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Total Belowground Carbon Allocation in a Fast-growing *Eucalyptus* Plantation Estimated Using a Carbon Balance Approach

Christian P. Giardina[1] and Michael G. Ryan*[2,3]

[1]*Department of Natural Resources and Environmental Management, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA;* [2]*US Department of Agriculture–Forest Service, Rocky Mountain Research Station, 240 West Prospect Street, Fort Collins, Colorado 80526, USA; and* [3]*Colorado State University, Department of Forest Sciences and Graduate Degree Program in Ecology, Fort Collins, Colorado 80523, USA*

Abstract

Trees allocate a large portion of gross primary production belowground for the production and maintenance of roots and mycorrhizae. The difficulty of directly measuring total belowground carbon allocation (TBCA) has limited our understanding of belowground carbon (C) cycling and the factors that control this important flux. We measured TBCA over 4 years using a conservation of mass, C balance approach in replicate stands of fast growing *Eucalyptus saligna* Smith with different nutrition management and tree density treatments. We measured TBCA as surface carbon dioxide (CO_2) efflux ("soil" respiration) minus C inputs from aboveground litter plus the change in C stored in roots, litter, and soil. We evaluated this C balance approach to measuring TBCA by examining (a) the variance in TBCA across replicate plots; (b) cumulative error associated with summing components to arrive at our estimates of TBCA; (c) potential sources of error in the techniques and assumptions; (d) the magnitude of changes in C stored in soil, litter, and roots compared to TBCA; and (e) the sensitivity of our measures of TBCA to differences in nutrient availability, tree density, and forest age. The C balance method gave precise estimates of TBCA and reflected differences in belowground allocation expected with manipulations of fertility and tree density. Across treatments, TBCA averaged 1.88 kg C m^{-2} y^{-1} and was 18% higher in plots planted with 10^4 trees/ha compared to plots planted with 1111 trees/ha. TBCA was 12% lower (but not significantly so) in fertilized plots. For all treatments, TBCA declined linearly with stand age. The coefficient of variation (CV) for TBCA for replicate plots averaged 17%. Averaged across treatments and years, annual changes in C stored in soil, the litter layer, and coarse roots (−0.01, 0.06, and 0.21 kg C m^{-2} y^{-1}, respectively) were small compared with surface CO_2 efflux (2.03 kg C m^{-2} y^{-1}), aboveground litterfall (0.42 kg C m^{-2} y^{-1}), and our estimated TBCA (1.88 kg C m^{-2} y^{-1}). Based on studies from similar sites, estimates of losses of C through leaching, erosion, or storage of C in deep soil were less than 1% of annual TBCA.

Key words: carbon budget; belowground carbon allocation; ecosystem respiration; root respiration; mycorrhizae; soil respiration; litterfall; soil carbon; fine root production; carbon sequestration.

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Current address for C. P. Giardina: USDA Forest Service, North Central Research Station, 410 MacInnes Drive, Houghton, Michigan 49931, USA
**Corresponding author*; *e-mail:* mgryan@fs.fed.us

Introduction

Plants allocate carbon (C) belowground to produce coarse and fine roots, for root respiration and exudates, and to support mycorrhizae (Raich and Nadelhoffer 1989). Total belowground C allocation (TBCA) is a large fraction of gross primary production (more than 30%) (Ryan and others 1994, 1997b; Gower and others 1996b). It can exceed aboveground net primary production (Law and others 1999) and provides the primary source of detrital C to mineral soil. Despite the significant role of TBCA in the C budget of terrestrial ecosystems, controls on TBCA are poorly understood. Net production of fine roots has been studied the most, using sequential coring, root ingrowth cores or screens (Caldwell and Virginia 1991), or fine root biomass derived from coring coupled with root growth and death from mini-rhizotrons (Steele and others 1997). These methods require assumptions that are not easily tested or have statistical problems (Caldwell and Virginia 1991), and the methods do not always agree (see, for example, Steele and others 1997). Also, these methods are labor intensive, so that sample sizes tend to be small and the means have large variances. The other components of TBCA (root respiration and exudates; mycorrhizal respiration and turnover) have been largely ignored, even though root respiration may be one to four times that of fine root production (Ryan and others 1997b) and greater than 50% of surface respiration (Ekblad and Högberg 2001; Högberg and others 2001).

Because of the uncertainty in estimates of fine root production and lack of estimates for other components of TBCA, Raich and Nadelhoffer (1989) proposed a "mass balance" approach to estimate TBCA that relies on conservation of mass. Here, we outline a similar approach that accounts for changes in C storage and advective losses.

Conservation of Mass and TBCA

Consider the belowground system to be bounded at the top of the litter layer. Through conservation of mass, outputs from the belowground system must equal inputs minus any change in storage over a defined time period. Therefore, C released from roots, mineral soil, and the litter layer through decomposition and respiration (surface carbon dioxide [CO_2] efflux or "soil" respiration, F_S) or export (erosion, leaching, or CH_4 efflux, F_E) must equal inputs from aboveground leaf, fruit, and twig litter (aboveground litterfall, F_A) plus belowground inputs (TBCA, the total of root respiration, carbohydrates used for mycorrhizae or exudates, and production of fine roots) minus any change in C stored in coarse or fine roots, litter, or soil per unit time (Δt):

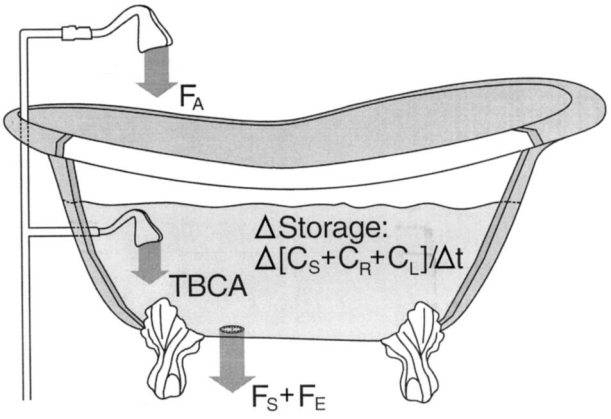

Figure 1. Analogy for mass balance approach to estimate TBCA. The flux of water into the tub from the underwater faucet (TBCA) can be calculated for any period of time by measuring the flux of water out of the tub ($F_S + F_E$), the flux into the tub from the faucet above water (F_A), and any change in water stored in the tub over the period ($\Delta [C_S + C_R + C_L]/\Delta t$). Through conservation of mass, the flux of water from the underwater faucet equals outputs minus inputs plus storage change. Similarly, TBCA = $F_S + F_E - F_A + \Delta [C_S + C_R + C_L]/\Delta t$.

$$F_S + F_E = \text{TBCA} + F_A - \Delta[C_S + C_R + C_L]/\Delta t \quad (1)$$

where C_S = carbon content of mineral soil, C_R = carbon content of root (coarse + fine) biomass, and C_L = carbon content of the litter layer. Put another way, any inputs must be respired, lost to leaching or erosion, or change storage.

Through conservation of mass, TBCA can be estimated by difference by measuring fluxes out of the soil–litter system (F_S and F_E), into the soil–litter system (F_A), and any change in C storage ($\Delta [C_S + C_R + C_L]/\Delta t$):

$$\text{TBCA} = F_S + F_E - F_A + \Delta[C_S + C_R + C_L]/\Delta t \quad (2)$$

The concept is the same as measuring the flow of water into a tub from a faucet under the surface of the water (Figure 1). Over some period of time, the flow from the underwater faucet (analogous to TBCA) equals the total water out of the drain (analogous to $F_S + F_E$) minus the total input from the faucet above the water (analogous to F_A) plus any change in the volume of water in the tub (analo-

gous to $\Delta[C_S + C_R + C_L]/\Delta t$). Conservation of mass dictates that the water entering via the underwater faucet can be measured, even though the water in the tub and the water flowing out of the drain is a mixture from both faucets. The primary obstacles to applying the C balance approach to TBCA are (a) measuring with reasonable precision the annual fluxes of inputs, outputs, and annual changes in C storage; and (b) the accumulation of error in calculating TBCA as a sum of many components, each with its own error.

Testing the TBCA Approach

In this study, we used this C balance approach to estimate TBCA in replicate stands of rapidly growing *Eucalyptus saligna* in a fully factorial experiment with two tree planting densities (10^4/ha and 1111/ha) and grown under two nutrient regimes (fertilization at planting only and continuous fertilization). Our first objective was to determine the response of TBCA to changes in fertility, tree planting density, and forest age. We hypothesized that (a) TBCA would decrease with fertilization because root growth, activity, and biomass decrease with greater nutrient availability (Keith and others 1997); (b) closely spaced trees would allocate more C belowground than widely spaced trees because of greater demand and competition for belowground resources (Binkley and others 1997); (c) in control plots, TBCA would increase with stand age as nutrient loss and sequestration in tree biomass reduce soil nutrient availability (Gower and others 1996a; Ryan and others 1997a); and (d) in fertilized plots, TBCA would remain constant through the study because growth would not be limited by nutrient availability (Gower and others 1996a).

A second objective was to evaluate how changes in C stored in roots, the soil, and the litter layer altered our estimates of TBCA. Raich and Nadelhoffer (1989) assumed that $\Delta[C_S + C_L + C_R]/\Delta t$ and F_E were zero; using published measurements of annual soil surface CO_2 efflux and litterfall for a wide variety of mature forest types, they estimated TBCA as F_S–F_A. Controversy surrounding the use of this simplified approach to estimate TBCA centers on the assumption that changes in C storage in soil, the litter layer, and roots can be ignored for forests that are not disturbed, irrigated, or fertilized (Gower and others 1996b; Nadelhoffer and others 1998). No study of TBCA has directly tested this assumption by quantifying rates of ΔC_S, ΔC_L and ΔC_R. For this objective, we hypothesized that changes in rates of C storage in soil, forest floor, and roots in a rapidly growing plantation forest (hereinafter simply ΔC_S, ΔC_L, and ΔC_R) would be large relative to the other components of Eq. (2).

Our final objective was to evaluate the accuracy of the C mass balance approach to estimating TBCA. We accomplished this by (a) estimating the variance for TBCA across replicate plots; (b) examining cumulative error associated with calculating TBCA; and (c) examining potential errors in technique and assumptions.

Methods

Study Site

The study site is a 2.5-ha plantation of *Eucalyptus saligna*, 13 km NNE of Hilo, Hawaii (19°50′28.1″N, 155°7′28.3″W). The site is at 350 m elevation and has a mean annual temperature of 21°C and an average annual rainfall of about 4000 mm (Binkley and others 1992). Soils are more than 2 m deep, acidic (pH 5–6 in water) and are classified as thixotropic, isothermic Typic Hydrudands in the Kaiwiki series. Sugar cane was cropped on the site for more than 80 years, with harvesting and replanting or ratooning every 2 years. From about 1920 onward, routine management of the soil included applications every 2 years of 85 kg N/ha, 75 kg P/ha, and 110 kg K/ha. After 1955, 700 kg/ha of lime was also added every 2 years. In 1993, the last sugar cane crop was harvested about 1 year before planting of *Eucalyptus* seedlings. The site was fallow for about 9 months and then, in February of 1994, plowed to turn under the developing vegetation (mostly C_4 grasses). Three months later, new regrowth was killed with a broadcast application of glyphosate herbicide (Roundup; Monsanto Company Agricultural Products, St. Louis, MO, USA). *E. saligna* seedlings were grown for 6 months in a greenhouse from a single, open-pollinated seed stock. Prior to planting in April 1994, seedlings were selected for uniform size (about 0.20–0.25 m in height). After 4 years of growth, tree height was about 25 m and woody biomass averaged 3.7 kg C/m^2 (J. H. Fownes, D. Binkley, M. G. Ryan unpublished).

The plantation contains 18 30 × 30 m plots. The experimental design has two treatments: two levels of tree spacing (1 × 1 m or 3 × 3 m, equal to 10^4 or 1111 trees/ha at planting) and three levels of fertilization (control, high fertilization, or delayed fertilization), organized in three randomized blocks. All plots received N+P+K+S+Ca+Mg in planting holes, followed by a broadcast application of the same at 7 months. Total fertilizer received during these two applications equaled 310 kg N/ha, 130 kg

P/ha, 260 kg K/ha, 125 kg Ca/ha, 23 kg Mg/ha, and 100 kg/ha micronutrients (5% Mn, 5% Zn, 5% Fe, 5% S, 1.5% Cu, and 0.5% B), the current recommendation for operational plantations. The high-fertilization (HF) treatment was designed to minimize nutrient limitations on *Eucalyptus* growth; from age 7 months to the end of this study, HF plots received quarterly applications 65 kg N/ha, 31 kg P/ha, 46 kg K/ha, and annual additions of 125 kg Ca/ha, 58 kg S/ha, 23 kg Mg/ha, and 100 kg/ha micronutrients (Binkley and Resh 1999). The delayed-fertilization treatment received the same fertilizer application as control plots until April 1998, after which they received the same applications as the high-fertilization plots. In this paper, we analyze and discuss only the control and HF treatments ($n = 12$ plots) because the delayed-fertilization treatment was not initiated until year 4.

Measuring Components of the Belowground C Budget

We estimated annual TBCA using Eq. (2), measurements of F_S, F_A, ΔC_S, ΔC_L, and estimates of ΔC_R from an allometric regression with aboveground biomass. We assumed that F_E was zero for our site (see discussion in Analysis of Errors and Assumptions, below). All measurements were taken within the interior 10 × 10 m area of the plots planted with 10^4 trees/ha or the interior 15 × 15 m of the plots planted with 1111 trees/ha.

Soil surface CO_2 efflux (F_S) and mineral soil temperature at 0.10 m depth were measured monthly at 15 locations along a transect running diagonally through each plot using a closed system (Field and others 1991), PPSystems CIRAS 1 gas analyzer (PPSystems, Haverhill, MA, USA) with a PPSystems soil respiration chamber (area = 7800 mm^2). Diel variation in soil temperature is less than 2°C in these shaded, moist soils, and for two 24-h measurement periods, F_S did not vary with time of day in any of the plots. Therefore, we estimated monthly F_S as measured rate (μmol m^{-2} s^{-1}) multiplied by 2.63×10^6 s, the number of seconds in 1 month.

Because recent studies (Le Dantec and others 1999; Janssens and others 2000) have shown that the PPSystems chamber can yield higher F_S than the LI-COR soil respiration chamber (LI-COR 6400-09; LI-COR, Lincoln, NE, USA), we compared the PPSystems chamber to the LI-COR chamber at our site. We measured F_S with both systems on 12 of our treatment plots, with eight subsamples per plot. For the LI-COR measurements, we inserted collars 2–3 cm into the soil at least 4 h prior to sampling, following the approach outlined in Norman and others (1997). For the PPSystems measurements, the chamber was inserted into soil and litter (approximately 1 cm) immediately prior to the flux measurement (the same method used throughout this 4-year study). For this comparison, plot means for F_S ranged from 3.1 to 6.4 μmol m^{-2} s^{-1} for the PPSystems and 2.8 to 6.0 μmol m^{-2} s^{-1} for the LI-COR chamber, and plot means were correlated between methods ($r = 0.70$, $P < 0.01$). Average F_S (4.2 μmol m^{-2} s^{-1} for PPSystems and 4.0 μmol m^{-2} s^{-1} for the LI-COR) did not differ ($P = 0.39$, paired-samples t-test), and a zero-intercept linear regression of plot means (intercept was not significant in a standard linear regression, $P = 0.26$) yielded PPSystems = 1.04 × LI-COR. Because of these results, we did not adjust estimates of F_S. In another field test, the PPSystems chamber and analyzer produced similar rates for F_S as the LI-COR Soil Respiration Chamber operating with a LI-COR 6200 (Arneth and others 1998).

Aboveground litter was trapped in eight trays (0.186 m^2 each) per plot. Each tray was set on the forest floor and collected monthly. At the time of collection, litter from each trap was composited by plot; dried at 70°C to constant weight; separated into leaf material or twig, branch, and bark material; and then weighed. Fresh foliage biomass averaged 50.6% C; fresh woody biomass averaged 48.2% C. Because most of the litter was leaf material, we assumed a C content of 50% for total litter. Litter decomposes rapidly in this wet tropical environment, so we corrected leaf litter weight for estimated decomposition in the trap (estimated leaf litter inputs = measured input × 1.15). To derive our estimate, we assumed that (a) litterfall was constant over the 30 days in the measurement period, (b) decay was a constant fraction of litter mass (exponential decay), and (c) decay rate of 0.0095/day equaled those measured over 1 month for 12 individually tethered leaves. All leaves were intact after 1 month, and mass loss ranged from 15% to 25%.

Forest floor standing crop was sampled in January of each year using eight subsamples (0.186 m^2 each) per plot. Samples were composited by plot; dried at 70°C to constant weight; separated into leaf and twig, branch, and bark components; and then weighed. We also assumed that mean C content in 1997 (53%) measured with a LECO CHN Analyzer (LECO, St. Joseph, MN, USA) applied to all years for forest floor material. We estimated ΔC_S as the annual difference in forest floor C mass for each plot.

Biomass of coarse roots was estimated from aboveground biomass (measured annually) using an allometric relationship between coarse root bio-

mass (more than 10 mm) and aboveground biomass (J. L. Stape, unpublished). The regression was developed using 18 *E. grandis x urophylla* trees (a hybrid of two species that are closely related to *E. saligna*) growing in plantations in Bahia State, Brazil. Precipitation for these plantations averaged 1600 mm/y, and biomass for trees used in the allometric relationship varied from 100 to 600 kg. For roots excavated down to 7 m, coarse root biomass (kg) = 0.154 × aboveground biomass (kg) (R^2 = 0.87, estimated for the zero-intercept regression as in Kvalseth 1985). We estimated the biomass of 2–10-mm roots as the proportion (21%) of 2–10-mm roots to roots larger than 10 mm reported in a study with *E. grandis* in Brazil (Reis and others 1985). Coarse root biomass for roots larger than 2 mm was thus 0.19 × aboveground wood + bark biomass. For nine *E. saligna* trees at a site near our study site, coarse root biomass (more than 2 mm) averaged 0.23 × aboveground wood + bark biomass R. Powers, and D. Binkley, unpublished). Allometric relationships for other species show similar values for the ratio of coarse root biomass to aboveground wood + bark biomass (0.22 in *Pinus radiata* [Jackson and Chittenden 1981] and 0.20 for *E. nitens* [Misra and others 1998]). Both these studies showed that the ratio was insensitive to growing conditions. We estimated ΔC_R as the annual difference in coarse root biomass in winter.

Fine root biomass (less than 2 mm diameter) was measured using three cores per plot in October 1995 and January 1996 (0–1.0 m) and 15 cores per plot in August 1999 (0–0.2 m). In October 1995, fine root biomass in 0–0.2-m soil represented approximately 60% of the fine root biomass in 0–1.0 m soil, and we used this ratio to estimate total fine root biomass in 1999. Because biomass changed less than 0.02 kg C m^{-2} y^{-1} from 1995 to 1999, fine root biomass was approximately 5% of total root biomass, and fine root biomass was not estimated in 1997 and 1998, we assumed that the annual change in C stored as fine roots was zero.

In March 2000, we examined mycorrhizal infection rates for fine roots in all plots. Fruiting bodies in the plantation were dominated by two species of mycorrhizal fungi, *Laccaria fraterna* and *Scleroderma verrucosum*. Root samples were taken from 10 0–0.20-m depth cores per plot. Cores were composited by plot and refrigerated immediately after sampling. Four days after sampling, fine roots were hand-picked from soils, washed with H_2O and H_2O_2, and stained according to the trypan blue method (Phillips and Hayman 1970). Percent infection was then determined by direct stereoscopic observation of stained roots.

Prior studies at nearby sites with similar soils showed that total soil C below 0.15 m did not differ between native forests, sugar cane plantations, and *Eucalyptus* plantations (Bashkin and Binkley 1998). Additionally, 80 years of intensive sugar cane cultivation (sugar cane has a C_4 photosynthetic pathway whereas trees have a C_3 pathway) did not alter the $\delta^{13}C$ of soil below 0.45-m soil depth (Bashkin and Binkley 1998). Therefore, we sampled soil C to 0.30-m depth and assumed zero change in soil C below 0.30 m. Soil was sampled at three permanently located sites per plot in April 1994, prior to planting, in January 1997, and in August 1999 (Binkley and Resh 1999; D. Binkley unpublished). We estimated ΔC_S for each plot as the difference in soil C for the sample period divided by period length in years.

Statistical Analysis

Our sample unit was the plot, and estimates of all components and TBCA were made for each plot using data collected on that plot. Differences in F_S, F_A, ΔC_R, ΔC_L, ΔC_S, and TBCA with time or related to tree density or fertility were assessed using repeated-measures analysis of variance (ANOVA) (year = repeated measure, and block, fertility regime, and tree density were main effects). Analyses were computed with SPSS procedure GLM (SPSS, Chicago, IL, USA). Because the interaction between tree density and fertility was not significant, we present means for the two tree planting densities and two fertility treatments rather than the four separate treatment combinations. The effects of subsampling and cumulative error on estimates of variance for TBCA are discussed in Analysis of Errors and Assumptions.

Results

Components of the TBCA Budget

Over 4 years of measurement, soil surface CO_2 efflux (F_S, or "soil respiration") averaged across treatments was 5.4 μmol m^{-2} s^{-1}. For all treatments, F_S was generally higher in summer than in winter, and peaked in year 1 (Figure 2). In years 1 and 4, control and HF plots had similar F_S rates in the summer; in years 2 and 3, control plots maintained higher summer efflux rates than the HF plots (Figure 2). Variation in monthly F_S was poorly correlated with soil temperature at 0.10-m depth ($r <$ 0.56, generally much lower), except for the control treatment with 10^4 trees/ha in year 1 (r = 0.75). Correlation coefficients were generally lower in the plots with 1111 trees/ha and in the HF treatment.

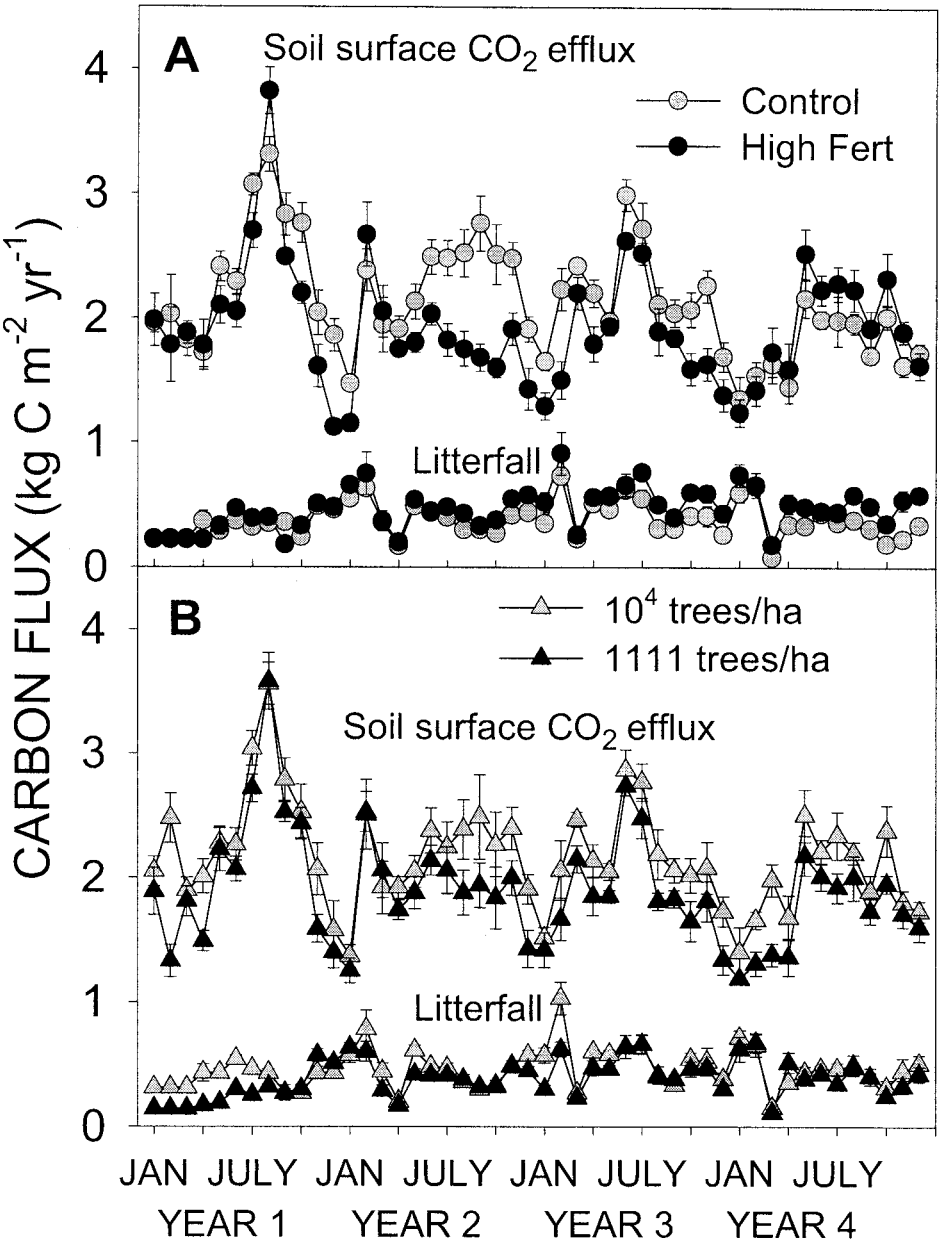

Figure 2. Monthly soil surface CO_2 efflux and litterfall by fertility treatment (**A** control, $n = 6$; high fertilization, $n = 6$) and stem density (**B** 10^4 trees/ha, $n = 6$; 1111 trees/ha, $n = 6$). Error bars are standard errors of means (SEM).

Annual total F_S was lower in continuously fertilized plots compared to controls (1.92 versus 2.14 kg C m^{-2} y^{-1}, $P < 0.01$) and higher in plots with 10^4 trees/ha compared to plots with 1111 trees/ha (2.17 versus 1.89 kg C m^{-2} y^{-1}, $P < 0.01$). Annual F_S declined linearly through the 4 years of measurements, from 2.23 kg C m^{-2} y^{-1} in year 1 to 1.84 kg C m^{-2} y^{-1} in year 4 ($P < 0.01$); there was no interaction between stand age and density ($P = 0.90$). By age 4, F_S no longer differed between the control and HF treatments ($P = 0.21$).

Annual total litterfall C (F_A) was greater in fertilized plots (0.47 versus 0.38 kg C m^{-2} y^{-1}, $P < 0.01$) and less in plots with lower stem density (0.38 versus 0.46 kg C m^{-2} y^{-1}, $P < 0.01$). F_A increased with stand age ($P < 0.01$), and the increase with age was greater for the fertilized stands. Monthly F_A was generally higher in the winter since the lower light removed foliage from the canopy. Monthly and annual F_A and F_S were not correlated ($r = 0.14$, $P = 0.28$ for monthly; $r = 0.10$, $P = 0.58$ for annual).

The estimated accumulation of C in coarse roots (ΔC_R) was larger than changes in soil or forest floor C. The annual change in C stored in roots (ΔC_R) averaged 0.21 kg C m^{-2} y^{-1}; it was greater in plots with

10^4 trees/ha and for the HF treatment and decreased with stand age ($P < 0.01$). Fine root biomass in 0–0.2-m depth soils for control plots was 0.07 ± 0.05, 0.06 ± 0.02, and 0.06 ± 0.01 kg C/m^2 in 1995, 1996, and 1999, respectively. Fine root biomass in HF plots in 1995, 1996, and 1999 was slightly lower: 0.04 ± 0.02, 0.06 ± 0.02, and 0.04 ± 0.01 kg C/m^2, respectively. Because fine root biomass was roughly constant within treatments for all years measured, we assumed that the change in fine root biomass was zero from 1995 (year 1) to 1999. Mycorrhizal infection rates were lower ($P < 0.01$) in the HF treatment (8.4%) than in controls (36.5%). The annual change in C in mineral soil (ΔC_S) averaged −0.01 kg C m^{-2} y^{-1}, with no difference between fertilization treatment ($P = 0.19$), stem density ($P = 0.91$), or sampling periods ($P = 0.63$). The annual change in forest floor C mass (ΔC_L) declined with stand age for all combinations of treatments ($P = 0.01$); the highest accumulation rates occurred in year 1 (0.11 kg C m^{-2} y^{-1}) and there was little change by year 4 (−0.01 kg C m^{-2} y^{-1}). The annual change in C stored in roots, soil, and the litter layer ($\Delta[C_S + C_R + C_L]/\Delta t$) did not vary with stand age ($P = 0.38$) (Figure 3).

Total Belowground Carbon Allocation

Over 4 years, TBCA in the HF treatment averaged lower than the control (1.76 versus 2.01 kg C m^{-2} y^{-1}), but the difference was not significant ($P = 0.06$). *Eucalyptus* allocated more C belowground in plots with 10^4 trees/ha than in plots with 1111 trees/ha (2.04 versus 1.73 kg C m^{-2} y^{-1}, $P = 0.03$). The interaction of fertilization and stem density was not significant ($P = 0.81$) (Figure 3). TBCA declined linearly with stand age for all density and fertility treatments, from 2.24 kg C m^{-2} y^{-1} in year 1 to 1.61 kg C m^{-2} y^{-1} in year 4 ($P < 0.01$); there was no interaction between stand age and stem density ($P = 0.49$) or fertility treatment ($P = 0.40$), indicating that the decline in TBCA with stand age was similar for all treatment combinations.

We quantified the effect of changes in C storage in soil, forest floor, and roots ($\Delta[C_S + C_L + C_R]/\Delta t$ in Eq. [2]) on TBCA by comparing TBCA estimated with the full model in Eq. (2) with TBCA estimated assuming zero change in C storage. Averaged across all treatments and years, assuming that $\Delta[C_S + C_L + C_R]/\Delta t = 0$ yielded estimates of TBCA that were 13.6% lower than estimates calculated from the full model (Eq. [2]). High-density plots in the HF treatment had the largest bias (−19%), and low-density control plots had the smallest bias (−10%), but there were no statistical differences in error rate between fertility ($P = 0.23$) or planting density treatments ($P = 0.62$). Annual increases in coarse roots were responsible for most of the increase in C storage. Including estimates of coarse root biomass in the calculation of TBCA (that is, assuming that $\Delta[C_S + C_L] = 0$ and TBCA $= F_S - F_A + C_R$) reduced the bias in TBCA to an average of a 2.1% underestimate across treatments.

The conservation of mass, C balance approach for estimating TBCA produced precise estimates for treatment means. The coefficient of variation (CV) for TBCA for replicate plots within a year ($n = 3$) ranged from 6% to 36% and averaged 17% over all treatments and years. The CV for TBCA was greater than the CV among replicate plots for the two largest components of TBCA (average CV for F_S and F_A was 7.9% and 9.1%, respectively), demonstrating the effect of "cumulative" error. As discussed below in Analysis of Errors and Assumptions, error or uncertainty calculated from plot-level estimates of TBCA incorporates subsample error and the "cumulative" error associated with summing F_S, F_A, ΔC_R, ΔC_L, ΔC_S to calculate TBCA.

Discussion

Components of the TBCA Budget

Soil surface CO_2 efflux at our site (1.61–2.51 kg C m^{-2} y^{-1}) exceeds the range of published values for tropical moist forests (0.89–1.45 kg C m^{-2} y^{-1}) (Raich and Schlesinger 1992). The large annual F_S in this study likely relates to the high productivity of forest plantations in the moist tropics (Binkley and others 1997). Annual F_S from nearby 16-year-old plantations of *E. saligna* (2.3 kg C m^{-2} y^{-1}) and *Albizia falcataria* (2.6 kg C m^{-2} y^{-1}) was also high (Binkley and Ryan 1998), suggesting that plantations of fast-growing trees may continue allocating large quantities of C belowground for many years. Warm temperatures (mean annual temperature of 21°C), high rainfall (around 4000 mm y^{-1}), fertile soils, and the absence of a dry season may also contribute to the high F_S at our site.

F_S was poorly correlated with soil temperature at 0.10 m, except in year 1 for the plots with 10^4 trees/ha. The lack of correlation with soil temperature and variation in the relationship under different growth conditions suggests that soil temperature does not strongly influence F_S at this site. Others have related variation in F_S to temperature (Raich and Potter 1995; Boone and others 1998; Raich 1998), but the lack of a strong seasonal temperature change at our site may allow other factors, such as photosynthesis and root activity (Fitter and others 1999; Ekblad and Högberg 2001; Högberg and others 2001; Janssens and others

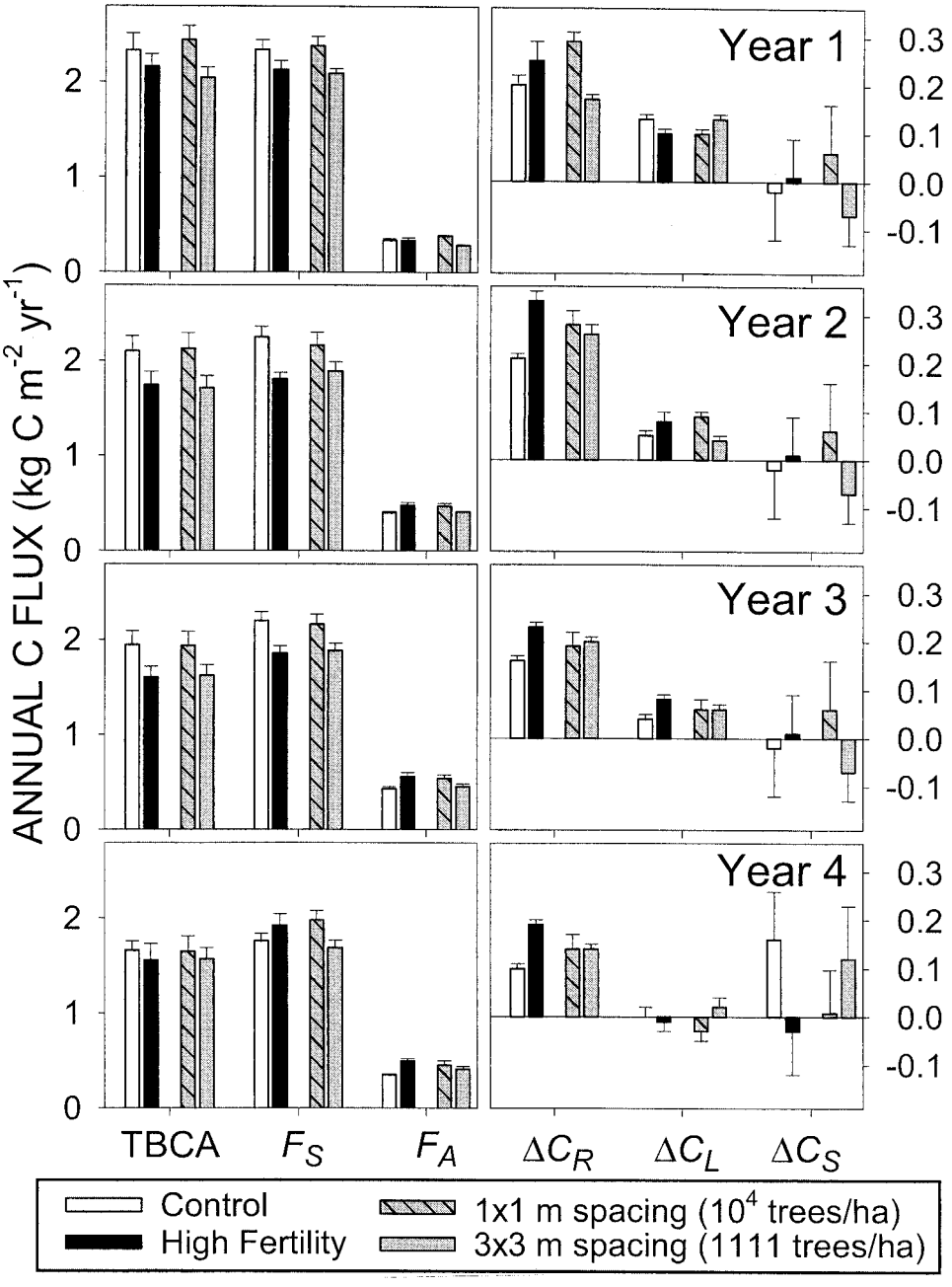

Figure 3. Components used in calculating the annual belowground C budget: means by fertility treatment (control, $n = 6$; high fertilization, $n = 6$) and stem density (10^4 trees/ha, $n = 6$; 1111 trees/ha, $n = 6$). Error bars are standard errors of means (SEM). F_S = surface CO_2 efflux, F_A = aboveground litterfall, and ΔC_R, ΔC_L, and ΔC_S = the annual change in C stored in coarse roots, litter layer, and mineral soil, respectively. Note different scales for right- and left-hand panels.

2001), to exert a stronger influence on F_S than soil temperature.

Annual changes in C storage in soil and forest floor were minor compared to F_S (average of 3% across treatments) and had little effect on estimates of TBCA (average of 2.1%). The small changes in these storage components relative to TBCA are notable because of the very rapid growth of *Eucalyptus* at this site. Despite large inputs of aboveground litter (0.42 kg C m^{-2} y^{-1}) and TBCA (1.88 kg C m^{-2} y^{-1}), forest floor mass stabilized at a low level within a few years after planting and total C in mineral soil changed little over the study period. Small changes in soil C also have been observed for nearby sites with similar soils and changes in land use (Bashkin and Binkley 1998).

Response of TBCA to Changes in Nutrient Availability, Tree Density, and Stand Age

If nutrient availability increases at a site, the amount of C required to acquire nutrients may decline as belowground resources become less limiting to plant growth than aboveground resources (Albaugh and others 1998; Keith and others 1997).

Changes in C allocation belowground can occur as a lowered flux, or as changes in the partitioning of photosynthesis, or both. Increased nutrient availability did not alter the flux of TBCA in the HF plots. In these plots, more coarse root biomass and perhaps greater root respiration rates because of higher nitrogen (N) content (Pregitzer and others 1998) offset lower fine root biomass and lower mycorrhizal infection rates. Increased nutrient availability did, however, dramatically lower partitioning of photosynthesis to TBCA from approximately 45% to approximately 35% (C. P. Giardina, M. G. Ryan, D. Binkley, J. H. Fownes unpublished). The greater TBCA in plots with high stem density may be a function of greater nutrient use and increased competition for belowground resources (Binkley and others 1997) or greater gross primary production in high-density plots (Janssens and others 2001).

We hypothesized that TBCA would increase with stand age in control plots as nutrient availability declined, but that TBCA would remain constant with stand age in HF plots. Instead, TBCA declined with stand age in all treatments, and the decline was similar for control and HF treatments until year 4. In an age sequence of lodgepole pine stands in Wyoming, declines in TBCA with stand age corresponded with declines in aboveground net primary productivity (ANPP) (Smith and Resh 1999). As with our study, the age-related decline in TBCA in the lodgepole pine forests was unrelated to soil nutrient availability (Olsson and others 1998).

Raich and Nadelhoffer (1989) constructed global-scale relationships between litterfall and F_S or TBCA. They speculated that these relationships would generally apply to mature forests but would not apply where changes in C storage were large (for example, plantations, disturbed forests, forests established on agricultural land). Using measured F_A as the independent variable, F_S and TBCA in our study were poorly estimated with these relationships (a paired samples *t*-test showed actual F_S and TBCA were different from estimates using the Raich-Nadelhoffer equation, $P < 0.01$) (Figure 4). Although changes in C storage have been invoked to explain why the Raich-Nadelhoffer equations failed at smaller scales (Gower and others 1996b; Nadelhoffer and others 1998), changes in C storage were too small to explain the failure of these global-scale relationships at our site (Figure 4). We are unsure why these relationships did not apply in our stands, but the predictions from the equation improved with stand age (Figure 4).

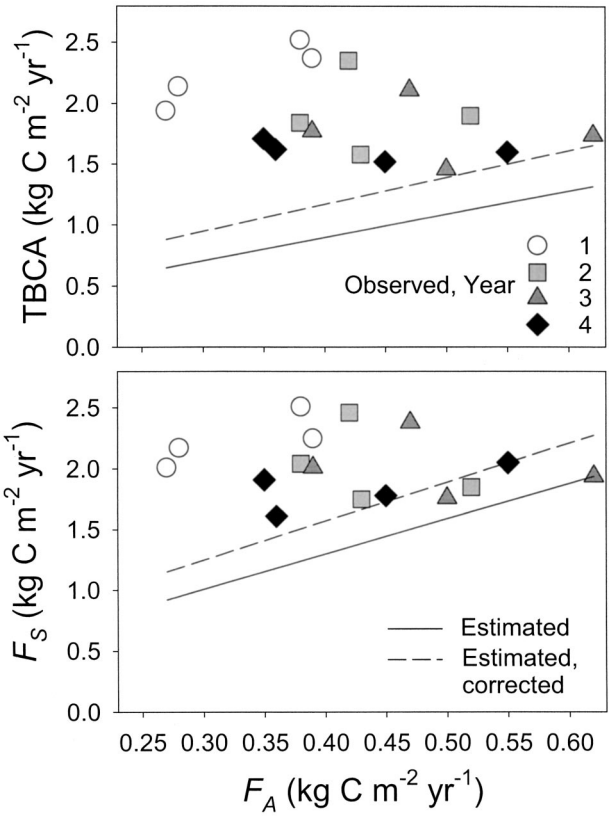

Figure 4. Observed annual total belowground C allocation (TBCA) and surface CO_2 efflux (F_S) versus annual litterfall (F_A). Values are means of treatment combinations (fertilization regime × seedling density) for each year; F_A was not correlated with TBCA ($r = -0.34$, $P = 0.19$) or F_S ($r = -0.17$, $P = 0.53$). Solid lines are TBCA and F_S estimated from a global data set (TBCA = 0.13 + 1.92 F_A; F_S = 0.13 + 2.92 F_A) (Raich and Nadelhoffer 1989). Dashed lines are TBCA and F_S estimated from the Raich-Nadelhoffer equations, corrected for changes in C storage in roots, soil, and forest floor.

Analysis of Errors and Assumptions

Two statistical problems are perceived to arise for estimating TBCA or its components: (a) subsamples are required to derive an estimate for a given plot, so that each plot-level estimate carries uncertainty and this uncertainty must increase the overall uncertainty in TBCA; and (b) because TBCA is a sum, its variance must be calculated as the sum of the variances of the individual variables plus the sum of the covariance between each pair of the variables to account for "cumulative" error.

Perceived problem (a) is easily resolved. The variance for a plot level estimate based on subsampling is:

$$V(\bar{\bar{y}}) = \frac{1 - n/N}{n} s_1^2 + \frac{n/N(1 - m/M)}{nm} s_2^2 \quad (3)$$

where s_1^2 = variance among plots not related to subsampling, s_2^2 = the subsample variance, n = the number of plots, N = the population of possible plots, m = the number of subsamples, and M = the number of possible subsamples (Cochran 1977). Because n/N is approximately 0 for field studies, subsample variance adds approximately 0 to the overall variance, and the plot-level variance is an excellent estimator of the true variance. We verified this conclusion using a simulation study and showed that for true replicate plots the standard deviation of replicate plots = the standard error of the subsample variance for an individual plot.

Perceived problem (b) does not, in fact, exist. The variance of the plot-level estimates of TBCA is mathematically equivalent to the sum of the variances of the individual variables plus the sum of any covariance between each pair of the variables (Mood and others 1974). With our data set, we verified this for individual treatments, treatment combinations, and annual means. To four significant digits, the variance of the plot-level estimates of TBCA equaled the sum of the variances of F_S, F_A, ΔC_S, ΔC_R, ΔC_L plus the sum of the covariance between each pair of the variables. In simple terms, the variance of the plot-level estimates of TBCA (which we use) contains all available information on subsampling error and "cumulative" error.

The change in coarse root biomass (ΔC_R) represented the largest change in C storage at our site, and we evaluated the potential error in using an allometric relationship to estimate changes in coarse root C that was developed from another site and for a closely related species of *Eucalyptus*. We estimated coarse root biomass (larger than 2 mm) as 0.19 × aboveground wood + bark biomass. This ratio was similar to that found for nine *E. saligna* trees at a nearby site (0.23) (R. Powers, and D. Binkley, unpublished) and similar to those of other species of trees (0.22 in *Pinus radiata* [Jackson and Chittenden 1981] and 0.20 for *E. nitens* [Misra and others 1998]). Because ΔC_R averaged 11% of TBCA, if the true ratio of coarse root biomass to aboveground wood + bark biomass was between 0.15 and 0.23, the potential error in TBCA from using an incorrect allometric would be less than 2%. Given the conservative nature of the ratio of coarse root biomass to aboveground wood + bark biomass for other plantations of fast-growing trees, we expect our estimates of C_R to be robust.

Although sampling for fine roots was not extensive and in 1999 was limited to the surface 0.2 m, fine root biomass in 0–0.20-m soil showed little or no change from 1995 to 1999. Further, because fine root biomass in the surface 1.0 m was small (around 0.06 kg C/m^2), even a 100% annual change in fine root standing crop would cause only a ±3% error in TBCA.

Soil C represents the largest belowground pool of C. For similar sites and soils, Bashkin and Binkley (1998) report 11 kg C/m^2 to 0.55-m depth. Our soils are approximately 1.5 m deep and if the C content from 0.55 to 1.5 m equals that in 0.4–0.55 m, as reported in Bashkin and Binkley (1998), total soil C at our site would be more than 20 kg C/m^2. Because TBCA averaged 1.9 kg C m^2 y^{-1}, relatively small annual changes in total soil C have the potential to have a large effect on TBCA. Therefore, precise estimates of soil C storage are needed to calculate TBCA. The potential sources of error in our estimate of ΔC_S are (a) subsample and plot-to-plot variability, which is already incorporated into estimates of TBCA; and (b) no information on ΔC_S below 0.3 m.

The potential error in TBCA from assuming $\Delta C_S = 0$ below 0.3 m is likely very small. For similar sites and soils, Bashkin and Binkley (1998) found no change in soil C for 0–0.55 m over 10–13-year periods. At our study site, ΔC_S was estimated from 1997 to 1999 for 0–0.45 depth and also found to be zero (D. Binkley unpublished). Decomposition of soil C below 0.5 m in our soils is extremely slow (Torn and others 1997), as indicated by the observation that 80 years of sugar cane cultivation failed to change the $\delta^{13}C$ signature from that found in native forests (Bashkin and Binkley 1998). Because detrital inputs are greatest near the soil surface and changes in soil C are highest in the upper soil layers (Bashkin and Binkley 1998; Richter and others 1999), the lack of change in soil C in the surface 0.3 m of soil strongly suggests no change below 0.3 m. For similar sites and soils, soil C content below 0.25 m in *Eucalyptus* forests is similar to that found in native forests (Bashkin and Binkley 1998), and the small difference suggests a ΔC_S rate of 0.003 kg C m^{-2} y^{-1}. Finally, rates of ΔC_S reported for other ecosystems are small (−0.09 to 0.1 kg C m^{-2} y^{-1}; average, 0.035 kg C m^{-2} y^{-1}) (Post and Kwon 2000) compared to our mean TBCA of 1.88 kg C m^{-2} y^{-1}. Using the largest rates of ΔC_S reported in Post and Kwon (2000) at our site would change TBCA by ±5%. Our soils do contain a lot of C, but it accumulated over thousands of years (Torn and others 1997).

We corrected measured litterfall for mass loss that occurred in our litter traps between measurements. To estimate a correction factor, we measured mass loss from tethered leaves. A study of decomposing *Eucalyptus* leaves (Bernhard-Reversat 1999) suggested that leaching probably accounts for less than

50% of the mass loss observed in our 1-month decomposition study. However, as long as the original mass of F_A is estimated accurately, TBCA will not be biased (Eq. [2]). Although the C lost from leaves in the litter traps between collections was likely partly respired and partly leached, neither outcome would alter our estimates of TBCA. This is because any C released from decomposition is measured as F_S and any C leached into the soil is measured as F_S if respired quickly, or as ΔC_S or ΔC_L if leachates remain in the soil or litter layer.

At our site, F_E (loss of C through leaching below 0.35 m, erosion off site, or CH_4 efflux) was likely negligible. Reported losses of dissolved organic C (DOC) and dissolved inorganic C (DIC) are very small for closed canopy forests (Raich and Nadelhoffer 1989; Raich 1998; Richter and others 1999; Campbell and others 2000; Fisher and Binkley 2000). Although tropical data are sparse, concentrations of DOC are typically low in forest soils (Fisher and Binkley 2000), and losses from temperate forest soils rarely exceed 0.01 kg C m^{-2} y^{-1} (Campbell and others 2000). We estimated the loss of DOC from our site using information on losses of dissolved organic nitrogen (DON) and the ratio of DON to DOC for nearby sites. For these sites, losses of DON rarely exceeded 5 kg N ha^{-1} y^{-1}, and maximum DOC:DON was 26 (Neff and others 2000). Using these maximum estimates gives a DOC loss of 0.013 kg C m^{-2} y^{-1} (less than 0.7% of TBCA); this value likely represents the maximum export rate for our site. The downward flux of DIC is also negligible. If we use a high DIC of 1.5 mg C/L (Fisher and Binkley 2000) and assume that 2 m of annual precipitation (50%) moves through the soil, losses of DIC from our site would be 0.003 kg C m^{-2} y^{-1} (less than 0.2% of TBCA). Most upland forests are small net consumers of CH_4 (less than 0.001 kg C m^{-2} y^{-1}) (Le Mer and Roger 2001).

Erosion under a closed canopy is minimal (Raich and Nadelhoffer 1989; Raich 1998; Richter and others 1999) because the canopy and forest floor layer intercept rainfall, greatly reducing the impact of raindrops on soil particle movement (Fisher and Binkley 2000). In forests, roots bind soil together, further reducing soil movement. Other Typic Hydrudand soils on the island of Hawaii were shown to have no erosion-related soil loss, even where soil was without the cover of litter or vegetation (El-Swaify and Dangler 1976), so our assumption of zero C loss in erosion seems reasonable.

Because F_S is the largest contributor to TBCA, annual estimates of F_S have the greatest potential for contributing error or bias to estimates of TBCA. Annual estimates of F_S have two potential sources of error or bias: (a) bias in individual flux measurements, and (b) errors in estimating annual fluxes from point measurements. True F_S in field measurements is unknown, and different methods produce estimates that can vary ±20% (Norman and others 1997). Although results from the method used in this study did not differ from the recommended standard (Norman and others 1997), additional study is needed to determine true F_S and assess measurement technology. Bias in extrapolation could occur if F_S varied strongly over a 24-h day, and measurements are routinely taken under conditions that do not represent the average. Our measurements were always taken during the daytime, but measurements over two 24-h periods showed no diel pattern (variation in soil temperatures was less than 2°C). Also, the annual pattern of low fluxes in winter and high fluxes in summer was repeated for all 4 years.

Changes in C Storage

An assumption that $\Delta[C_S + C_L + C_R] = 0$ will give biased estimates of TBCA, particularly in aggrading forests, where storage may be increasing rapidly. In general, changes in total soil C content following shifts in land use appear small (Richter and others 1999; Post and Kwan 2000). Therefore, if changes in soil C and forest floor mass are much smaller than F_S, as appears to be the case for forests, then a simplified C balance approach to estimating TBCA (using measurements of F_S, F_A, and C_R) may be reasonably accurate for most forests (less than 3% bias in our study), even those that are young or recently disturbed. We recommend additional study to assess the role of changing C storage in estimates of TBCA.

Applicability to Other Ecosystems

Based on these analyses, we believe that the C balance method can be applied to other, more complex ecosystems, including sites where F_S and F_A are currently collected or have been collected routinely (see for example, Raich and Nadelhoffer 1989). Variance among replicate plots or stands for plot-level estimates is not large, even for nonplantation ecosystems, and replicate stands increase the power of inference. For example, when Smith and Resh (1999) estimated TBCA for a replicated chronosequence of *Pinus contorta*, they found that the CV for their replicate plots was approximately 15%, comparable to the CV found in this study. For an individual site, precision can be gained by increasing the number of subsamples (Veldkamp and Weit 1994), and compositing and paired resampling can lower the variance for estimates of ΔC_S. In ecosys-

tems that are more strongly seasonal in temperature and soil moisture and have large diel temperature variability, measurements of F_s would need to capture that variability (Keith and others 1997). Finally, changes in pools measured over long time periods will be more accurate than measurements of short-term change.

The C balance approach does not provide information about the size of individual components of TBCA (that is, mycorrhizal respiration, root respiration, dry matter production). Knowing the fraction of TBCA partitioned to dry matter production and to autotrophic respiration is important because root detritus enters the soil C cycle, whereas CO_2 from respiration escapes directly to the atmosphere. However, information on belowground allocation is scarce, particularly for the tropics (Clark and others 2001), and the C balance approach can set an upper limit for components derived using other methods (Raich and Nadelhoffer 1989). Estimates of TBCA also can be used with measures of ANPP and aboveground respiration to estimate gross primary production (Ryan 1991; Ryan and others 1997b).

Conclusions

The C balance method yielded precise estimates of TBCA, with an average coefficient of variation of 17% for replicate plots. This rapidly growing *Eucalyptus* plantation had very high annual rates of F_s and allocated substantially more C belowground than has been reported for native forests. High tree density increased TBCA throughout the study, whereas fertility had no significant effect on TBCA. TBCA declined linearly with stand age, and this decline was not influenced by tree density or fertilization regime.

Despite an annual input of over 2.3 kg C m^{-2} y^{-1} as F_A and TBCA, changes in C stored in litter and soil were less than 3% of TBCA, indicating that much of TBCA was quickly returned to the atmosphere as F_S. At our site, measuring changes in C stored in soil and the litter layer was not difficult, and the C balance method of estimating TBCA could be applied in other forest ecosystems. If ΔC_S and ΔC_L cannot be determined, our data suggest that site-specific measurements of F_S, F_A, and ΔC_R may provide reasonable estimates of TBCA (bias of less than 3%), even if the site is young or disturbed.

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