First-Rotation Changes in Soil Carbon and Nitrogen in a *Eucalyptus* Plantation in Hawaii

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ABSTRACT

We measured soil changes through a full rotation of a *Eucalyptus saligna* (Sm.) plantation. We hypothesized that accretion of C from *Eucalyptus* trees (C_3–derived carbon, C_3–C) would be balanced by an equal loss of older soil C derived from sugarcane (*Saccharum officinarum* L.) agriculture (C_4–derived C, C_4–C). We also hypothesized that large additions of N-containing fertilizer would increase C accretion by increasing the rate of C addition and decreasing the rate of C loss. The low spatial variability of the soil and the intensive sampling design provided precise tests of these hypotheses. Soil C averaged 13.8 kg m^{-2} for the O horizon plus the 0- to 45-cm depth mineral soil, with no change through the rotation [95% confidence interval (CI) $\pm$0.057 kg m^{-2} yr^{-1}], supporting the first hypothesis. Significant gains of C_3–C (0.136 kg m^{-2} yr^{-1}) balanced the losses of C_4–C (0.144 kg m^{-2} yr^{-1}). The second hypothesis was tested in the field using three levels of repeated, complete fertilization (including N at rates of 300, 700, and 1600 kg N ha^{-1}), and in laboratory incubations with N addition. Addition of N had no effect on the accumulation of soil N and C_3–C, nor on the rate of loss of older C_4–C, refuting the second hypothesis. This first-rotation forest plantation was not able to increase soil C, even with heavy fertilization. These results contrast markedly from the soil changes under the influence of N-fixing trees, indicating that the effect of N fixation on soil C derives from factors other than N supply.

How rapidly does soil content of C and N change in forest soils? Rates of change in soil organic matter and its C content are important for sustaining soil fertility, for sequestering (or releasing) C to the atmosphere, and as a key component for estimating belowground production using C mass balance (Raich and Nadelhoffer, 1989; Giardina and Ryan, 2002). Detecting rates of change in soil C and N can be almost impossible in forest soils with extremely high spatial variation. For example, Johnson (1995) carefully sampled 60 locations within the rocky soil of a watershed at the Hubbard Brook Experimental forest, and found that the coefficient of variation in soil N content was about one-third of the mean among soil pits. Repeated sampling with 60 pits across the watershed would not detect significant changes in soil N content unless the change exceeded about 700 kg ha^{-1} N (about 10% of the total N). Soils with such extreme variability can provide only weak tests of hypothesized changes across time or responses to experimental treatments. Detection of changes is much more precise in forest soils with low spatial variability. Richter and Markewitz (2001) characterized the change in ecosystem N content across time at the Calhoun Experimental Forest, and changes of 300 kg ha^{-1} N could be detected with a 95% confidence with eight replicate plots.

Even when precise soil C measurements are possible, predicting soil C storage is complicated by at least two other factors: the turnover time of different soil C pools, and interactions between soil N accumulation and soil C storage. For example, at the Calhoun site, precise soil C measurements confirmed that these forest soils were a C sink, but ^{14}C measurements showed that C was mainly accumulating in rapidly cycling pools that were only short-term sinks for C (Richter and Markewitz, 2001). Soils beneath N-fixing trees typically accumulate C faster than those under other types of trees (Kaye et al., 2000; Resh et al., 2002), but only a few studies have examined the response of soil C pools to fertilization in forests (Canary et al., 2000; Homann et al., 2001).

We tested two hypotheses about rates of change in soil C and N contents across time in a *E. saligna* plantation in Hawaii with low spatial variation in soil properties. *Eucalyptus* plantations cover more than 40 million ha of land in the tropics; intensive silviculture leads to very rapid rates of growth, and 6- to 8-yr rotations (Brown et al., 1997, FAO 1999).

The prior land use on this site was sugarcane agriculture, which provided a ^{13}C signature that allowed changes in soil C to be partitioned to gains of C from *Eucalyptus*, and losses of older (sugarcane-derived) C. Our first hypothesis was based on the results from a comparison of soils under sugarcane agriculture and *Eucalyptus* forestry (Bashkin and Binkley, 1998): Total soil C would not change through an 8-yr rotation of *Eucalyptus* forestry because gains of new C from *Eucalyptus* would be offset by equal losses of older soil C. The changes in C would also vary by depth, with gains of C derived from *Eucalyptus* concentrated in the top 30 cm, and losses of older soil C from the entire profile. The second hypothesis was based on changes in soil C under the influence of N-fixing *Falcataria moluccana* (Miq.) Barneby & J.W. Grimes at a nearby location on the same soil series (Kaye et al., 2000, Resh et al., 2002): Large additions of N from fertilizer would increase total soil C content by increasing the rate of accretion of *Eucalyptus*-derived C, and decreasing the rate of loss of older soil C.

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Published in Soil Sci. Soc. Am. J. 68:1713–1719 (2004).

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Abbreviations: $\delta^{13}C$, the per mil difference between the carbon-13 content of the sample and the Pee Dee belemnite standard; C_3–C, C_3–derived carbon; C_4–C, C_4–derived carbon; CI, confidence interval.

MATERIALS AND METHODS

Site Description

The study site is a 4-ha plantation of *E. saligna*, 13 km north of Hilo, HI (19° 50′ 28.1″ N, 155° 7′ 28.3″ W) at 350 m elevation with a mean annual temperature of 21°C and an average rainfall of about 4 m yr^{-1} (Binkley and Resh, 1999). The slopes are modest (<5%), with deep (>2 m), acidic (pH 5–6 in water) soils of the Kaiwiki thixotropic, isothermic Typic Hydrandept series. Sugarcane was cropped on this site for >80 yr. Other work in this plantation includes characterization of soil change in the first 2.5 yr of the rotation (Binkley and Resh, 1999), belowground C allocation (Giardina and Ryan, 2002), hydraulic limitation of tree growth (Barnard and Ryan, 2003), and a complete C budget for the rotation (Ryan et al., 2004). The last sugarcane crop was harvested about 1 yr before the planting of *Eucalyptus* seedlings (in May 1994). The site was fallow for about 9 mo, and then plowed to turn under the developing vegetation. The experimental design consists of 18 30- by 30-m plots with trees at two spacings (1 × 1 m and 3 × 3 m) receiving three fertilization regimes in three completely randomized blocks; herbicide treatments prevented the development of any understory vegetation. Total aboveground woody biomass averaged about 133 Mg ha^{-1} at 8 yr; heavily fertilized plots had about 50% more stem mass than control plots. The mass of coarse roots was about 22 Mg ha^{-1} (= 1.1 kg C m^{-2}). We did not measure the N content of the trees, but on the basis of relationships from similar plantations in Brazil (Goncalves et al., 1997), we expect the N content of aboveground biomass would average about 400 kg ha^{-1} N.

The control fertilization regime involved fertilization with N, P, K, and Ca in holes at 1- by 1-m spacings, and a broadcast application of the same amount at 7 mo. Total fertilizer application to the control plots (representing current operational rates) was 310 kg ha^{-1} N as urea, 130 kg ha^{-1} P, and 125 kg ha^{-1} Ca as triple-superphosphate, 260 kg ha^{-1} K as potassium chloride, and 100 kg ha^{-1} Granusol 2GB5 micronutrient fertilizer (5% Mn, 5% Zn, 5% Mg, 5% Fe, 1.5% Cu, and 0.5% B; American Minerals, King of Prussia, PA). The medium fertilization treatment matched the control, with additional fertilization beginning at age 3 yr, with quarterly applications for 3 yr of 65 kg ha^{-1} N, 31 kg ha^{-1} P, 46 kg ha^{-1} K, and annual additions of 125 kg ha^{-1} Ca, 58 kg ha^{-1} S, 23 kg ha^{-1} Mg, and 100 kg ha^{-1} Granusol micronutrients. The total fertilizer application for the medium treatment was 700 kg ha^{-1} N, 300 kg ha^{-1} P, 580 kg ha^{-1} K, 350 kg ha^{-1} Ca, 35 kg ha^{-1} Mg, and 300 kg ha^{-1} micronutrients. The high fertilization treatment matched the fertilization of the control plots at 0 and 7 mo, followed by quarterly fertilization for 5 yr, with total applications of 1600 kg ha^{-1} N, 680 kg ha^{-1} P, 1320 kg ha^{-1} K, 830 kg ha^{-1} Ca, 70 kg ha^{-1} Mg, and 600 kg ha^{-1} micronutrients.

Soil Pool Changes

Soils were sampled four times during the 8-yr rotation. Three permanent sampling locations were established in each of the 18 plots at the time of planting (April 1994). The first soil sampling involved excavation of small pits to a nearly 40-cm depth, with samples taken by trowel from the 0- to 15-cm depth, and the 15- to 30-cm depth. Bulk density (oven dry mass of soil per volume) was determined from samples of known volume taken from the midpoint of each depth. The soil is almost rock-free, and no correction for rock content was necessary. The upper 30 cm of the soil was a loose, Ap horizon as a result of >50 yr of plowing. The 30- to 45-cm depth was also an Ap horizon, similar but was more compacted as subsoil plowing occurred only once every 5 to 10 yr during the past 20 yr.

Subsequent soil samplings at age 2.5, 5.5, and 8.0 yr were done with a Giddings soil corer (i.d. 53 mm; Giddings Machine Co., Windsor, CO), with a plastic sleeve lining to allow for precise separation of soil depths (0–15, 15–30, and 30–45 cm) and determination of bulk density. Six samples (30 by 30 cm) were taken annually of the O horizon and composited within plots. Soil sampling occurred about 3 mo after the most recent fertilizer application (for fertilized plots).

Soils were air dried and 2-g subsamples ground and acidified with 10 mL of 0.2 *M* HCl to remove any residual carbonates from previous fertilization or liming treatments, and neutralized with drops of 1 *M* NaOH. Changes in total C and N were based on the concentrations measured in the carbonate-free samples (determined with a LECO 1000 CN analyzer), and bulk density. The ratios of C isotopes were used to estimate rates of gain of C from *Eucalyptus*, and loss rates of older soil C derived from sugarcane. Carbon isotopes were measured with a VG isochrom-NA stable isotope ratio mass spectrometer (VG, Middlewich, UK) at Colorado State University for the 0- and 2.5-yr sampling, and by the Stable Isotope Facility at the University of California at Davis for the 5.5- and 8.0-yr sampling. A quality assurance experiment showed high precision in $\delta^{13}C$ (the per mil difference between the ^{13}C content of the sample and the Pee Dee belemnite standard) between the laboratories, with no bias. The proportion of total soil C derived from sugarcane was calculated (after Vitorello et al., 1989) as

$$\%C_4 = (\delta - \delta_o/\delta_c - \delta_o) \times 100,$$

where δ is the $\delta^{13}C$ of the soil sample, δ_o is the $\delta^{13}C$ of soil samples with no C from C_4 plants, and δ_c is the $\delta^{13}C$ of C_4 (sugarcane) plant material. The value for soil samples with no C input from C_4 plants was taken as −25.5‰, based on values from native Hawaiian forests within a few kilometers of this site (Bashkin and Binkley, 1998). The $\delta^{13}C$ of sugarcane material was taken as −11.5‰ (Bashkin and Binkley, 1998). The percentage of the soil C derived from C_3 plants (including old C from the presugarcane period and new *Eucalyptus* C) was calculated as:

$$\%C_3 = 100 - \%C_4.$$

We expected minimal changes across 8 yr with the soil C derived from native C_3 vegetation more than 80 yr ago before the site was converted to agricultural use, so the change in C_3–C should be determined almost solely by the dynamics of C derived from the *Eucalyptus* trees.

Incubation Experiment

We measured the effects of fertilization and soil temperature on the decomposition of C derived from sugarcane and *Eucalyptus* trees using 6-mo laboratory incubations. Surface soils (0 to 15 cm) collected at age 5.5 yr (December 1999) from the control and high fertilization plots from both planting densities were stored at 4°C for 6 wk before the incubation. For sugarcane and tree isotopic endpoints, we collected surface soils from one active sugarcane field adjacent to our plantations, and one *Eucalyptus* plantation that had never been cultivated (i.e., native forest was directly converted to *Eucalyptus* plantations). These isotopic endpoints had no site or plot replicates, and any reported variance comes from lab replicates (we incubated two subsamples from each endpoint site). We composited all subsamples from a given plot, dried (105°C) a 10-g subsample of the composite to constant mass to determine water content, and incubated a 75-g (fresh mass)

subsample of the composite at field capacity and 21.5°C. From control plots, we incubated two additional subsamples, one augmented in the lab with the equivalent of 3.3 g m^{-2} N as aqueous NH_4NO_3 immediately before bringing the soils to field capacity, and a second (with no N addition) incubated at 25°C. The lab fertilization rate was equivalent to two field fertilizations of 0.005 kg N m^{-2}, assuming 30% of that N stays in the surface mineral soil. The soils placed in plastic cups and sealed in airtight 1-L jars fitted with Leur valves for headspace sampling. Approximately 20 mL of deionized water were placed in the bottom of each jar to prevent soil drying. Every two weeks this water was changed and the soil brought to field capacity with deionized water.

The decomposition of sugarcane and tree C was estimated by capturing CO_2 in the headspace of the incubation jars. Incubation jars were flushed with CO_2–free air at 1, 6, 20, 41, 48, 62, 92, 123, and 159 d until the headspace contained <15 μmol CO_2 mol^{-1} air and sealed for 1 to 5 d (longer at the end of the incubation). The concentration of CO_2 in the headspace was then determined using an infrared gas analyzer (LICOR-6200, Lincoln, NE). The headspace was sampled by first mixing with a 35-mL syringe and then sampling 2 mL with a 10-mL syringe. Three sealed jars without soil were used as blanks to ensure that all changes in headspace CO_2 were from microbial activity. Atmospheric pressure, air temperature, jar volume, sampled gas volume, and dry soil mass were used to convert headspace CO_2 concentration to g C. The headspace CO_2 was also analyzed at 1, 6, 20, 48, and 92 d for $\delta^{13}C$. The percentage of headspace C derived from sugarcane or trees was calculated with the equations above. The total C, C_4–C, and C_3–C evolved from the soil during the incubation period (159 d for total C, and 92 d for C_3– and C_4–C) was interpolated between flux rate measurements by fitting exponential decay curves to plots of C flux rate vs. incubation time ($r^2 \geq 0.95$ for all curves).

Statistical Analyses

Changes in total soil C, C derived from C_3 plants, C derived from C_4 plants, and total soil N were evaluated as a repeated measures ANOVA using fixed effects of soil depth, sample year, tree spacing, and fertilization (with compound symmetry for covariance structure, and Kenward-Roger degrees of freedom method, $P = 0.05$; SAS version 8.2, Proc Mixed). We also tested the value of the repeated sampling for determining the effects of spacing and fertilization at the end of the rotation (Year 8), with an ANOVA of Year 8 values with Year 0 values as covariates.

The incubation experiment was also analyzed by repeated-measures ANOVA using fixed effects of fertilization level (control, fertilized in the field, and fertilized in the laboratory), incubation temperature (two levels: 21.5°C for all fertilization treatments, and 25°C for control soils), and incubation time. Initially, we also analyzed for the effect of tree spacing, but this effect was never statistically significant ($P > 0.18$) and was not a component of our incubation hypotheses, so we pooled data from both planting densities before incubation analyses. The total respired C, respired C_3–C, and respired C_4–C were also analyzed by a standard ANOVA with N fertilization level and incubation temperature as main effects. Pairwise comparisons among means for factors with significant F values were made using Fisher's LSD tests.

RESULTS

Total soil C in the 0- to 45-cm soil averaged 13.5 kg m^{-2}, with no significant change across 8 yr (Fig. 1, mean change = −0.30 kg m^{-2}, 95% CI = ±0.45 kg m^{-2}). Over an 8-yr period, the 95% CI on the rate of change was

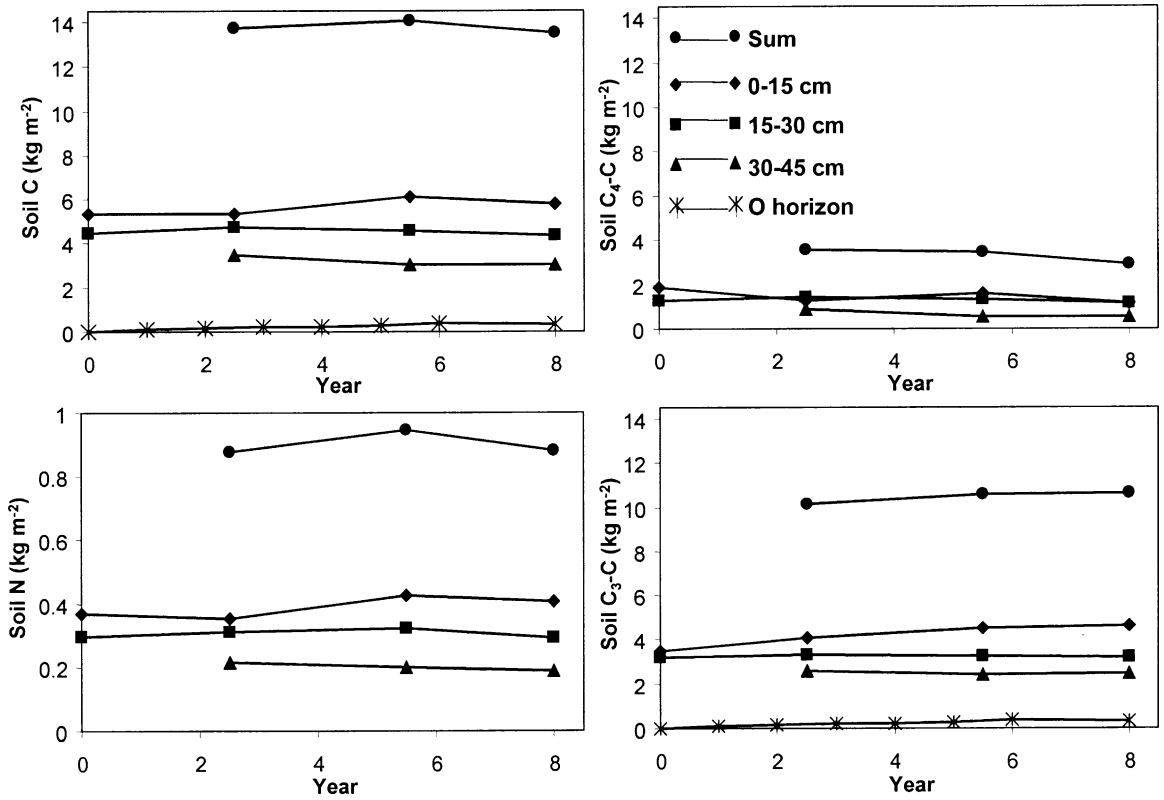

Fig. 1. Soil content of C (upper left), C derived from C_4 plants (upper right), C derived from C_3 plants (lower right), and N (lower left). Points are averages of 18 plots, with each plot as the average of three cores. The 30- to 45-cm depth was not sampled in Year 0. Soil C did not change across time, as the increase in C_3–derived C was matched by the loss of C_4–derived C. Soil N did not change.

0.057 kg C m^{-2} yr^{-1}. Soil C declined significantly with depth ($P < 0.0001$), with the 0- to 15-cm depth containing almost twice as much C as the 30- to 45-cm depth. The coefficient of variation more than doubled with depth, owing primarily to the decrease in C content rather than to the increase in variance. The interaction of year and depth was highly significant ($P < 0.0001$), with increasing C in the 0- to 30-cm depth across time, and decreasing C in the 30- to 45-cm depth. The effects of tree spacing and fertilization were not significant ($P > 0.2$), and showed no interaction with other factors.

The O horizon was absent at the time of planting, and increased to 0.31 kg m^{-2} after 8 yr for an average rate of change of 0.0039 kg C m^{-2} yr^{-1} ($P < 0.0001$). The significant increase in the O horizon was too small to provide any overall trend for the entire soil (O horizon plus 0–45 cm of mineral soil).

The pool of C derived from C_3 plants increased by an annual rate of 0.136 kg m^{-2} yr^{-1} ($P < 0.0001$, 95% CI = $\pm$0.030 kg m^{-2} yr^{-1}). The C_3 pool also differed among soil depths, and depth interacted with year ($P < 0.0001$), with large increases in the 0- to 15-cm depth across time, little change in the 15- to 30-cm depth, and a slight decrease in the 30- to 45-cm depth. The pool of C derived from C_4 plants declined at an annual rate of 0.144 kg m^{-2} yr^{-1} ($P < 0.0001$, 95% CI = $\pm$0.030 kg m^{-2} yr^{-1}), and again the depth $\times$ year interaction was significant (with greater losses from the 0- to 15- and 30- to 45-cm depths than the 15- to 30-cm depth). As with total soil C, no effects (or interactions) of tree spacing or fertilization were significant ($P > 0.2$).

Surprisingly, soil N content was not affected by fertilization ($P = 0.99$). The average rate of change in the mineral soil N pool was about 0.5 g N m^{-2} yr^{-1} (95% CI = $\pm$4.2 g m^{-2} yr^{-1} N) with no overall change through the rotation. This rate of change is small compared with the amount of N fertilizer added (ranging from 31 g m^{-2} N for the control treatment to 160 g m^{-2} N for the high fertilization treatment), as well as the approximate N content of the trees (on the order of 40 g m^{-2} N). Within the rotation, the only significant trend across time was an increase of about 22.6 g m^{-2} N between Years 2.5 and 5.5, followed by a decline of 20.8 g m^{-2} N by Year 8.0. The O horizon increased to a total N content of 26 g m^{-2} N at age 8 yr, a rate of increase of 3.2 g m^{-2} yr^{-1} N. We expect that most of the fertilizer N leached from the soil into deeper horizons or to streams; a few measurements of gaseous N loss demonstrated relatively high rates relative to other soils (on the order of 0.3 g m^{-2} yr^{-1}; P. Matson, C. Giardina, 1999, unpublished data), but very low rates relative to the dominant fluxes in the N cycle in this forest.

The second statistical analysis of soil pool sizes used a nonrepeated measures ANOVA with only the end-of-rotation 8-yr values, testing whether Year 0 values were useful covariates in detecting the effects of depth or treatments. The Year 0 covariate significantly improved the model for C_4–C ($P = 0.002$) and N ($P = 0.02$), but not for total C or C_3–C.

The laboratory incubations showed rapid initial rates of CO_2 production, dropping to less than half the initial rates within 4 wk (Fig. 2). The rate of CO_2 production for the high-temperature incubation exceeded the rates for all other incubations, and the effects of N addition (either from the N-fertilized field soil, or the N addition in the laboratory to control soils) were not significant. The $\delta^{13}C$ of the CO_2 declined across time for all treatments (and even slightly for the endpoint soils), dropping by about 5‰. The C_4–C contributed about 75% of the CO_2 production at the beginning of the incubation in the Year 5.5 soils, declining by about half after 2 mo. The cumulative total loss of C did not differ among the control, field-fertilized, and laboratory-fertilized treatments (Fig. 3), but the higher temperature incubation increased the total C loss by about 40%. The lack of

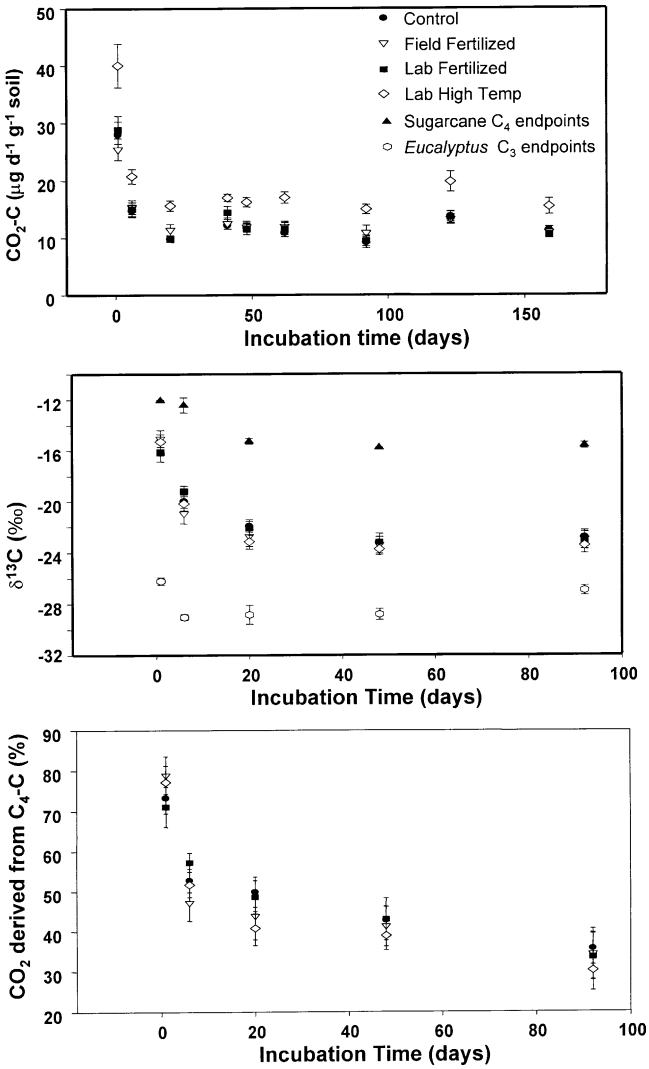

Fig. 2. The CO_2 released in laboratory incubations showed declining rates across time (top graph; bars are standard errors), as well as declining $\delta^{13}C$ (the per mil difference between the carbon-13 content of the sample and the Pee Dee belemnite standard; middle graph), and proportion of CO_2 derived from C_4 sugarcane (bottom graph). The rate of CO_2 release was not affected by N addition (either in the field or laboratory), but the rates were higher at 25°C (high temperature) than at 21.5°C (all others). The middle graph also shows the $\sigma^{13}C$ for a nearby *Eucalyptus* plantation that was never cropped with sugarcane (providing a pure C_3 signal), and a site that was currently in sugarcane land use (providing a C_4 endpoint).

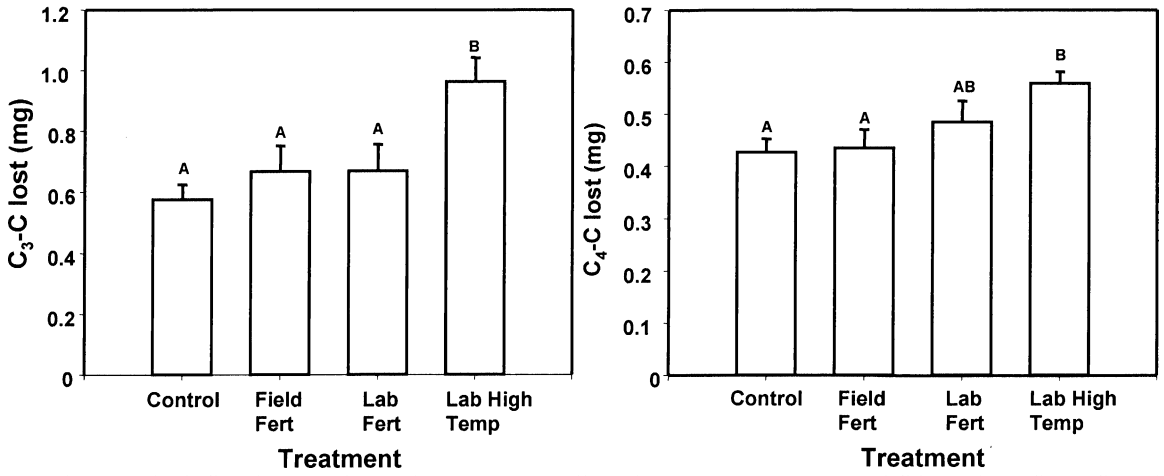

Fig. 3. Cumulative loss of soil C during 3-mo incubations showed no effect of added N on the release of C from either C_3– or C_4–derived pools, and the release from both pools was increased at the higher temperature (25°C) relative to the other treatments (21.5°C). Bars with the same letter do not differ at $P = 0.05$ (error bars are standard errors).

effect of N treatment was apparent for the loss of C derived from both C_3 and C_4 plants.

DISCUSSION

Our first hypothesis was strongly supported; a lack of change in total soil C resulted from equivalent losses and gains of C_4–C and C_3–C. The total belowground C allocation (TBCA) in this plantation averaged about 1.880 kg m^{-2} yr^{-1} across years and treatments (Giardina and Ryan, 2002); the net increase of C_3–C of 0.136 kg m^{-2} yr^{-1} indicated that about 7% of the C allocated to the belowground ecosystem accumulated during the 8-yr period, and 93% was lost as CO_2. Given that the net increase in C_3–C was almost precisely balanced by the loss of C_4–C (0.144 kg m^{-2} yr^{-1}), the TBCA could be calculated with good precision simply by accounting for the soil CO_2 efflux, the rate of aboveground litter input, and the rate of increase in coarse root mass (Giardina and Ryan, 2002). In fact, even the full 95% CI around the mean rate of change (0.057 kg C m^{-2} yr^{-1}) represented just 3% of the total belowground C allocation, too small to contribute substantially to the overall experimental variance among plots and treatments in TBCA. The increment of C in coarse root mass was about 0.14 kg C m^{-2} yr^{-1}, representing the largest component of C sequestration in the soil as a result of afforestation.

If the rate of gain and loss for the entire C pool from 0 to 45 cm averaged 0.14 kg C m^{-2} yr^{-1}, the turnover time of the C in this soil would be on the order of 100 yr. We expect the actual turnover rate for the entire soil C pool is somewhat longer than 100 yr, as 80+ years of sugarcane agriculture in this area depleted the soil pool of C_3–C by only 40% (Bashkin and Binkley, 1998).

Our second hypothesis was strongly refuted by the results of both the field measurements of soil pools across time, and by the laboratory incubations. The addition of N fertilizer had no effect on total soil C, the accretion of new C_3–C, or the loss of older C_4–C. The laboratory incubations showed no effect of N (added over time in the field, or in a single application in the laboratory) on the release of CO_2 from pools derived from either C_3 or C_4 plants. These results contrast sharply with the effects of the N-fixing *Falcataria* trees, where N accumulated at a rate of 13.5 g m^{-2} yr^{-1} for 17 yr, with associated accretion of C of 0.118 kg m^{-2} yr^{-1} ($P < 0.01$, Kaye et al., 2000). The experimental design in the present study would have detected a significant fertilizer effect on C storage if the effect were even half as large as the N-fixer effect. About 2/3 of the increase in soil C under the N-fixer was derived from new C added by the trees, and about 1/3 resulted from greater retention of older C derived from sugarcane. The contrasting effects of N fixation and N fertilization (on the same soil series, at locations within 3 km) indicates that N supply was not the apparent driver of the effect of the N-fixing trees on soil C. We expect that the increased storage of C under the influence of the N-fixing trees resulted from the indirect effect of the trees on the soil communities; the soils under the N-fixing trees had several-fold more worms (Zou, 1993), as well as greater bacterial biomass and lower fungal biomass (Garcia-Montiel and Binkley, 1998), than the soils under *Eucalyptus*. Canary et al. (2000) and Homann et al. (2001) looked for changes in soil C accumulation in response to large additions of N fertilizer in Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], but high spatial variability limited detectability to >15% change in C, which exceeded any effect of the N fertilizer.

How do our changes in soil C compare with other forest soils? Bashkin and Binkley (1998) measured soil C in locations near the present study site, contrasting sugarcane agriculture with *Eucalyptus* plantations. They found no significant changes in total soil C with afforestation (+0.020 kg C m^{-2} yr^{-1}, $P = 0.78$); this lack of change resulted from offsetting losses of C_4–C (−0.148 kg C m^{-2} yr^{-1}, $P = 0.03$), and gains of C_3–C (0.162 kg C m^{-2} yr^{-1}, $P = 0.01$). Gains of C_3–C were greater in the upper soil layers, and losses of C_4–C were distributed more evenly with depth (from 0–55 cm). The apparent

reforestation effect from the land-use comparison was remarkably similar in both rates and vertical pattern to the within-site changes we measured in the present study. Both studies found very low and nonsignificant changes in total soil C, and the rates of change in C pools derived from C_4 and C_3 plants were well within the CI of the soil C changes in the present study. Post and Kwon (2000) summarized the available literature on rates of change in soil C with afforestation, and found an average increase of about 0.034 kg C m^{-2} yr^{-1}. The minimum detectable change (95% CI) in our study across 8 yr was 0.057 kg C m^{-2} yr^{-1}, so our site appears to be consistent with the global average from Post and Kwon (2000). However, two-thirds of the reforestation studies cited by Post and Kwon (2000) sampled to 30 cm or less, so their average increase would just about match the upper bound of our 95% CI for 0- to 30-cm depth. Moreover, a one-tailed hypothesis that soil C increased at our site by 0.034 kg C m^{-2} yr^{-1} in the 0- to 30-cm depth (based on Post and Kwon's average) would be rejected ($P < 0.01$), so we conclude that the rate of C change in our site was less than the global average summarized by Post and Kwon (2000). Guo and Gifford's (2002) metaanalysis of estimates of change in soil C with afforestation found an 18% (95% CI from 9 to 27%) increase in soil C, far exceeding our 95% CI (0–45 cm) of 3.3%, again indicating that our rate of change was unusually low compared with other sites. Most of the studies used by Guo and Gifford had both lower total soil C and lower precision than the present study; the lower percentage change in soil C could derive from either a truly lower (near 0) rate of change in the present study, or from unwarranted assumptions in the metaanalysis.

The experimental design of this project provided high precision in our tests for changes with treatments and across time. If we tested only for the effects of treatment (fertilization and thinning) on soil C and N after 8 yr, with no prior sampling, the precision of tests would have been lower for some variables than with the repeated sampling that included a Time 0 value for each plot. The detection of treatment effects for total N and C_4–C were improved by using Year 0 pool sizes as covariates, although the Year 0 covariates were not helpful for total C or C_3–C. The use of prior plot averages as covariates is often more critical when dealing with high-variation soils. For example, Rothe et al. (2002) tested the 19-yr effects of N-fixing red alder (*Alnus rubra* Bong.) on steep slopes in Oregon; a repeated-measures design with the initial plot average of soil N was able to detect a significant increase in soil N under the influence of alder, whereas a simple ANOVA on the post-19-yr plot averages could not detect the alder effect. They found that inclusion of the initial plot averages reduced the minimum detectable treatment effects by one-third (for C) to two-thirds (for N).

Was the soil in the *Eucalyptus* plantation C saturated? Six et al. (2002) reviewed the processes underlying the accumulation of C in soils. They suggested that physicochemical characteristics inherent to soils define the maximum protective capacity of these pools, which limits increases in soil C. The physicochemical processes include binding of organic molecules onto mineral particles, the occlusion of organic matter inside organomineral aggregates, and simple biochemical recalcitrance of the organic matter. Bashkin and Binkley (1998) estimated that the soils in this area experienced a net loss of about 1.72 kg C m^{-2} (13% of total C) as a result of land use conversion from native forest to sugarcane agriculture. The physicochemical processes that allowed the accumulation of this larger amount of soil C before agriculture do not appear to currently allow substantial net increases in soil C; such a hysteresis effect may cloud discussions of C saturation of soils.

The influence of fertilizer N on the accumulation of C in nonforest soils appears to vary substantially among studies and sites. Gregorich et al. (1996) found that three decades of N fertilization increased the labile pool (light fraction) of C in a soil under corn (*Zea mays* L.) agriculture by twofold, with no increase in nonlabile pools, and no change in the turnover rate of the C. Gregorich et al. (2001) also found that rotations of corn with legumes showed much larger increases in soil C (relative to unfertilized corn monocultures) than fertilization of corn monocultures. Ludwig et al. (2003) found that fertilized plots cropped with corn showed a 2.5 kg C m^{-2} increase in total soil C; only 14% of this extra C was derived from corn, indicating a substantial reduction in turnover rate of older (more than 40 yr old) soil C. In contrast, Reicosky et al. (2002) found that soil C did not differ between control and fertilized fields of corn after 30 yr of treatment, and Campbell et al. (1991) also found a similar lack of response of soil C to fertilization of winter wheat (T*riticum aestivum* L.) systems. In an alpine soil, Neff et al. (2002) found that N fertilizer increased stable C storage in an alpine soil and decreased C stored in labile pools. The diverse results of these N addition experiments indicate that no single model (or set of processes) is likely to describe all situations.

We conclude that experimental tests of rates of change in forest soil C can provide high-precision estimates of rates of change on sites with relatively low spatial variance in soil C, including the effects of forest management practices during moderately short time periods. This site showed a precise, near-zero rate of change in total soil C that resulted from relatively rapid rates of loss of older soil C and accumulation of new soil C. The balance between C loss and C gain appears to be malleable, but the drivers of the balance need to be examined more thoroughly. Addition of inorganic N fertilizer did not affect the rate of either C loss or C gain, in contrast to the large effect of N-fixation on reducing C loss and increasing C gain (Kaye et al., 2000; Resh et al., 2002). We suspect that the difference between N fertilization and N fixation may lie in the differences in effects on soil biology more than soil chemistry, and this suspicion needs substantial experimentation before we can describe with confidence the role of soil biology in explaining variable rates of C loss and gain in forest soils (Binkley and Giardina, 1998). Precise conclusions about rates of change in forest soil will de-

pend on our commitment to well-designed, long-term studies (Richter and Markewitz, 2001).

ACKNOWLEDGMENTS

This research was supported by NSF Grants DEB93-06356, DEB-9708521, and DEB-9816006, and McIntire-Stennis appropriations to Colorado State University. We thank our coinvestigators Jim Fownes, Randy Senock, and Christian Giardina for overall collaboration on the *Eucalyptus* project; Rudy King for assistance with statistical analysis; and Rod Chimner, Sigrid Resh, Margot Kaye, Jose Luis Stape, Wendy Estes, Cindy Shimada, Carol Volk, and Jennifer Clark for help in the lab and field.

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