is addressed in the Results and Discussion section. In the N-budget method for forests, it is assumed that N losses to the atmosphere from volatilization are small and negligible, or offset by fixation (Nadelhoffer and others 1985), which was tested here for grasslands where grazing by domestic livestock results in volatile losses from urine and feces, animal harvest, and animal transport of N may have an impact on N-budgets.

Carbon Flow or Balance

This method was developed by Raich and Nadelhoffer (1989) and Nadelhoffer and others (1998) also as an alternative to traditional biomass coring methods, and was considered to be an estimate of the upper-bounds of root production. The equation on an annual carbon basis is: root carbon allocation = soil respiration − aboveground litter fall + (leaching, erosion loss + change in root C − change in soil C). All data were from the CO_2 study site non-chambered controls described above. Soil respiration estimates were from Pendall and others (2003) and aboveground litter production estimates were from Morgan and others (2004) assuming all herbaceous ANPP becomes litter over winter in this temperate grassland, and using the standard biomass to carbon conversion constant. All other variables were considered at steady state (see Lauenroth 2000).

Regression Model

A regression model for global application was developed by Gill and others (2002) to estimate root production from commonly available environmental variables based on root biomass data from the literature and a derived root turnover coefficient. The equation for predicting root biomass is: root biomass = (0.79 * aboveground peak standing crop new growth [or ANPP]) − [33.3 * (mean annual temperature + 10)] + 1290 g/m^2, and root production = root biomass * root turnover, where turnover = $0.2884e^{0.046 \text{ mean annual temperature}}$.

Simulation Model

The estimate presented here is from a precursor to the widely used CENTURY model. Inputs to the model were largely from data collected during the International Biome Project, and processes included net photosynthesis, translocation to crowns and roots, death of plant parts, production of litter, respiration, and decomposition (Parton and others 1978).

Results

Across all methods the estimates of root production for shortgrass steppe ranged from a minimum of −349 to a maximum of +1699 $g/m^2/y$ for isotope dilution and coring sum of increments, respectively (Table 1). Traditional coring sum of increments also gave minimum values of zero when statistical constraints were used to define a significant increment. The carbon flux method gave high estimates relative to others. All other methods gave estimates ranging from 52 to 720 $g/m^2/y$. Root production estimates derived from simulation and regression models were similar to those obtained using minirhizotron and isotope decay, except for minirhizotron when using the average length calculation method.

A comparison of root production estimates from root ingrowth concentric coring, root ingrowth donuts, and minirhizotron from this shortgrass steppe site appear rather contradictory (Table 1). Root production estimates by ingrowth concentric coring were much higher than ingrowth donuts, and these were higher and lower, respectively, than those by minirhizotron. However, the ingrowth concentric coring authors used a 0.147 mm mesh sieve to retrieve roots, whereas all other studies at this site used a 0.5 mm mesh sieve. This later discrepancy could be a large component of the difference between estimates by

Table 1. Mean, Maximum, and Minimum Estimates of Root Production ($g/m^2/y$ Organic Matter) for Shortgrass Steppe Based on Various Methods and Ways of Calculating Data

Method	Mean	Min	Max	Years and (N)	Study
Coring sum of increments					
HSD statistics	220	0	1238	1985–1997 (13)	Milchunas and Lauenroth (2001)
STD statistics	263	0	1431	1985–1997 (13)	Milchunas and Lauenroth (2001)
No statistics	449	100	1699	1985–1997 (13)	Milchunas and Lauenroth (2001)
Pulse-isotope turnover[1]					
Not soil ^{14}C adjusted[2]	170	145	194	1986–1995 (10)	Milchunas and Lauenroth (1992, 2001)
Soil ^{14}C adjusted[2]	217	184	248	1986–1995 (10)	Milchunas and Lauenroth (2001)
Two-phase life span[3]	185	157	211	1986–1995 (10)	calc. from Milchunas and Lauenroth (2001)
Pulse-isotope dilution[1]	321	−349	627	1985–1988 (4)	Milchunas and Lauenroth (1992)
Root ingrowth concentric coring	472	460	485	2000–2001 (2)	McCulley and others (2005)
Root ingrowth donut	93	52	113	1998–2001 (4)	Milchunas and others (2005a)
Decomposition adjusted[6]	118	66	144	1998–2001 (4)	calc from Milchunas and others (2005a, b)
Minirhizotron					
By max length[7]	191	87	320	1997–2001 (5)	Milchunas and others (2005b)
By avg length[7]	414	254	720	1997–2001 (5)	Milchunas and others (2005b)
By regression[7]	160	96	288	1997–2001 (5)	Milchunas and others (2005b)
Rhizotron excavation window[4]	527	351	703	1973 (1)	Ares (1976)
Early simulation model of carbon flow[5]	189	155	223	– (2)	Parton and others (1978)
Regression model (global environmental variables)[4]	210	188	307	3 different models	Gill and others (2002)
Nitrogen budget[4]	65–315[5]		–		Calc. from data in King and others (2004); Schimel and others (1985); NADP[5]
With gaseous and animal fluxes	61–304				See text for additional references
Carbon flux[4]	1107		–		Calc. from data in Pendall and others (2003); Morgan and others (2004)

All values normalized to a depth of 40 cm based on biomass of 1170 g/m^2 from Milchunas and Lauenroth (2001) and depth distribution from Leetham and Milchunas (1985).
[1]^{14}C was used in all isotope work at this site, but it is assumed that ^{13}C could be substituted, although backgrounds levels would be much greater and more variable than for ^{14}C.
[2]Adjusted or not adjusted for ^{14}C contamination in soil embedded in roots. Most isotope studies do not adjust for this factor (see ''Discussion'' section). Difference between these estimates and those reported in Milchunas and Lauenroth (1992, 2001, Table 2) are because values here are for 0–40 cm depth data and using the standard 1170 g/m^2 as in all calculations in this table, and those used for comparison with biomass coring in the earlier publications focused on the 0–20 cm depth.
[3]See Figure 1C. Calculated using 2/3 of roots in phase 1 with a turnover time (X-axis intercept) of 5.4 years and 1/3 of roots in long-lived phase 2 with turnover time of 9.6 years, and based on soil ^{14}C corrected data. This double regression method was used to better represent the two different life-span groups than could a single regression.
[4]Calculated using data from citations and additional details in methods section.
[5]See ''Methods'' section.
[6]Adjusted for a root disappearance rate of 27% based on minirhizotron numbers of roots lost within 365 days of birth (see ''Discussion'' for details).
[7]Both maximum length and average length methods use new root length growth plus either average root total length or individual root maximum length during the annual period to calculate turnover. The regression method uses the regression of new growth:loss ratio, that is, the time for these two processes to equilibrate after tube installation.

the two ingrowth methods, eliminates the concentric core data from direct comparison, but also underscores the need for standard methods in all root separation from soil studies in order for reasonable comparisons to be made (Table 2 hand pick versus wet sieved). It is possible that the size differences in the ingrowth areas of the concentric core and the donut studies also contributed to differences in the estimates. The ingrowth donut method produced conservative estimates relative to minirhizotron and isotope turnover (Table 1), but relative treatment estimates were similar between ingrowth and minirhizotron under identical experimental conditions (Table 2).

The method of calculation resulted in a 28% difference between average estimates within the isotope turnover method, and 159% difference within the minirhizotron method (Table 1). Within a method of calculation, differences between minimum and maximum annual values were also greater for minirhizotron compared to isotope turnover. Mean values for the minirhizotron calculated by the regression method and the isotope turnover calculated by the two-phase life span method were very close, differing by less than 16%, and these two methods both use a regression approach. The isotope turnover two-phase life span estimate is adjusted for both soil isotope contami-

Table 2. Relative CO_2 Treatment Effects within Various Methods for Root Growth or Standing Biomass

Variable-Method*	CO_2 treatment						Reference
	Control	Ambient	Elevated	Ratio A/C	Ratio E/C	Ratio E/A	
Root length growth (mm/tube/y) By Minirhizotron	369	372	565	1.01	1.53	1.56	Milchunas and others (2005b)
Root biomass growth (BNPP g/m^2/y) By Ingrowth donut	93	101	152	1.09	1.63	1.63	Milchunas and others (2005a)
Root biomass (g/m^2) By Hand-picked[2]	623	574	710	0.92	1.14	1.24	King and others (2004)
Root biomass (g/m^2) By Wet-sieved[2]	943	949	1006	1.01	1.07	1.06	LeCain and others (2006)

Estimates of BNPP by minirhizotron are different from those obtained by ingrowth donut (Table 1) but relative CO_2 treatment effects are similar. Root biomass by hand picking is lower than by wet sieving, and results in different relative CO_2 treatment effects.
[1]Growth and BNPP are averages of 5 years for minirhizotron and 4 years for ingrowth donuts.
[2]Biomass hand-picked from large cylinders was from late autumn of year 5 of CO_2 treatment and wet-sieved monolith from early spring of year 6.

nation and is based on two regressions, one for short-lived roots and another for long-lived roots (Figure 1A, C). The minirhizotron regression method is the only one of the three estimates that is not based only on new-length growth and some estimate of total length present to obtain turnover and does not require equilibration of new root growth and old root loss, but is based on calculating the time necessary for these two processes to equilibrate and considers this the turnover time (Figure 1B, Table 1). The most common method of calculating minirhizotron data, the average length method, uses new length growth and average total length present during the year to obtain turnover. This method resulted in substantially higher estimates of root production than the minirhizotron by regression of new growth:loss ratio method and isotope turnover methods. Differences among methods of calculation in both minirhizotron and isotope data are described in more detail in the ''Discussion'' section.

Discussion

The approach in this section will be to combine the differences among methods at this shortgrass steppe site and other comparisons reported in the literature with a compilation of known biases of each method (Table 3). Where possible, the direction of each bias within each method has been listed in Table 3 for comparison with estimates of root production among the methods to establish which particular biases may be generally more or less important. The order of methods in the discussion will be to first eliminate a method with the most apparent problems. Order is also based on a focus on direct rather than indirect methods.

Isotope Dilution

The negative values for isotope dilution are clearly nonsensical (Table 1), and led Milchunas and Lauenroth (1992) to suggest abandonment of this method. The critical assumption for the dilution method from pulse labeling that is likely not to be met is that of attaining a uniform labeling of tissue age classes. This is not an assumption for isotope decay from pulse labeling (Table 3) or bomb-atmospheric isotope labeling. However, isotope dilution is used in a variety of non-root-production methods, and should be viewed with caution if the same assumption is necessary as is in this case.

Traditional Biomass Coring

Very high values from biomass coring methods are predicted from analytical and modeling assessments of estimates because decrements in biomass between dates are ignored (Persson 1978; Singh and others 1984; Lauenroth and others 1986; Biondini and others 1991; Sala and others 1988). Zero values occurred in 8 out of 13 years based on a long-term coring data set (Milchunas and Lauenroth 2001), and have been reported by other researchers (Hansson and Andrén 1986; Kurz and Kimmins 1987; Fogel 1990). The critical assumption that is not met in biomass coring methods is the independence in timing of production and decomposition (Table 3); both occur simultaneously to various extents and can lead to low or zero values. Elimination of zero values can be achieved only by use of no or very liberal statistical constraints (Table 1), but this means the values are based on noise. The potential for high values has been mathematically proven, and is more likely where production is low compared to standing

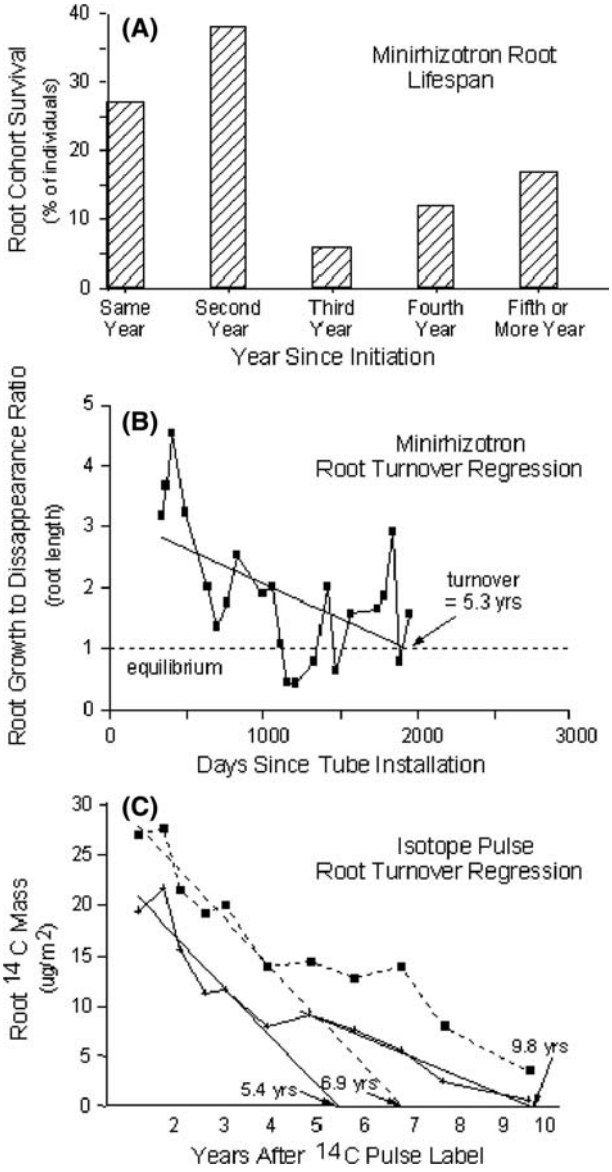

Figure 1. **A** The proportion of roots (%) initiated during one year that disappeared that year or in subsequent years through five years of tracking cohort survivorship using minirhizotron. **B** Root turnover based on minirhizotron equilibration of root growth-loss. **C** Root turnover based on regression calculations from ^{14}C loss after pulse labeling. *Dashed lines* represent data and regression for roots ash corrected, but not corrected for ^{14}C in the ash embedded in the roots. *Solid lines* represent data and regressions corrected for ^{14}C in the ash embedded in the roots and divided into two separate regressions for a relatively rapid loss of label and a second distinctly slower phase of isotope loss. Turnover times for all regressions are estimated as the *X*-axis intercept. **A** and **B** from Milchunas and others (2005b) and C from Milchunas and Lauenroth (2001).

biomass (Sala and others 1988). Thus, although average values may appear reasonable, the zero values and proven artificially high values certainly cast doubt on bases for a valid mean, and biomass coring methods should also be abandoned as estimators of root production. There are two important deductions from the above. A large proportion of current root-production literature is still based on biomass coring, but is useful only for the biomass data. Other sequential sampling approaches, such as for aboveground production by movable cages, are also subject to positive bias due to ignoring decrements while summing increments (McNaughton and others 1996).

Carbon Flow or Balance

The high value obtained with the carbon flow method (Table 1) is to be expected. Developers of this method suggest it as a way of placing upper bounds on root production for a particular community, and it has been used to examine some important cross-site comparison questions and global syntheses on total belowground carbon flux (Raich and Nadelhoffer 1989; Nadelhoffer and others 1998; Litton and others 2007; Litton and Giardina 2008). Hendricks and others (2006) reported annual belowground-C allocation values that were higher than even total-biomass production values (not just C) for roots based on minirhizotron estimates. There are a number of potential biases associated with this method (Table 3), possibly the greatest of which is the difficulty in obtaining accurate annual estimates of soil respiration (Fahey and others 1999). Potential biases of this method will not be discussed further here because the focus of this article is primarily on direct methods. However, one aspect of this method not addressed in previous literature is the issue of crowns of grasses. Crowns are the perennial organs that are the interface between aboveground leaves and stems and belowground roots and are approximately half above- and half belowground. The carbon flow method is a balance approach that was developed for forests, where grasses may or may not be a significant component, and subtracts off aboveground leaf-litter production. Annual production or loss of crowns may be more difficult to estimate than that of roots, because estimates may only be made accurately by isotope methods (see discussion of crowns below in isotope section). Not accounting for perennial

Table 3. Root Production Methods and their Potential Known Biases and the Direction of Over- or Under Estimation

Method	Factors causing positive bias	Factors causing negative bias
	Factors causing unknown or potentially either biases	
Minirhizotron		
	length/diameter primary units, not biomass; production requires conversion	
	initial plant injury	
	root architecture influence on interception of plane: + or - depth dependent	
	modified temperature/light along tube	
	equilibration periods differ with system; low initial decomposition and greater initial growth	
	tube bending altering single location calibration	
	digitizing tedious, with potential id/drawing error	
	tube or soil movement; + loss, turnover	
	tube separation from soil; + growth	
	growth along artificial plane; + length	
		growth after last sample grazed/decomposed before next sample
Root ingrowth concentric core and mesh bag		
	low competition in root-free soil	
	root proliferation after severing	
	cloth may restrict entry by herbivores larger than mesh	
		grazing and decomposition loss between sampling periods
		size of ingrowth area outside spatial range of roots, soil volume not at equilibrium with outside in terms of new growth
		mesh and mesh size constraint to root entry
	missing center and coring into non-root-free filled area	
	sifting soil may alter nutrient mineralization rates and microbial activity	
	soil bulk density difference, and that effect on soil water dynamics	
	root architecture influence on interception of plane: + or - depth dependent	
	altered profile horizons	
	requires root separation from soil, errors depend on method	
Root ingrowth donuts		
	same as other ingrowth above except without: there is no outer uncored area, and no additional coring required post-instillation	
^{14}C or ^{13}C turnover		
	label in solubles must be fixed in structural tissue prior to starting estimate; + label translocation to root, and respiration losses, negative if decomposition of label during period	
	age class and quality of tissue labeled is constant thru time (a uniform label at pulse time is Not an assumption)	
	estimate an integration over several years	
	requires root separation from soil, errors depend on method	
		labeled soil contamination
Coring sum of increments		
	unreliable, but biases listed due to common use of this method	
		production-decomposition simultaneous
	liberal statistical constraint	
	biological and sampling variance accumulates due to using only positive increments	
	grazing loss not accounted for	
	cannot separate live from dead or species accurately, which can affect peaks/troughs	
	requires root separation from soil, errors depend on method	
Excavation window		
	same as minirhizotron except: temperature light effects greater, plane for architectural bias is vertical, replication difficult, more of process is manual	
Nitrogen budget		
	accurate estimates of N-mineralization, leaching, atmospheric deposition,	
	and allocation to perennial tissue all difficult and each with many potential biases	
	requires root separation from soil	
	all measured mineralized N taken up by plants	
	N limits growth	
	steady state conditions	
	retranslocation of N from senescing roots is low	
Carbon flux		
	this method is considered an upper limit estimate only	
	accurate estimates of annual soil respiration difficult	
	all assumptions involved in estimating aboveground annual inputs	
	respiration includes root and secondary trophic faunal not related to primary tissue fluxes	
	steady state	
^{14}C dilution		
	Unreliable due to requirement of uniform isotope labeling of tissue age classes	

Magnitudes of errors are not implied. The number of potential biases is not indicative of accuracy because magnitudes differ and can be additive or offsetting. An estimate of exudation and sloughing inputs is only possible with isotope techniques.

crowns can be part of the reason for overestimations by this method in some ecosystems. The high estimates obtained by this method for other reasons would pose problems for comparing root production *across methods* within forests, and the importance and variability of crowns in grasslands may limit *within method* comparisons of grasslands as has been done for forests.

Nitrogen Budget

The N-budget estimates for root production at this site (Table 1) vary only due to the two different sources of estimates for aboveground and root N concentration and biomass. Estimates of atmospheric inputs, leaching losses, and volatile losses may not be available for many sites, and estimates of N-mineralization are subject to a number of problems (Ruess and others 1996; Fahey and others 1999; Hendricks and others 2006). Atmospheric inputs can be large compared to other fluxes (Burke and others 2008). Volatile losses are usually assumed to be small (Nadelhoffer and others 1985) and estimates are often lacking for particular sites. Even when these factors are held constant there can still be a range of variability in estimates of root production due to estimates of root N concentration (Aber and others 1985; Nadelhoffer and Raich 1992; Lauenroth 2000). Clark (1977) estimated that live roots in shortgrass steppe may generally have an N concentration of 1.1%, but detrital and senescent roots may reach 2.5% N. Milchunas and others (2005a) observed new roots growing into ingrowth donuts ranged annually from 0.9 to 2.5% N. Roots obtained by coring contain a mix of old and new roots, and the proportions and N concentration of each may vary seasonally.

In grasslands, large herbivores can affect nitrogen fluxes through volatilization from urine and feces and through animal harvest (Lauenroth and Milchunas 1991). To the calculation of root production by the N-budget method, we added the following fluxes in units of g $N/m^2/y$: −0.4 as N_2, −0.22 as NO_x, −0.0142 as N_2O, −0.2 as NH_3 from senescing vegetation, −0.01 as NH_3 from animal urine volatilization, and −0.087 from animal harvest (Schimel and others 1985; Lauenroth and Milchunas 1991; Mosier and others 2008; Burke and others 2008). Adding these fluxes had little effect on estimates of root production. All the above fluxes add to less than the additional loss from animal redistribution of N out of uplands to water tank, fence-line, and so on areas (−1.09 g $N/m^2/y$—Senft 1983). This would have a similar, small, but additive effect if included in the estimates in Table 1 for volatile losses.

There is a problem of a potentially large crown component in grasslands, as described above for the carbon flow method. Crowns are perennial organs as are tree stems and trunks. Measuring annual N allocation to crowns may be similarly difficult to estimating annual N allocation to woody biomass in forests. It is possible that crown increments could be estimated by changes in tiller density. However, the root production estimates for the nitrogen budget in Table 1 include crowns in the belowground root estimate. Placing crowns with the belowground component is just as appropriate as including it in the aboveground component, but means that the low estimates in Table 1 must be particularly low relative to other methods that do not have crowns included in the root estimate.

Regardless of the potential biases examined above, the N-budget gave reasonable estimates of production in this shortgrass steppe ecosystem. However, the low estimate was lower than those for minirhizotron and isotope decay, and the high estimate was higher than for the other two methods except for the minirhizotron when calculated by average root length. This raises questions concerning estimates of root production based on the N-budget method, and estimating N-mineralization and plant uptake have been raised by others as problems in this indirect method (Ruess and others 1996; Hendricks and others 2006).

Modeling by Regression or Simulation

It is noteworthy that estimates by these methods at this shortgrass steppe site were generally comparable to those obtained using minirhizotron and isotope decay (Table 1), especially those using preferred methods of calculation (described below). This is also interesting from a historical perspective in the case of simulation modeling. The simulation model used (Parton and others 1978) was a precursor to the widely used CENTURY model (Parton and others 1987, 1988). Estimates of root production available at that time were based on the biomass coring method, and the biomass coring estimates averaged a high of 422 $g/m^2/y$ (Sims and Singh 1978) compared to the model estimate of 189 $g/m^2/y$. The model estimates indicated a problem with coring methods. A regression model was developed by Gill and others (2002) based on a global data set on root biomass and commonly available environmental variables and a derived root turnover coefficient. For the regression model, the shortgrass steppe site was used as an indepen-

dent validation site for the overall model. Although independent, this may have had some influence on the choice of the sub-method that estimated root production at 210 g/m^2/y, for inclusion in the final recommended model, out of three potential sub-methods.

Root Ingrowth Concentric Coring and Root Ingrowth Donut

The root ingrowth method was developed by Hendrickson and Veihmeyer (1931), Lund and others (1970), Persson (1978, 1979), and Steen (1983, 1991). In the traditional method, a mesh bag is placed in a cored or augered hole and filled with soil sieved free of roots. After a period of time when new roots grow into the mesh ''stocking,'' near-surface roots are cut free and deeper roots removed by pulling the mesh stocking out or by complete excavation of the stocking. Soils in native grasslands and shrublands are usually too hard (compared to tilled cropland or high organic matter forest soil) for such removal by pulling, making complete excavation difficult and a concentric coring method has most often been adopted (See ''Methods'' section and Jordan and Escalante 1980; Neill 1992; McCulley and others 2005). The root donut method was developed for use in studies with limited sampling space for long-term destructive sampling (such as elevated CO_2 sites), and to span a larger representation of areas under and between plants than that represented by a single point-in-space core (see ''Methods'' section and Milchunas and others 2005a). New root growth into the donut comes from a surface directly next to the undisturbed soil, unlike the sampling of a smaller area than that filled when using the concentric coring method. The donut method utilizes the same hole/location multiple years, whereas new holes are installed for each time period sampled in the concentric core method.

Root ingrowth biases must have been perceived to be more serious than traditional sequential biomass coring methods, or the majority of root production estimates in the past may not have been done by the biomass coring method. Both root ingrowth methods are less labor intensive than sequential biomass coring because sampling is less frequent with the former. No waiting period for equilibration is necessary as for the minirhizotron method (but see below), waiting for stabilization of labile carbon as for the isotope decay method, or long period of sampling as for the isotope decay method (sometimes many years, see descriptions below). In addition, ingrowth methods require no specialized equipment; making it inexpensive as well as fast. The bias most often indicated in the literature as a potential problem is an unusually high proliferation of new root growth into the competition-free space resulting in an overestimation of root productivity (Table 3). Sifting of the soil to remove roots may alter subsequent N-mineralization and microbial activity. The artificial repacking of the soil may alter bulk density and may not reconstruct the natural profile changes with depth. All these factors may cause bias in production estimates in either direction and of an unknown amount. Several other problems are similar to many other methods. For example, new growth after the last sampling date that undergoes decomposition or herbivory before the next sampling will be missed. This is the same with the minirhizotron method, but not isotope decay, although increased frequency of sampling is more feasible with minirhizotron.

There are two other potential problems with root ingrowth methods that are overlooked in many reviews. One concerns an architectural bias. This was pointed out in reference to minirhizotron tubes (Pages and Bengough 1997) rather than with respect to ingrowth structures, and probably varies with depth in the profile and may act differently in the two methods. These biases are due to different probabilities of roots that tend to grow in particular directions intercepting a surface oriented or placed in a particular angle (Figure 2). Another often-not-mentioned potential bias of ingrowth methods concerns the sensitivity of the estimate to the relationship between size of the root-free ingrowth volume and the spatial and temporal range of roots (but see Jordan and Escalante 1980; Hertel and Leuschner 2002; Milchunas and others 2005a). This can best be portrayed using a hypothetical exaggeration. If my root-ingrowth area is the area of a house in a grassland with small individuals, then roots will never reach the center of the area and there will always be competition-free space where water and nutrients may move from, but productivity per unit area will still be calculated based on the entire ingrowth area, thereby resulting in an underestimation of productivity. To place this in a more practical framework, the larger the size of the ingrowth area, and the smaller the spatial range of roots and/or speed of expansion, the greater the area and or longer the time that incoming roots are in a competition-free unoccupied zone. Hertel and Leuschner (2002) compared root ingrowth cores (outer-filled and inner sampled the same diameter) with a large ingrowth chamber method and concluded that the size of ingrowth

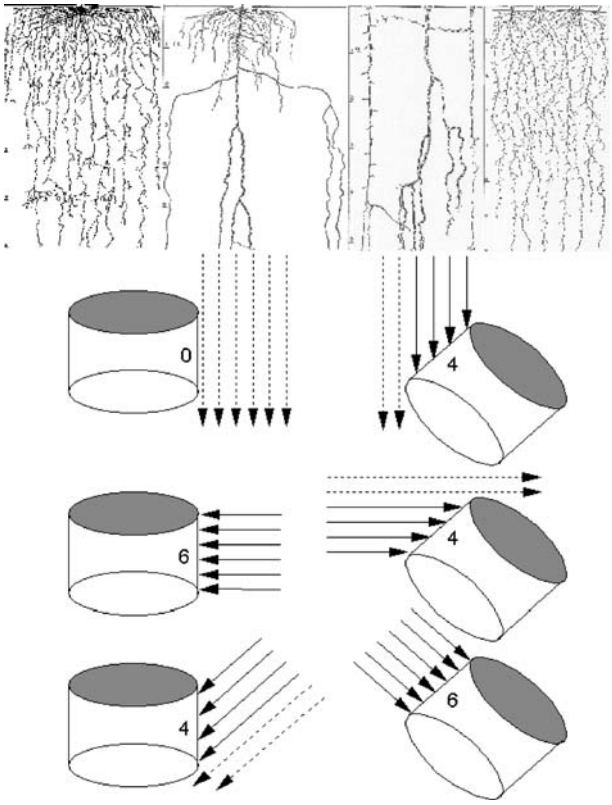

Figure 2. Root architectures of various forb species and illustration of probability of roots growing in different horizontal–vertical directions intercepting a surface positioned at different angles. Root diagrams from Weaver (1958) from left to right for *Gutierrezia sarothrae*, *Chrysopsis villosa*, *Echinacea pallida*, and *Aster ericoides*. The first two species have a large proportion of horizontally growing roots, whereas the latter two show relatively greater vertical growth direction. Numbers on cylinders representing either minirhizotron tubes or root ingrowth cores are the intercepts out of a possible evenly spaced six.

area used was an important factor. The smaller cores gave a larger estimate of production than the larger chambers.

Another potential factor causing bias in the root ingrowth method concerns a possibility for roots to proliferate after being severed during instillation (see references in Joslin and Wolfe 1999). ''Root pruning'' of woody perennials in horticulture is done to improve re-establishment after transplanting by stimulating new root growth (Geisler and Ferree 1984), and most studies have been on woody species. In a study in wheat and barley, root growth patterns were not affected by cutting injury to roots during installation of the mesh bags, based on examination of root-length-density around mesh bag walls and bulk soil and on different acclimation periods after disturbance prior to opening bags to ingrowth (Steingrobe and others 2001). A marsh grass study by Neill (1992) suggests just the opposite to a proliferation after severance effect. Estimates of root production from the sum of several short-term ingrowth bags (representing multiple episodes of root severing) were less than estimates from a single long-term bag, suggesting that the disturbance inhibited proliferation. If decomposition had been a predominant factor in the long-term bags, then the long-term bags would have had lower values. Hertel and Leuschner (2002) observed that it took 12 months after installing ingrowth cores before any new ingrowth occurred in an old-growth forest. This suggests a delay in root growth after disturbance rather than proliferation after severing, and has also been reported by Vogt and others (1998) in forests. These observations are opposite from that observed by Hendricks and others (2006), where short-term ingrowth cores gave consistently higher estimates of production than long-term ingrowth cores at several forest sites. Bias due to severance disturbance and that due to decomposition between sampling intervals may offset and vary with species and environment.

Studies and cross-method comparisons have assessed the magnitude of the above potential problems, although both are rather limited given the length of time ingrowth methods have been available. Most comparisons have been between ingrowth and biomass sequential coring, which we will not examine here. Steingrobe and others (2000) directly tested several assumptions of the ingrowth method in oilseed-rape crops using 4 cm diameter ingrowth bags inserted at a 45° angle. These authors found that lower soil bulk density inside ingrowth cores than outside did not affect root production values, but higher inside bulk densities reduced ingrowth. Adding P inside ingrowth cores did not affect root growth rates, but additional available N increased ingrowth root length density. Soil inside ingrowth cores appeared to quickly adjust to outside soil moisture, whereby short-term root growth did not differ with initial moisture differences. However, several studies clearly indicate that opposite or similar results are possible for some of the above. Increased root growth into favorable microsites of increased nutrient availability and reduced competition has been clearly demonstrated (Fitter 1976; Eissenstat and Caldwell 1988). On the other hand, a lack of response in root expansion into competition free space has also been reported for the dominant perennial grass at this shortgrass steppe site,

based on ^{14}C labeling of individual plants with and without neighbor removal (Coffin and Lauenroth 1991).

There are a few papers that compare a root ingrowth method with a different non-biomass coring method or direct method at the same site or study. Steele and others (1997) reported production values for ingrowth by concentric coring that were up to two to four times less than for minirhizotron. However, the inner ingrowth cores were half the diameter of the original cored holes that were filled with root-free soil which could result in an underestimation by this method, and minirhizotron tubes were only left for four months to equilibrate before starting measurements which could result in an overestimation bias by that method (Table 3). Ingrowth cores that were left in one year and others for two years (both estimates of root production calculated on an annual basis) gave estimates of production that were greater, equal, or less depending on plant community. It is not clear whether the one versus two years in the ground estimate differences were due to abiotic differences between years, plant community differences in decomposition within periods, or rates of expansion into the smaller inner concentric sampling area. Higgens and others (2002) found that the concentric core ingrowth method (6 cm outer vs. 2.3 cm inner core) yielded estimates of root production that were 2- to 3-fold greater than peak-standing-crop of root biomass, whereas minirhizotron estimates of root production were only about 50% greater than peak-crop biomass. This result of greater production by ingrowth compared to minirhizotron is opposite from the Steele and others (1997) result. Minirhizotron tubes in the Higgens and others (2002) study were allowed to equilibrate for 8 months, but problems were reported with tube contact with soil and production was based on new-length growth. These authors suggested that differences between ingrowth and minirhizotron methods could be due to artificially low root turnover near the minirhizotron tubes or stimulated production into ingrowth cores, and speculated that the latter was more likely. Tierney and Fahey (2001, 2002) and Fahey and Hughs (1994) observed that root production estimates by root ingrowth concentric coring (outside 5 cm inside 1.9 cm) were lower than those from minirhizotron. Independent methods indicated significant decomposition of roots over short periods in this northern hardwood forest, which is common in many other mesic systems. Estimates of root production by standard ingrowth were 254 g/m^2/y compared to 508 g/m^2/y when corrected for decomposition between periods (Fahey and Hughs 1994). Tierney and Fahey (2001) considered a best estimate based on minirhizotron at this site was 303 g/m^2/y, which was higher than the ingrowth method but not as high as the decomposition corrected value. These authors used root longevity rather than root length data from the minirhizotron to estimate productivity to avoid the potential bias of growth across the artificial plane of the tubes. This important concept is evaluated further in the minirhizotron section below. Hendricks and others (2006) provide the most thorough cross-site evaluation of ingrowth concentric coring (10 cm outside 7.6 cm inside) versus minirhizotron methods to date. Fine root production by root ingrowth in hydric, mesic, and xeric longleaf pine sites was estimated to be 1069, 1409, and 2015 g/m^2/y compared to 4618, 1906, and 2295 g/m^2/y, respectively, based on minirhizotron. The difference between methods decreased with increased aridity, as the ratio of ingrowth to minirhizotron estimates went from 0.23, 0.74, to 0.88. Tube equilibration after installation was explicitly tested based on comparisons of root-length production and mortality (see further discussion of this important concept below in minirhizotron section) and therefore was not a bias factor in this comparison. However, the independent validation procedure of Hendrick and Pergitzer (1993) indicated that production and mortality estimates from the minirhizotron were representative of bulk soil, and production and mortality are measured independently. In all but one study above, root ingrowth estimates were lower than those obtained by minirhizotron. Hendricks and others (2006) also reached the conclusion that ingrowth methods were generally conservative estimates of root production.

The low production estimate by root ingrowth donut compared to minirhizotron and isotope turnover suggests the size of ingrowth area may be an important factor. The size factor in this context refers to the large volume of soil relative to root biomass occupancy and, therefore, the amount of growth/volume is low. Size of the ingrowth area may be isolated as the factor, because other factors different between the two methods would cause an opposite directional bias (Table 3). Further, the minirhizotron by regression method uses both new growth and new loss, thereby accounting for decomposition plus herbivory, yet still yields higher values than ingrowth donuts. Even more compelling evidence suggesting that ingrowth underestimates production comes from the decomposition adjusted values. The loss of roots during the first

year of root ingrowth data was estimated from minirhizotron tubes placed approximately one to two meters away from each ingrowth replicate and during the same year. Loss of the minirhizotron cohort of roots born in the first year of ingrowth was only 27% in this semiarid environment (Figure 1A). Adjusting root ingrowth estimates of root production for losses of roots that were initiated that same year increased the average production estimate from 93 to 118 $g/m^2/y$, and this small increase still results in an underestimate compared to minirhizotron. The only other potential biases in the ingrowth method are those that can cause either positive or negative bias (Table 3). The architectural bias could be an additional significant effect on data from this site, but concerns associated with bulk density and soil used in constructing the ingrowth profile were minimized by packing and using nearby native soil rather than sand or other artificial medium.

These within-site shortgrass steppe comparisons together with other comparisons in the literature strongly suggest that ingrowth estimates of root production tend to be low relative to other methods. The assessment at our grassland site also suggests ingrowth methods are highly sensitive to the size of the ingrowth area, and that architectural bias could also play a role. This is based on the elimination of other important potential biases either due to their opposite actions or by adjusting other methods for bias that would act in the same direction to result in underestimation. Root density inside ingrowth volumes, as a rule, never reaches the outside density because if it did decomposition would be a major factor resulting in underestimations, just as it is for biomass coring methods. New root growth could theoretically reach a per-volume equilibrium as in outside soil, but the specific volume (size) of ingrowth to use and the timing when growth rates are equilibrated can never be known. Hertel and Leuschner (2002) commented that the ideal ingrowth cores should have a very small size to minimize delays in the re-colonization of invading roots. This needs further direct testing, and is counter to the general perception that growth into a competition-free, nutrient available medium would be the over-riding factor and lead to overestimations by ingrowth methods. Root ingrowth volumes placed at different orientations may be used to further test the influence of the architectural factor for both ingrowth and minirhizotron methods (Figure 2).

On the other hand, root ingrowth remains a simple, straightforward, low labor, and low cost method. Our use of both root ingrowth donuts and minirhizotron in the same CO_2 experiment produced very similar relative differences among treatments (Table 2). Factors such as architectural bias, low competition space, and artificial profile horizons may produce relative differences under different circumstances than the treatments studied at this shortgrass site, and insertions at angles may be optimized as with minirhizotron tubes (see below). Ingrowth methods remain an easy reasonable approach to indexing relative differences in root growth among treatments within a study, but appear to be limited when comparing across methods that differ substantially and when absolute values are sought for cross-site synthesis. Some methods that may not accurately quantify BNPP may be acceptable for assessing experimental responses to treatments.

Minirhizotron and Rhizotron Excavation Window

Rhizotron excavation windows are included in this primarily minirhizotron section because windows were the precursor to the technologically advanced minirhizotron, and because there are some similarities as well as comparative differences between the two. Windows are essentially large, but vertical, minirhizotrons where individual roots are followed by hand drawings and hand calculations of growth and loss. The important similarity yet difference between the two that may be potentially interesting for comparative purposes concerns our question of the effect of an artificial surface a minirhizotron tube presents to a growing root. A growing root hits the curved-plane surface of the tube and, instead of passing through the plane, is forced to grow across it (Figure 2). The additional length of growth across that artificial surface may inflate new-length growth estimates, causing overestimation of root production (Table 3). The estimate of root production from excavation windows at this shortgrass steppe site is higher than the estimates from minirhizotron methods (Table 1). There are potentially other reasons for the difference between the two methods: there was essentially no equilibration period before starting measurements at the excavation window site and angles of the planes were different (Ares 1976). However, estimates of decomposition of roots were also higher by rhizotron window than by minirhizotron, suggesting some contributing factor associated with the artificial surface (altered water flow, and so on), rather than the equilibration period or angle/root architectural bias. This would tend to support recent considerations of using root-life-span

rather than new-root-length growth to calculate turnover from minirhizotron to bypass problems associated with the artificial surface (Tierney and Fahey 2001). However, there are two very important limitations when using root life-spans to calculate turnover. First, the life-span calculation method was not done using data from this site because of the very long life span of some roots in this system (Figure 1A). The option of using life-span calculations may depend on the plant community being studied, with less possibility of use for systems with roots of high longevity that would require very long studies. This may be related to an arid-mesic gradient. Second and also related to the speed of root dynamics in a particular system, the use of minirhizotron life-span calculations for turnover would present some additional problems for annual or short-term estimates of root production. There may be time-lags between a period that is stressful for root growth and the period in which mortality and/or loss appear (Milchunas and others 2005b). A potential asynchrony between growth and demography dynamics as observed by minirhizotron could affect timing of estimates, but would have less effect with increasing time interval of interest. Lifespans are estimated from demographic modeling for different cohorts over time (Ruess and others 2003; Eissenstat and Yanai 1997), whereas turnover is usually derived from annual estimates of new length growth and average biomass values. Minirhizotron methods can give good estimations of lifespan and decomposition for different time or age cohorts using demographic modeling methods. However, Tierney and Fahey (2001) show that methods of calculating root production based on life-span methods can vary from 274 to 401 $g/m^2/y$ depending on use of single or multiple cohorts and root numbers, length, or mass. Also, the type of material used to create tubes can have a greater effect on lifespan than on growth estimates (Withington and others 2003).

The importance of the method of calculating minirhizotron turnover is illustrated by the range of values from 160 to 414 $g/m^2/y$ for this shortgrass site based on the same minirhizotron data set and the same root biomass data to convert turnover to production (Table 1). The low value by regression of new growth:loss ratios indicates the importance of the equilibration period and/or taking into account the longer-lived roots and the equilibration of not only new growth but also the time lag in mortality. The regression method is the only one of the three estimates that is not based only on new-length growth and some estimate of total length present to obtain turnover (Figure 1B, Table 1). The regression method presented in Milchunas and others (2005b) does not require equilibration of new root growth and old root loss, but is based on calculating the time necessary for these two processes to equilibrate and considers this the turnover time. The regression method therefore bypasses the need for equilibration that may take many years in systems with long life spans of roots. In this way, it is similar to the suggested life-span method (Tierney and Fahey 2001) that also bypasses the artificial surface bias (inflationary effects of growth [or decomposition] alone across the artificial surface). The regression method may be an alternative to the life-span method for systems where life-spans are very long, and the life-span method may be appropriate for systems with fast turnover. The minirhizotron regression method estimate of 160 $g/m^2/y$ root production is very close to the best estimate by isotope turnover—two-phase life-span method of 185 $g/m^2/y$ (discussed in ''Isotope Turnover'' section). However, the regression method needs further testing in systems with long root longevities, because of the variability in growth and loss at this shortgrass steppe site that experiences frequent drought periods and precipitation pulses. Alternatively, short-term minirhizotron observations may be capable of providing reasonable production estimates if the general form of the root survivorship curve or the age-frequency distribution can be determined for the particular plant community.

Both the 191 and 414 $g/m^2/y$ root production estimates are based on exactly the same new-length growth values, but vary in the means of calculating the total length values (Table 1). The average length method of calculating a total length value, or some variation of averaging, is possibly the most common approach. The ''average'' method used is total root length for each tube, for each period within a year, averaged across periods for each year. The maximum total length method is based on individual root maximum length during each year. The maximum length ever recorded over all periods during that year of each individual root within a tube is summed, and that value used as the total length in the calculation of turnover = new growth/total length. This method was considered in the CO_2 study because of the non-steady-state, aggrading conditions under elevated CO_2 that can effect the average length method, and was more in-line with isotope turnover methods. However, calculations based on hypothetical situations indicate the average-length method is theoretically most accurate under general conditions.

This means there is a very large discrepancy between the regression method and the new-length growth method using average standing lengths to calculate turnover (Table 1, 160 vs. 414 g/m^2/y, respectively). The latter estimate is the usual way of calculating minirhizotron data, but produces very high values of root production.

An important point from these comparisons is that root production values based on different ways of calculating the same data from the same method can vary tremendously. Data from comparisons here support the important suggestion by Tierney and Fahey (2001) that life-span calculations may avoid a critical potential bias in the minirhizotron method based on the effect of growth into and across an artificial surface, but also suggests qualifications or potential limitations to this approach that may be positively related to decreasing rates of root dynamics of the particular ecosystem. In systems where roots have very long life-spans, calculation methods based on life-spans may not be possible except after many years of monitoring because equilibration periods can be longer than the commonly recommended one year. The period necessary for equilibration can be estimated during data collection periods by the regression method (Figure 1B) or similar calculations in the validation procedure of Hendrick and Pergitzer (1993) and illustrated in Hendricks and others (2006). Estimates based on life-span may also vary considerably due to method of calculation. Comparisons between minirhizotron and root ingrowth methods in the section above suggest that, even given the variability within the minirhizotron method, minirhizotron tends to overestimate root production when compared to ingrowth. Comparisons of minirhizotron with isotope turnover and bomb isotope methods, and some additional bias factors for minirhizotron listed in Table 3, are discussed in the next section. The comparisons here suggest that high values can result from artificial surfaces that roots grow across in rhizotron excavation windows and minirhizotrons. Methods of calculating minirhizotron data based on regression of growth and decomposition ratios to an equilibration time (Milchunas and others 2005b) or by root life-span calculations (Tierney and Fahey 2001) may avoid this apparently large problem. Additional testing of these calculation methods is necessary.

Isotope Turnover

Before discussing BNPP estimates by isotope turnover, it is important to note that isotope methods are the only means of estimating crown production. Crowns are a very important biomass component of grasslands. Crown biomass varies among 10 North American grasslands and ranges from 175 to 300 g/m^2 compared to a root biomass range of 143–2177 g/m^2 (Sims and Singh 1978 appendix III). Long-term estimates of crown biomass averaged 430 g/m^2/y at our grassland site (Milchunas and Lauenroth 1992). Because of slow turnover however, Milchunas and Lauenroth (2001) estimated crown production of 57 g/m^2/y compared to 181 g/m^2/y for roots at this shortgrass steppe site using ^{14}C decay. Although crowns represent a large carbon pool in grasslands, this is the only estimate of crown production we are aware of in the literature, except for estimates based on seasonal biomass peak-trough (max–min) methods that were discounted above for roots (biomass coring method). Isotope methods are also the only methods considered here that may also estimate exudation and sloughing components of carbon capture by plants.

There are two distinctly different primary methods of estimating root production from isotope labeling, and further variations within each (disregarding isotope dilution covered above). One method is based on pulse labeling plots using CO_2 of either ^{14}C or ^{13}C enrichment (Dahlman and Kucera 1965, 1967; Milchunas and Lauenroth 1992, 2001) and calculating isotope turnover. The other primary method uses longer-term uptake of label, either from elevated levels of $^{14}CO_2$ in the global atmosphere due to atomic bomb testing to estimate Δ^{14}C (Gaudinski and others 2001; Trumbore and Gaudinski 2003) or to estimate δ^{13}C values from uptake during continual labeling in elevated CO_2 studies (Matamala and others 2003). Assumptions among methods of pulse versus continuous isotope labeling can be very different but not always recognized in the literature.

In pulse-label methods, the critical assumption of a uniform label throughout plant tissue that fails in the isotope dilution method is not an assumption in the isotope turnover method (Table 3, Milchunas and Lauenroth 1992, 2001). Dahlman and Kucera (1968) and many others clearly show that label from a pulse is preferentially translocated to more actively growing tissue. The isotope turnover method is based on the decomposition of roots that were initially heavily labeled with isotope, with production being the inverse of decomposition and non-steady state conditions accounted for by changes in root biomass between time periods (Milchunas and Lauenroth 1992, 2001). The number of years required for complete turnover is obtained by regressing isotope loss over time, and

dividing the X-intercept time by an estimate of root biomass. Data used in the regression need to be only those collected after stabilization of labile (soluble) label into structural tissue that can't be exuded, translocated, or respired (Milchunas and others 1985). There are no artificial surfaces or disturbances prior to final destructive sampling. Other than the usual problems concerning separation of roots from soil (often underestimated, requiring standardization), there are only a few other potential factors that can cause bias (Table 3). The most critical assumption is that age class and quality of tissue labeled is constant through different seasonal times of labeling. Thus, a *non*-uniform label is expected, and younger more heavily labeled roots will age and progress through the death and decomposition process. The assumption is that the progression of life-death-decomposition will be the same for the group of cohorts pulse labeled at different times of the year. This important concept requires some explanation. When an established plant is pulse labeled, most if not all existing living cohorts of roots are labeled, not just one newest cohort. There is, however, what may be called a cohort bias, whereby label assimilation efficiency decreases with increasing age or growth/maintenance requirements. Similar to the suggestion by Tierney and Fahey (2001) that minirhizotron estimates based on life-span of roots should use multiple cohorts to avoid seasonal effects on survival (see ''Discussion''), multiple labelings are a means to minimize differences between cohort-biased labeling (Milchunas and Lauenroth 1992). Problems with the assumption of similar life-death-decomposition of different seasonal cohorts may be more likely in forest communities with woody lifeforms (Ruess and others 2003, 2006) than in grasslands where root initiation and loss lack seasonal pattern but are more related to periods of drought that sporadically vary seasonally and annually without consistent pattern (Milchunas and others 2005b). Isotope labeling requires placing clear tents over vegetation during the labeling process, and therefore turnover methods are not practical in plant communities with large canopies such as shrublands and forests (Fahey and others 1999). An additional drawback to isotope turnover is the long period of time necessary to obtain the data necessary for regression. This would be a problem in slow moving systems such as the shortgrass steppe, but less so where loss of label is more rapid. However, this is somewhat of a limitation for minirhizotron methods as well where equilibration periods can be longer than originally estimated (Milchunas and others 2005b) and life spans of roots can be longer than previously thought (Eissenstat and Yanai 1997; Gaudinski and others 2001, see discussion above on minirhizotrons, and isotope turnover estimates of seven-plus years in Milchunas and Lauenroth 1992, 2001).

In contrast to pulse labeling, a relatively more continuous labeling is achieved from the bomb atmospheric $\Delta^{14}C$ method and the $\delta^{13}C$ from the elevated CO_2 experiment method which results in a more uniform labeling of cohorts, but other assumptions and problems are that the age of roots is normally distributed, differences in labile nonstructural components are not accounted for (as in the discussion of pulse isotope dilution above), and in the case of $\delta^{13}C$ from elevated CO_2 experiments the increase in growth due to the CO_2 treatment (Gaudinski and others 2001; Tierney and Fahey 2002; Luo 2003; Matamala and others 2003; Trumbore and Gaudinski 2003). In a theoretical, mathematical approach to assessing some of the potential bias factors, Luo (2003) reports that errors in the bomb $\Delta^{14}C$ or $\delta^{13}C$ elevated CO_2 method estimates of root longevity may vary from 25 to 806%. Based on these references, it has generally been concluded that these isotope methods overestimate root turnover times (long turnovers that result in underestimates of production) and minirhizotron methods underestimate root turnover times (short turnovers that overestimate production). When only turnover is estimated, the loss of very fine roots during separation from soil in isotope studies but not with minirhizotrons is an additional factor causing the observed discrepancy between the two (Table 3). The age cohorts sampled in longer-term, continuous labeling may underestimate the rapidly turning over first-order roots (Ruess and others 2006). The potential for large biases within the bomb $\Delta^{14}C$ or $\delta^{13}C$ elevated CO_2 methods exist, as well as for large differences between these isotope methods and minirhizotron estimates of root turnover. Tierney and Fahey (2002) observed estimates of fine root turnover by bomb $\Delta^{14}C$ were 54% less than those based on minirhizotron using median longevity calculations and were 67% less when calculated by new length growth and standing length. Gaudinski and others (2001) found differences in mean life span between methods of from a few months to 2 years for minirhizotron compared to 3–18 years for bomb $\Delta^{14}C$. Both studies indicate substantial differences between minirhizotron and continuous isotope labeling methods.

However, literature concerning the bomb $\Delta^{14}C$ and the elevated CO_2 $\delta^{13}C$ method have more or less ignored the isotope pulse-label turnover

method in discussions of differences among isotope and other root production methods (Gaudinski and others 2001; Tierney and Fahey 2002; Luo 2003; Matamala and others 2003; Trumbore and Gaudinski 2003), possibly because of the potential differences between continuous and pulse labeling methods. The focus of this article is on a comparative approach among methods. Although we have not used the bomb $\Delta^{14}C$ method at this shortgrass steppe site and the elevated CO_2 $\delta^{13}C$ method is not used here because adjusting to control levels of root production depends on the method of root production used (Table 1), a comparison of the isotope pulse-label turnover method and continuous isotope labeling methods here may provide insight when viewed in terms of the differences in potential bias factors.

Before comparing isotope and minirhizotron methods, it first should be clear that both isotope and minirhizotron mean estimates of root production vary within each respective method depending on how the data were calculated (Table 1). This is particularly true for the minirhizotron, where mean estimates range from 160 to 414 g/m^2/y (discussed above). The isotope pulse turnover methods of calculation produce root production estimates that range from averages of 170 to 185 to 217 g/m^2/y. All three estimates are based on regression of isotope loss through time to obtain the X-intercept of turnover time, after all isotope in labile/soluble forms had been incorporated into structural tissue. Differences among the three ways of calculation within the pulse isotope turnover method are as follows. The soil-isotope non-adjusted values are estimates of loss that are ash corrected to an organic matter basis, but the C-isotope in that ash is not corrected for because the roots with the embedded soil are oxidized in the analyses. This is the usual way of reporting values for isotope concentrations; the assumption is that the isotope in the soil contamination is low especially when roots were floated out of soil (washed) as in this study. However, soil around and in roots can contain carbon recently exudated by plants as well as that incorporated into more recalcitrant forms. The soil-isotope corrected values were done because Milchunas and Lauenroth (2001) observed a second phase in isotope turnover (Figure 1C) that could have been due to the isotope that was in embedded soil rather than actual root material or because a small proportion of roots live a very long time (several other possibilities were eliminated). At that time, the possibility of some grass roots living up to 9.6 years (Figure 1A) seemed questionable, especially given the generally fine nature of grass roots (Milchunas and others 2005b). However, recent work from bomb $\Delta^{14}C$ studies also suggest that some roots may be long-lived (Gaudinski and others 2001; Tierney and Fahey 2002; Matamala and others 2003; Trumbore and Gaudinski 2003), as was suggested earlier by Eissenstat and Yanai (1997). Therefore, the two-phase life span estimate (Table 1) is based on two regressions (one for each phase, or short-lived versus long-lived roots, Figure 1C) and applying that turnover time to the proportion of the total initial isotope pool that each represents. The adjustment for isotope in soil embedded in roots had a slightly larger effect on the estimate of root production (raised estimate 47 g/m^2/y) than did accounting for long-lived roots (lowered estimate 32 g/m^2/y).

The ways of calculating data within isotope turnover or minirhizotron methods have some important implications for cross method comparisons at our grassland site as well as comparisons that have been made between bomb $\Delta^{14}C$ and minirhizotron methods at other locations. The root production estimate of 170 g/m^2/y that is not soil-isotope adjusted and is based on first phase roots is possibly most similar to bomb $\Delta^{14}C$ estimates, and the 414 g/m^2/y from minirhizotron is possibly most similar to minirhizotron estimates from the literature based on new root growth (see minirhizotron section above). These estimates conform to the general conclusions in the literature that isotope methods overestimate root turnover times (long turnovers that underestimate production) and minirhizotron methods underestimate root turnover times (short turnovers that overestimate production) (Gaudinski and others 2001; Tierney and Fahey 2002; Matamala and others 2003; Trumbore and Gaudinski 2003). The minirhizotron estimate by regression (equilibrium of loss/gain) takes the longer-lived root pool into account and is also low (160 g/m^2/y—slow turnover), but regression was noisy (low r^2) and sensitive to slope (Figure 1B). The most logical comparison at this site is between the pulse-isotope turnover with the two-phase life span calculation (185 g/m^2/y) that takes *both* short- and long-lived roots into account and the minirhizotron by regression estimate (160 g/m^2/y, rationale described in detail above), and these estimates are very close. However, the two-phase life span estimate is adjusted for both soil isotope and long-lived roots, and the greatest effect of the two adjustments was due to the soil rather than root lifespan. Because our pulse-isotope turnover method here has accounted for long-life span *and* is based on structural tissue only (all label in labile/soluble/non-structural components had been incorporated

into structural material before calculating turnover) the higher estimates by minirhizotron new growth/average length method can only be due to factors not discussed in bomb $\Delta^{14}C$ studies. The faster turnover/higher productivity estimates from minirhizotrons compared to isotope turnover methods (max length, two phase) could be due to (1) fine roots lost during soil root separation (especially first-order roots, see ingrowth comparison where sieve size differed, and Table 2 root hand pick versus flotation), (2) growth over artificial surface as discussed in minirhizotron versus rhizotron comparison above, or (3) movement or bending of the tube can artificially increase turnover estimates. Although there was no loss of tube contact with the soil at this site (tubes could not be moved side-to-side or up or down), the freeze thaw or other soil-profile shifting factors resulted in tubes bending whereby the camera casing rubbed hard against bends in the tube almost preventing deep insertion by the fifth year. This can cause both loss and gain of visibility of roots through soil even if they did not grow or decompose. This can affect minirhizotron estimates based on life-span calculations as well, and is one of the factors concerning the artificial surface that estimates based on life-span of individuals does not overcome. Minirhizotron estimates based on root life-span that do not wait long enough to determine the extent of phase two, long-lived roots may also overestimate production. Minirhizotron estimates of root production in slow-moving systems will require long-term studies to obtain reasonable estimates of root life-spans or will need to use the growth to loss regression to equilibration time approach.

In general, very large differences in root production estimates were observed within minirhizotron and pulse isotope turnover methods based on how the data were calculated. However, when logical means of calculation and adjustment were chosen within a method, the estimates between the two methods were very reasonably comparable. Minirhizotron estimates by the regression method were only slightly greater than those from pulse-isotope turnover, but nowhere near as great as the differences discussed above between minirhizotron and bomb $\Delta^{14}C$ or elevated CO_2 $\delta^{13}C$ methods. It is important to note here, however, that the coarse root component in forests will be much greater than in grasslands, leading to greater discrepancies between bomb $\Delta^{14}C$ and minirhizotron estimates in forests, especially when the minirhizotron estimates are based on calculations that are subject to the bias from growth across the artificial surface or tube movement. Tierney and Fahey (2002) were able to reconcile some of these differences by considering differences in life-spans of roots, as comparisons were here for pulse isotope and minirhizotron estimates. Further narrowing the gap between minirhizotron and any isotope estimate may include correction for soil-isotope levels in roots. The pulse-isotope turnover method can be adjusted for long-lived roots, but the lack of long-term data for other systems precludes a good evaluation of this method. The long-term nature of the pulse-isotope turnover method, and the problems of use in communities with high physiognomy may preclude it from practical application for routine measurements of root production, but additional tests for comparative method purposes are certainly warranted. This method is perhaps based on the least severe assumptions (Table 3), largely based on it's in situ nature and the only artificial aspect being the separation of roots from soil that is also necessary for even minirhizotron conversion-to-mass based estimates. Methods of adjusting production estimates for long-lived roots, as in the pulse isotope method, will be necessary to correct for this bias in bomb $\Delta^{14}C$ or elevated CO_2 $\delta^{13}C$ methods as well.

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REFERENCES

Aber JD, Melillo JM, Nadelhoffer JK, McClaugherty CA, Pastor J. 1985. Fine root turnover in forest ecosystems in relation to quality and form of nitrogen availability: a comparison of two methods. Oecologia 66:317–21.

Ares J. 1976. Dynamics of the root system of blue grama. J Range Manage 29:208–13.

Arnone JAIII, Zaller JG, Spehn EM, Niklaus PA, Wells CE, Körner C. 2000. Dynamics of root systems in native grasslands: effects of elevated atmospheric CO_2. New Phytol 147:73–85.

Biondini ME, Lauenroth WK, Sala OE. 1991. Correcting estimates of net primary production: are we overestimating plant production in rangelands? J Range Manage 44:194–8.

Burke IC, Mosier AR, Hook PB, Milchunas DG, Barrett JE, Vinton MA, McCulley RL, Kaye JP, Gill RA, Epstein HE, Kelly RH, Parton WJ, Yonker CM, Lowe P, Lauenroth WK. 2008. Biogeochemistry of soil organic matter and nutrient dynamics of shortgrass steppe ecosystems. In: Lauenroth WK, Burke IC, Eds. Ecology of the shortgrass steppe: a long-term perspective. New York: Oxford University Press. p 306–41.

Caldwell MM, Camp LB. 1974. Belowground productivity of two cool desert communities. Oecologia 17:123–30.

Clark FE. 1977. Internal cycling of ^{15}Nitrogen in a short-grass prairie. Ecology 58:1322–32.

Coffin DP, Lauenroth WK. 1991. Effects of competition on spatial distribution of roots of blue grama. J Range Manage 44:68–71.

Dahlman RC, Kucera CL. 1965. Root productivity and turnover in native prairie. Ecology 46:84–9.

Dahlman, RC, Kucera CL. 1967. Carbon-14 cycling in the root and soil components of a prairie ecosystem. In: Nelson DJ, Evans FC, Eds. Symposium on radioecology. Publication Number 272, U.S. Atomic Energy Commission, Radiation Ecology Section, and Clearinghouse for Federal Scientific and Technical Information. p 652–60.

Dahlman RC, Kucera CL. 1968. Tagging native grassland vegetation with carbon-14. Ecology 49:1199–203.

Eissenstat DM, Caldwell MM. 1988. Seasonal timing of root growth in favorable microsites. Ecology 69:870–3.

Eissenstat DM, Yanai RD. 1997. The ecology of root lifespan. Adv Ecol Res 27:1–60.

Fahey TJ, Bledsoe CS, Day FP, Ruess RW, Smucker AM. 1999. Fine root production and demography. In: Robertson GP, Bledsoe CS, Coleman D, Sollins P, Eds. Standard soil methods for long-term ecological research. New York: Oxford University Press. p 437–55.

Fahey TJ, Hughs JW. 1994. Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, NH. J Ecol 82:533–48.

Fitter AH. 1976. Effects of nutrient supply and competition from other species on root growth of *Lolium perenne* in soil. Plant Soil 45:177–89.

Fogel R. 1990. Root turnover and production in forest trees. Hortscience 25:270–3.

Hansson AL, Andrén O. 1986. Below-ground plant production in a perennial grass ley (*Festuca pratensis* Huds.) assessed with different methods. J Appl Ecol 23:657–66.

Kurz WA, Kimmins JP. 1987. Analysis of some sources of error in methods used to determine fine root production in forest ecosystems: a simulation approach. Can J For Res 17:909–12.

Gaudinski JB, Trumbore SE, Davidson EA, Cook AC, Markewitz D, Richter DD. 2001. The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon. Oecologia 129:420–9.

Geisler D, Ferree DC. 1984. Response of plants to root pruning. Hort Rev 6:155–88.

Gill RA, Kelly RH, Parton WJ, Day KA, Jackson RB, Morgan JA, Scurlock JMO, Tieszen LL, Castle JV, Ojima DS, Zhang XS. 2002. Using simple environmental variables to estimate belowground productivity in grasslands. Global Ecol Biogeogr 11:79–86.

Hendrick RL, Pergitzer KS. 1993. Applications of minirhizotrons to understand root function in forests and other natural ecosystems. Plant Soil 185:293–304.

Hendricks JJ, Hendrick RL, Wilson CA, Mitchell RJ, Pecot SD, Guo D. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. J Ecol 94:40–57.

Hendricks JJ, Nadelhoffer KJ, Aber JD. 1993. Assessing the role of fine roots in carbon and nitrogen cycling. Trends Ecol Evol 8:174–8.

Hendrickson AH, Veihmeyer FJ. 1931. Influence of dry soil on root extension. Plant Physiol 6:567–76.

Hertel D, Leuschner C. 2002. A comparison of four different fine root production estimates with ecosystem carbon balance data in a *Fagus-Quercus* mixed forest. Plant Soil 239:237–51.

Higgens PAT, Jackson RB, Des Rosiers JM, Field CB. 2002. Root production and demography in a California annual grassland under elevated atmospheric carbon dioxide. Global Change Biol 8:841–50.

Jordan CF, Escalante G. 1980. Root productivity in an Amazonian rain forest. Ecology 61:14–18.

Joslin JD, Wolfe MH. 1999. Disturbance during minirhizotron installation can affect root observation data. Soil Sci Soc Am J 63:218–21.

King JY, Mosier AR, Morgan JA, LeCain DR, Milchunas DG, Parton WJ. 2004. Plant nitrogen dynamics in shortgrass steppe under elevated atmospheric carbon dioxide. Ecosystems 7:147–60.

Lauenroth WK. 2000. Methods of estimating belowground net primary production. In: Sala OE, Jackson RB, Mooney HA, Howarth RW, Eds. Methods in ecosystem science. New York: Springer-Verlag. p 58–71.

Lauenroth WK, Hunt HW, Swift DM, Singh JS. 1986. Estimating aboveground net primary production in grasslands: a simulation approach. Ecol Model 33:297–314.

Lauenroth WK, Milchunas DG. 1991. The shortgrass steppe. In: Coupland RT, Ed. Natural grasslands, introduction and western hemisphere. Ecosystems of the World 8A. Amsterdam: Elsevier. p 183–226.

LeCain DR, Morgan JA, Milchunas DG, Mosier AR, Nelson JA, Smith DP. 2006. Root biomass of individual species, and root size characteristics after five years of CO_2 enrichment on native shortgrass steppe. Plant Soil 279:219–28.

Leetham JW, Milchunas DG. 1985. The composition and distribution of soil microarthropods in the shortgrass steppe in relation to the soil water, root biomass, and grazing by cattle. Pedobiologia 28:311–25.

Litton CM, Raich JW, Ryan MG. 2007. Carbon allocation in forest ecosystems. Global Change Biol 13:2089–109.

Litton CM, Giardina CP. 2008. Below-ground carbon flux and partitioning: global patterns and response to temperature. Functional Ecol 22:941–54.

Lund ZF, Pearson RW, Buchanan GA. 1970. An implanted soil mass technique to study herbicide effects on root growth. Weed Sci 18:279–81.

Luo Y. 2003. Uncertainties in interpretation of isotope signals for estimation of fine root longevity: theoretical considerations. Global Change Biol 9:1118–29.

Matamala R, Gonzalez-Meler MA, Jastrow JD, Norby RJ, Schlesinger WH. 2003. Impact of fine root turnover on forest NPP and soil C sequestration potential. Science 302:1385–7.

McCulley RL, Burke IC, Nelson JA, Lauenroth WK, Knapp AK, Kelly EF. 2005. Regional patterns in carbon cycling across the Great Plains of North America. Ecosystems 8:106–21.

McNaughton SJ, Milchunas DG, Frank DA. 1996. How can primary productivity be measured in grazing ecosystems? Ecology 77:974–7.

Milchunas DG, Lauenroth WK. 1992. Carbon dynamics and estimates of primary production by harvest, C^{14} dilution, and C^{14} turnover. Ecology 73:593–607.

Milchunas DG, Lauenroth WK. 2001. Belowground primary production by carbon isotope decay and long-term root biomass dynamics. Ecosystems 4:139–50.

Milchunas DG, Lauenroth WK, Singh JS, Hunt HW, Cole CV. 1985. Root turnover and production by ^{14}C dilution: implications of carbon partitioning in plants. Plant Soil 88:353–65.

Milchunas DG, Mosier AR, Morgan JA, LeCain D, King JY, Nelson JA. 2005a. Root production and tissue quality in a shortgrass steppe exposed to elevated CO_2: Using a new ingrowth method. Plant Soil 268:111–22.

Milchunas DG, Morgan JA, Mosier AR, LeCain D. 2005b. Root dynamics and demography in shortgrass steppe under elevated CO_2, and comments on minirhizotron methodology. Global Change Biol 11:1837–55.

Morgan JA, Mosier AR, Milchunas DG, LeCain DR, Nelson JA, Parton WJ. 2004. CO_2 enhances productivity but alters species composition and reduces forage quality in the Colorado shortgrass steppe. Ecol Appl 14:208–19.

Mosier AR, Parton WJ, Martin RE, Valentine DW, Ojima DS, Schimel DS, Burke IC. 2008. Soil-atmosphere exchange of trace gases in the Colorado Shortgrass Steppe. In: Lauenroth WK, Burke IC, Eds. Ecology of the shortgrass steppe: a long-term perspective. New York: Oxford University Press. p 342–72.

Nadelhoffer KJ. 2000. The potential effects of N deposition on fine root-production in forest ecosystems. New Phytol 147:131–9.

Nadelhoffer KJ, Aber JD, Melillo JM. 1985. Fine roots, net primary production, and soil nitrogen availability: a new hypothesis. Ecology 66:1377–90.

Nadelhoffer KJ, Raich JW. 1992. Fine root production estimates and belowground carbon allocation in forest ecosystems. Ecology 73:1139–47.

Nadelhoffer KJ, Raich JW, Aber JD. 1998. A global trend in belowground carbon allocation: comment. Ecology 79:1822–5.

Neill C. 1992. Comparison of soil coring and ingrowth methods for measuring belowground production. Ecology 73:1918–21.

Norby RJ, Jackson RB. 2000. Root dynamics and global change: seeking an ecosystem perspective. New Phytol 147:3–12.

Pages L, Bengough AG. 1997. Modeling minirhizotron observations to test experimental procedures. Plant Soil 189:81–9.

Parton WJ, Schimel DS, Cole CV, Ojima DS. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Sci Soc Am J 51:1173–9.

Parton WJ, Stewart JWB, Cole CV. 1988. Dynamics of C, N, P and S in grassland soils: a model. Biogeochemistry 5:109–31.

Parton WJ, Singh JS, Coleman DC. 1978. A model of production and turnover of roots in shortgrass prairie. J Appl Ecol 15:515–41.

Pendall E, Del Grosso S, King JY, LeCain DR, Milchunas DG, Morgan JA, Mosier AR, Ojima DS, Parton WA, Tans PP, White JWC. 2003. Elevated atmospheric CO_2 effects and soil water feedbacks on soil respiration components in a Colorado grassland. Global Biogeochem Cycles 17:15-1–13.

Persson HA. 1978. Root dynamics in a young Scots pine stand in central Sweden. Oikos 30:508–19.

Persson HA. 1979. Fine-root production, decomposition and mortality in forest ecosystems. Vegetation 41:101–9.

Raich JW, Nadelhoffer KJ. 1989. Belowground carbon allocation in forest ecosystems: global trends. Ecology 70:1346–54.

Ruess RW, Hendrick RL, Burton AJ, Pregitzer KS, Sveinbjornssön B, Allen MF, Maurer GE. 2003. Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska. Ecol Monogr 73:643–62.

Ruess RW, Hendrick RL, Vogel JG, Sveinbjörnsson B. 2006. The role of fine roots in the functioning of Alaskan boreal forests. In: Chapin FSIII, Oswood MW, Van Cleve K, Viereck LA, Verbyla DL, Eds. Alaska's changing boreal forest. New York: Oxford University Press. p 189–210.

Ruess RW, Van Cleve K, Yarie J, Viereck LA. 1996. Contributions of fine root production and turnover to carbon and nitrogen cycling in taiga forests of the Alaskan interior. Canadian Journal of Forest Research 26:1326–36.

Sala OE, Biondini ME, Lauenroth WK. 1988. Bias in estimates of primary production: an analytical solution. Ecol Model 44:43–55.

Schimel DS, Stillwell MA, Woodmansee RG. 1985. Biogeochemistry of C, N, and P in a soil catena of the shortgrass steppe. Ecology 66:276–82.

Senft RL. 1983. The redistribution of nitrogen by cattle. Ph.D. dissertation, Colorado State University, Fort Collins, 198 pp.

Sims PL, Singh JS. 1978. The structure and function of ten western North American grasslands. III. Net primary production, turnover and efficiency of energy capture and water use. J Ecol 66:573–97.

Singh JS, Lauenroth WK, Hunt HW, Swift DM. 1984. Bias and random errors in estimators of net root production: a simulation approach. Ecology 65:1760–4.

Steele SJ, Gower ST, Vogel JG, Norman JM. 1997. Root mass, net primary production and turnover in aspen, jack pine and black spruce forests in Saskatchewan and Manitoba, Canada. Tree Physiol 17:577–87.

Steen E. 1983. Usefulness of the mesh bag method in quantitative root studies. In: Atkinson D, Ed. Plant root growth, an ecological perspective Brit Ecol Soc Special Publ No 10. London: Blackwell Scientific Publications. p 75–86.

Steen E. 1991. Variation of root growth in a grass ley studied with a mesh bag technique. Swedish J Agric Res 14:93–7.

Steingrobe B, Schmid H, Claassen N. 2000. The use of the ingrowth core method for measuring root production of arable crops B influence of soil conditions inside the ingrowth core on root growth. J Plant Nutr Soil Sci 163:612–22.

Steingrobe B, Schmid H, Claassen N. 2001. The use of the ingrowth core method for measuring root production of arable crops B influence of soil and root disturbance during installation of the bags on root ingrowth into the cores. Eur J Agron 15:143–51.

Swinnen J, Van Veen JA, Merckx R. 1994a. Losses of ^{14}C from roots of pulse-labeled wheat and barley during washing from soil. Plant Soil 166:93–9.

Swinnen J, Van Veen JA, Merckx R. 1994b. ^{14}C pulse-labeling of field-grown spring wheat: an evaluation of its use in

rhizosphere carbon budget estimations. Soil Biol Biochem 26:161–70.

Swinnen J, Van Veen JA, Merckx R. 1994c. Rhizosphere carbon fluxes in field-grown spring wheat: model calculations based on ^{14}C partitioning after pulse-labeling. Soil Biol Biochem 26:171–82.

Swinnen J, Van Veen JA, Merckx R. 1995. Root decay and turnover of rhizodeposits in field-grown winter wheat and spring barley estimated by ^{14}C pulse labeling. Soil Biol Biochem 27:211–17.

Tierney GL, Fahey TJ. 2001. Evaluating minirhizotron estimates of fine root longevity and production in the forest floor of a temperate broadleaf forest. Plant Soil 229:167–76.

Tierney GL, Fahey TJ. 2002. Fine root turnover in a northern hardwood forest: a direct comparison of the radiocarbon and minirhizotron methods. Can J For Res 32:1692–7.

Trumbore SE, Gaudinski JB. 2003. The secret lives of roots. Science 302:1344–5.

Vogt KA, Vogt DJ, Bloomfield J. 1998. Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. Plant Soil 200:71–89.

Weaver JE. 1958. Classification of root systems of forbs of grassland and a comparison of their significance. Ecology 39:393–401.

Withington JM, Elkin AD, Bułłaj B, Olesińnski J, Tracy KN, Bouma TJ, Oleksyn J, Anderson LJ, Modrzyńnski J, Reich PB, Eissenstat DM. 2003. The impact of material used for minirhizotron tubes for root research. New Phytol 160:533–44.