DIVISION S-3—SOIL BIOLOGY & BIOCHEMISTRY

Use of Carbon-13 and Carbon-14 to Measure the Effects of Carbon Dioxide and Nitrogen Fertilization on Carbon Dynamics in Ponderosa Pine

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ABSTRACT

Soil C sequestration in predicted, future elevated CO_2 environments will be important to atmospheric CO_2 levels, soil tilth, and fertility. An elevated CO_2 study with ponderosa pines (*Pinus ponderosa* Laws) grown in chambers produced above ground vegetation with a $\delta^{13}C$ of $-44\permil$ and roots with $-42\permil$. This together with carbon dating made it possible to follow soil C dynamics. Fifty percent of the California upland soil C, resistant to acid hydrolysis, was designated as the resistant fraction. Carbon dating showed the mean residence times of this fraction to be 400 to 1500 yr greater than the total soil C for the horizons sampled. Young ponderosa pines grown in CO_2 chambers produced negligible leaf litter. There were 32% more roots in the presence of either added N or double CO_2 but 77% more in the presence of both. Root-derived soil C was equivalent to 10% of the root C after the 6-yr growth period. Analysis of laboratory CO_2 evolution during extended incubation showed the active soil C pool represented 1 to 2% of the soil C with a field-equivalent mean residence time (MRT) of 24 to 53 d. The slow pool represented 46 to 52% of the C with MRT of 24 to 67 yr depending on treatment and soil depth. Analysis of the $^{13}CO_2$ label during incubation from the elevated CO_2 treatments, showed the root-derived ^{13}C of the active fraction to have residence times similar to those of the total soil non labeled C at ≈35 d. Root-derived C of the slow pool at 10 yr MRT turned over three to four time as fast as the general soil C. The ^{13}C of the light fraction (LF), showed it to be most closely associated with the active pool. The particulate organic matter (POM) was part of the slow pool as determined with incubation.

THE INCREASE IN CONCENTRATION OF CO_2 in the earth's atmosphere by 1.5 mL L^{-1} annually together with increases in atmospheric N deposition in many parts of the world has the potential for altering numerous ecosystem processes (Hungate et al., 1997; Watson et al., 1990; Trabalka, 1985). Elevated CO_2 and N fertilization treatments should increase below ground production and root and microbial respiration, if other resources (moisture, nutrients, etc.) are not limiting (Johnson et al., 1994; Strain and Thomas, 1992). Growth of loblolly pine (*Pinus taeda* L.) in 560 mL L^{-1} CO_2 in the Duke FACE experiment resulted in a 25% increase in production (DeLucia et al., 1999). This was reflected in a 15% increase in litter fall in the second year of exposure to elevated CO_2 (Allen et al., 2000) but no statistically significant differences in fine root production, microbial biomass, or plant chemistry. Growth in elevated CO_2 can lead to changed plant species composition and litter quality and quantity (Oene et al., 1999). This together with changes in other constituents can alter decomposition of litter and C cycling back to the atmosphere (Strain and Thomas, 1992). Increases in N can act through enhanced plant growth, changes in litter quality, and either positive or negative alteration of in situ decomposition rates (Fogg, 1988). The response of trees to elevated atmospheric C and N fertilization is especially important in that forests are considered to be major potential C sinks in global change scenarios (Schimel, 1995; Fan et al., 1998). Atmospheric N may enhance both tree growth and soil C sequestration; it can also contribute to forest decline (Nadelhoffer et al., 1999). Elevated CO_2 concentration in the soil atmosphere has resulted in increased ecosystem C uptake but also has increased the rate of C cycling (Hungate et al., 1997).

Soils may also act as C sinks under elevated CO_2 partial pressures (Leavitt et al., 1994; Dixon et al., 1994). The changes in soil organic carbon (SOC) concentrations can best be measured with experiments that measure differences in SOC pool sizes and fluxes in response to management. Acid hydrolysis, together with C dating of the resistant fraction, can be used to determine the old resistant (C_r) fraction of SOC (Campbell et al., 1967; Martel and Paul, 1974; Trumbore, 1993). Carbon dating applied to a range of North American grassland soils (Paul et al., 1997) has shown the MRT of SOC to be dependent on climate, landscape position, soil management, and soil forming processes. The SOC pool resistant to acid hydrolysis is generally 1200 yr older than the total SOC; MRT also increases rapidly with depth. Curve fitting of the CO_2 respiration data is used to estimate the pool sizes and decomposition rate constants of the active (C_a) and slow (C_s) pools (Collins et al., 2000; Paul et al., 2000). Long-term mineralization of soil C by microorganisms in extended laboratory incubation allows the soil biota and its enzymes to act on the soil constituents, resulting in CO_2 evolution. Plotting the CO_2 efflux data on the basis of per unit time helps to determine the in situ availability of C to the soil microbiota and provides statistical parameters (Hess and Schmidt, 1995).

Anthropogenic CO_2 used in elevated atmospheric

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CO_2 experiments often has a natural ^{13}C label that is useful in determining the input of plant C to the soil and the turnover rate of soil fractions such as the LF, POM, and the C associated with silt and clay (Gregorich and Janzen, 1996). Analysis of the $^{13}CO_2$ evolved during incubation makes it possible to separate the C pool dynamics of the recently added, labeled C from that of the in situ SOC.

The availability of soils from a 6-yr elevated CO_2 study on ponderosa pine (Johnson et al., 1997) made it possible to measure below ground C incorporation and the dynamics of SOC. The open top chamber study examined the effects of elevated CO_2 together with N fertilization on ponderosa pine seedlings. Carbon dating of the total soil and the non hydrolyzable fraction, extended incubation and curve analysis of the evolved CO_2 and ^{13}C analysis of biophysical soil fractions were used to determine the fate of root derived C in the elevated CO_2 environment with and without supplemental N. Knowledge of the relative contribution of roots, turnover rates of C and its distribution to the different pools in the soil from elevated CO_2 environment is necessary in decision making for global change scenarios. The available tracer also make possible much more quantitative information of the role and characteristics of SOM in forestry and pedogenesis.

MATERIALS AND METHODS

Site and Soil Characteristics

The study site was located at the U.S. Forest Service Institute of Forest Genetics in Placerville, CA. Experimental design and sample collection were described by Johnson et al. (1997). Mean separation, of non transformed samples, was tested by least squares means options of SAS general linear model procedure (SAS PROC GLM, SAS, 1995). The soil is an Aiken clay loam, oxidic, mesic Xeric Haplohumult derived from andesite with Ap (0–18 cm), Bw (18–30), and Bt (30–60) horizons. It has pH of 6.0 in water and a bulk density of 1.2 g cm^{-3} to the 60-cm depth. Yearly mean temperature is 14.1°C. The uniformity of the soil within the experimental plot was determined by intensive sampling prior to the initiation of the chamber establishment (Johnson et al., 1997). Ponderosa pine seeds were sown in the spring of 1991 in each of the 21 locations within each chamber. The experimental design included three replicates with three levels of N (0, 10, and 20 g m^{-2} yr^{-1} of N applied in early spring as ammonium sulfate) and four levels of CO_2 treatments (ambient, no chamber; ambient, chambered; 525 mL L^{-1} CO_2; and 700 mL L^{-1} CO_2). Treatments were applied in open top chambers watered using a timed stand pipe and low pressure spray heads. The soils of the intermediate, 10 g m^{-2} yr^{-1} of N and 525 mL L^{-1} CO_2, were not analyzed for this paper. Soil cores were taken from three transects (rings). The first transect was a circle of 1-m diam in the center of the plot. The second transect was a ring of 50 cm outside the center circle. The third ring was the area outside the second ring. Three soil cores of 2-cm diam were randomly taken from each ring. Cores of the same depth from one transect were bulked together, mixed, and air-dried.

Carbon-13 Tracer Methodology

The CO_2 used in the open-top chamber experiment was produced from natural gas (primarily fossil methane) that was

^{13}C depleted. We analyzed $\delta^{13}C$ contents of above and below ground plants and soil C pools after growing pine trees under the ^{13}C-depleted CO_2 condition for 6 yr. The following equation was used in the calculation of the derivation of soil C after ^{13}C analysis.

% soil C from enriched CO_2 =

$$\left[\frac{\delta\ ^{13}C\ \text{chamber soil} - \delta\ ^{13}C\ \text{ambient chamber soil}}{\delta\ ^{13}C\ \text{pine roots} - \delta\ ^{13}C\ \text{ambient chamber soil}} \right] \times 100$$

Laboratory Methods

Dry soil samples were mixed and sieved through a 2-mm sieve to remove plant residues. The LF of the 0- to 18-cm depth was measured by adding 10 g soil to a 100-mL beaker and dispersed with 40 mL of NaI solution (SG ≈1.70 g cm^{-3}) (Janzen et al., 1992). The floating material (LF) was finely ground <150 μm and analyzed for total C and $\delta\ ^{13}C$ with a Model 2020 Europa mass spectrometer (PDZ Europa, Crewe, UK). After the LF was removed, the soil samples were washed three times with 50 mL of distilled water to remove the NaI then dispersed in 30 mL of 5 g L^{-1} sodium hexametaphosphate by shaking for 15 h on a reciprocal shaker (Cambardella and Elliot, 1992). Following dispersion, the soil samples were passed through a 53-μm sieve and thoroughly rinsed with water to remove silt and clay. The POM plus sand retained on the sieve was oven dried at 60°C overnight. The soil slurry passing through the sieve was fractionated to silt and clay by sedimentation and decantation. The sand plus POM and the silt and clay were ground to pass a 250-μm screen and analyzed for total C and $\delta\ ^{13}C$ (Harris and Paul, 1991).

Laboratory incubations were conducted with three field soil replicates from all depths. These were air dried and sieved to pass a 4-mm screen. Triplicate, 100 g air dry-sieved soil samples of each field replicate were weighed into 1-L canning jars, and adjusted to 550 mL kg^{-1} water holding capacity (Paul et al., 2000). Water holding capacity was estimated by the volumetric soil water method (Elliot et al., 1994). Mineralizable SOC was measured during a 187-d laboratory incubation at 37°C in the dark. Carbon dioxide was trapped in NaOH (2 mL, 2 M in a 20 mL vial). Control jars contained no soil. The CO_2 traps were replaced initially at 5-d intervals for the surface horizon and at 10-d intervals for the subsurface samples and later after longer periods. The rate of CO_2 evolution for each interval was measured by titration of excess NaOH in the base traps to pH 7.0 with 0.2 M HCl after the addition of 2 mL of 2 M $SrCl_2$ (Harris et al., 1997) to precipitate carbonate as $SrCO_3$. Dried $SrCO_3$, containing 200 mg C, was weighed into tin capsules and analyzed for total C and $\delta\ ^{13}C$. Vanadium pentaoxide (5 mg) was added to enhance combustion.

The CO_2 evolved over time was used to estimate the size and kinetics of the SOC pools. Pool sizes and dynamics were based on a model with three pools that decompose according to first order kinetics with the following equation:

$$C_{(t)} = C_a e^{-k_d t} + C_s e^{-k_s t} + C_r e^{-k_r t}$$

Where; $C_{(t)}$ = total soil C pool at Time t; C_a, k_a represent the active pool; C_s, k_s = slow pool; C_r, k_r = resistant pool.

The resistant pool (C_r) was determined by hydrolysis with hot, 6 M HCl. The nonhydrolyzable fraction (C_r pool) was washed and C dated to determine its MRT and analyzed for C content and $\delta\ ^{13}C$ (Leavitt et al., 1994). The field MRT, obtained by carbon dating, was transformed to an equivalent laboratory value (Q_{10} correction) for insertion in the three pool model. The size and turnover rates of the active fraction

Table 1. The $\delta^{13}C$ and ^{14}C age for non-chamber soils at Placerville, CA.

| Depth | Total soil | | Nonhydrolyzable | | |
	$\delta^{13}C$	^{14}C age	Fraction of total soil C	$\delta^{13}C$	^{14}C age
cm	‰	Yr BP† ± SE	%	‰	Yr BP ± SE
0–18	−25.2 (0.02)‡a*	478 ± 58	52.4 (2.32)a	−26.2 (0.09)a	985 ± 55
18–30	−24.7 (0.11)b	765 ± 45	47.0 (1.86)a	−26.0 (0.09)a	1480 ± 63
30–60	−24.3 (0.02)c	540 ± 55	47.8 (4.74)a	−27.2 (0.69)a	2013 ± 60

* Values within a column, followed by the same letter, are not significantly different at $P = 0.05$.
† BP is before present.
‡ Standard error of the mean in parentheses.

(C_a) and slow pool (C_s) were determined by non-linear regression (SAS PROC NLIN, SAS, 1995) of the rate of change of CO_2 evolution with time (Paul et al., 2000). Data were fit to the above, first order kinetics model where C_s was constrained as $(C_{SOC} - C_r - C_a)$. In first order reactions at steady state, $MRT = k^{-1}$. The MRT from the laboratory incubation at 37°C were scaled to the average field temperature (14.1°C) by assuming a Q_{10} of 2 $(2^{(37-14.1)/10} = 4.9)$.

RESULTS

Mean Residence Times of Soil and The Resistant Fraction

The total SOC of the soil outside the chambers had ^{14}C ages of 478 yr in the surface with no change at depth (Table 1). The proportion of the SOC resistant to hydrolysis in these soils (47–52%) is similar to that of other grasslands (Follett et al., 1997) with the nonhydrolyzable C being greatest at the surface. The residue of hydrolysis had a 500 yr greater MRT in the surface horizon and 1500 yr at the 30- to 60-cm depth. The $\delta^{13}C$ values of the soil not exposed to elevated CO_2 (Table 1) showed its grassland heritage with possibly a few C_4 plants with $\delta^{13}C$ values at −25.2‰ for the surface soils and −24.3‰ at depth. The residue of acid hydrolysis, was 1.0‰ more negative at the surface and 2.9‰ more negative in the subsurface relative to the total soil. Acid hydrolysis of other soils usually produces a more nega-

tive $\delta^{13}C$ value for resistant fractions than found here. The MRT changes with depth and with hydrolysis also are not as great as those in other grasslands (Paul et al., 1997) leading us to believe the Placerville soils have lower levels of aromaticity and possibly less horizonation than other soils.

Kinetics of the Active and Slow Pool

Soil organic C levels were not changed significantly by either N fertilization or elevated CO_2 treatment of the seedling trees (Table 2). The more sensitive biological assay utilizing CO_2 evolution, showed the surface horizons of treated soils to evolve CO_2 at levels (Fig. 1) that corresponded to differences in plant growth (Table 3). Nitrogen fertilization by itself produced a similar amount of CO_2 upon soil incubation as growth in the elevated CO_2 treatment without N (1080 mg C kg^{-1} soil) equivalent to 5.95% of the total SOC. Elevated atmospheric CO_2 with 20 g N m^{-2} yr^{-1} yielded the highest CO_2 evolution of 1314 mg C kg^{-1} soil equivalent to 6.85% of the SOC. There were no significant changes in the total amount of CO_2 evolved from the lower depths (Fig. 1).

The CO_2 evolution rates, when plotted on a unit time basis for curve fitting and statistical purposes (data not shown), showed a sharp change in slope at 15 to 50 d incubation at 37°C, that demarcated the C_a and C_s pools.

Table 2. Total C pool sizes and C-mineralization kinetics derived from extended laboratory incubations.

| Depth | SOC | Active C | | | Slow C | | |
		C_a pool	C_a/SOC	Field MRT†	C_s pool	C_s/SOC	Field MRT
cm	——— g kg^{-1} ———		%	d	g kg^{-1}	%	yr
350 µL L^{-1} CO_2 & 0 g N m^{-2}							
0–18	18.05 (0.27)‡ef	0.13 (0.003)bcd	0.70	34ab	8.47 (0.13)a	46.93	34abc
18–30	15.25 (0.67)cd*	0.10 (0.009)abc	0.67	24a	7.98 (0.36)a	52.33	39abc
30–60	7.27 (0.09)a	0.13 (0.001)abc	1.77	53b	3.68 (0.05)c	50.47	39ac
350 µL L^{-1} CO_2 & 20 g N m^{-2}							
0–18	18.41 (0.45)f	0.17 (0.011)de	0.90	39ab	8.60 (0.21)a	46.73	27ab
18–30	14.87 (0.55)bc	0.13 (0.019)bcd	0.91	37ab	7.75 (0.31)a	52.09	35abc
30–60	6.31 (0.36)a	0.07 (0.019)a	1.05	30ab	3.23 (0.17)c	51.19	29bc
700 µL L^{-1} CO_2 & 0 g N m^{-2}							
0–18	17.50 (0.953)def	0.20 (0.021)e	1.15	39ab	8.14 (0.25)a	46.48	28ab
18–30	12.52 (0.90)b	0.12 (0.012)bcd	1.00	30ab	6.51 (0.49)b	52.00	34abc
30–60	6.34 (0.49)a	0.08 (0.018)ab	1.30	38ab	3.23 (0.25)c	50.94	42bc
700 µL L^{-1} CO_2 & 20 g N m^{-2}							
0–18	19.30 (1.28)f	0.27 (0.033)f	1.38	46b	8.93 (0.58)a	46.25	24a
18–30	15.68 (1.59)cde	0.15 (0.003)cd	0.95	41ab	8.16 (0.84)a	52.05	49c
30–60	7.26 (0.37)a	0.12 (0.028)abcd	1.59	40ab	3.58 (0.17)c	50.65	67d

* Values within a column followed by the same letter are not significantly different at $P = 0.05$.
† MRT is mean residence times converted to field rates using a Q^{10} of 2;$(2^{(37-t)/10})$; where t = mean annual temperature.
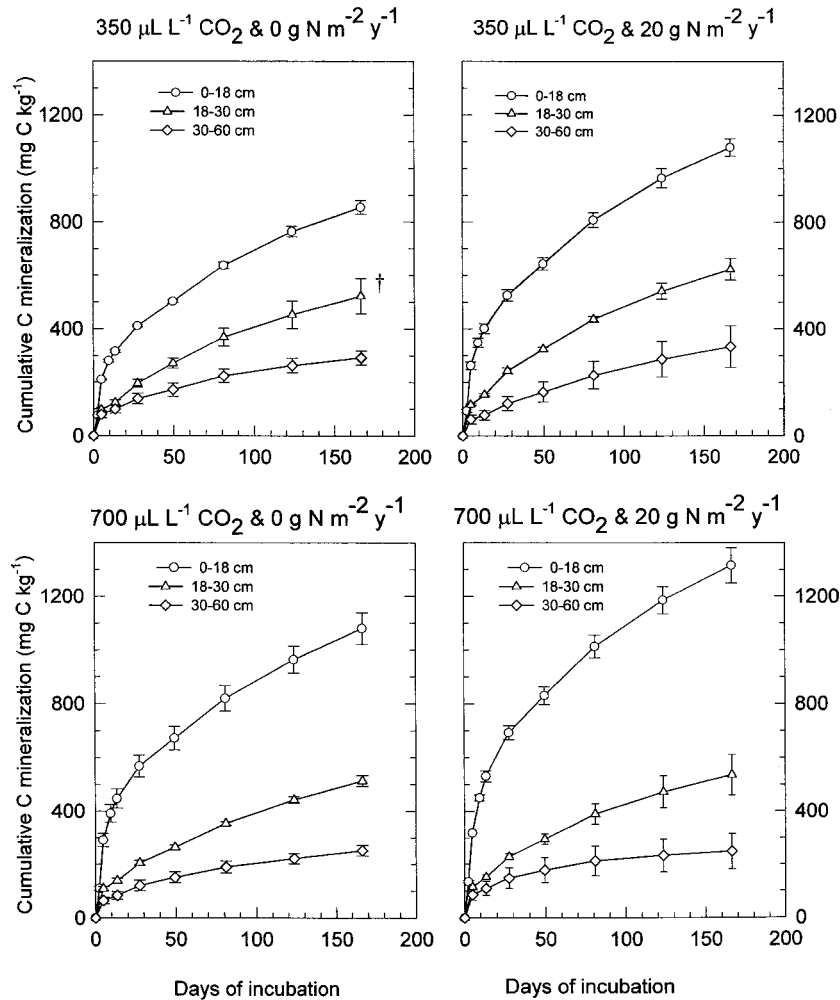‡ Standard deviations in parentheses.

Fig. 1. Carbon dioxide-C mg kg^{-1} soil mineralized in Placerville, CA († ± 1 SE).

Pool sizes and decomposition rate constants determined by curve fitting were converted to MRT (Table 2). The C_a pool represented 0.67 to 1.59% of the total SOC. The control treatment had the smallest C_a pool size. The C_a pool was smallest as a percentage of SOC in the intermediate depth. The C_s pool in the surface soil accounted for 46% of the SOC. The intermediate depth had a C_s pool size of 52% of the total C with the lowest depth sampled being somewhat smaller. The MRT of the C_s pool at 24 to 34 yr in the surface soil was very much older than that of the C_a pool. It generally increased with depth especially in the 700 mL L^{-1} CO$_2$ and 20 g m^{-2} yr^{-1} N treatment where it was 67 yr.

Table 3. Above- and belowground plant production and ^{13}C of juvenile ponderosa pine.

	350 μL L^{-1} CO$_2$		700 μL L^{-1} CO$_2$	
	0 g N m^{-2}	20 g N m^{-2}	0 g N m^{-2}	20 g N m^{-2}
Aboveground wt. g m^{-2}	9 517	12 590	12 649	16 932
Aboveground %C	52.5	53.9	54.9	54.7
Aboveground ‰^{13}C	−29.8	−29.3	−44.1	−44.3
Belowground wt. g m^{-2}	1 456	1 926	1 935	2 590
Belowground %C	29.2	29.4	26.7	24.4
Root C g C m^{-2}	425	566	516	632
Below ground ‰^{13}C	−28.4	−29.3	−42.7	−41.7

The Use of Carbon-13

The δ ^{13}C values of the juvenile ponderosa pine not exposed to the label inherent to elevated CO$_2$ were more negative at −28.3 to −29.6‰ (Table 3) than the soil. Needles of the elevated CO$_2$ treatments (−39 to −43‰) were more variable (data not shown) than the trunk and bark which showed consistent δ ^{13}C values averaging −44.2‰. The δ ^{13}C value of the roots was −42.2‰ for the elevated CO$_2$ treatment averaged over the two N levels. The δ ^{13}C values of soil in the open top chambers were analyzed in concentric rings within the various chambers and found to be uniform. The δ ^{13}C values, in Table 4, for the ambient CO$_2$ plots, with and without N, were very similar to those of the unchambered controls at all depths showing that there was no chamber effect.

The elevated CO$_2$ treatment of 700 μL L^{-1}, with and without added N, produced significant changes in the soil ^{13}C signal of the top two horizons. This was used, after root removal, to calculate the incorporation of the ^{13}C into SOC (Table 4). Observation of the plots during the 6 yr growth showed no discernible leaf litter. These pines, planted from seed, normally retain their needles for at least 5 yr. The soil label, therefore, can be attrib-

Table 4. The δ ^{13}C of soil in the open top elevated CO_2 chambers, percentage soil C from labeled and root-derived carbon.

	Depth (cm)		
	0–18	18–30	30–60
	δ ^{13}C		
	‰		
350 μL L^{-1} CO_2 & 0 g N m^{-2}y^{-1}	−25.0 (0.05)cde*	−24.7 (0.05)e	−24.2 (0.09)fg
350 μL L^{-1} CO_2 & 20 g N m^{-2}y^{-1}	−25.1 (0.09)†cd	−24.9 (0.05)de	−23.9 (0.09)g
700 μL L^{-1} CO_2 & 0 g N m^{-2}y^{-1}	−25.7 (0.08)ab	−25.2 (0.03)cd	−24.3 (0.36)f
700 μL L^{-1} CO_2 & 20 g N m^{-2}y^{-1}	−26.0 (0.10)a	−25.3 (0.07)bc	−24.9 (0.01)fg
	Soil C from label		
	%		
700 μL L^{-1} CO_2 & 0 g N m^{-2}y^{-1}	3.7	2.8	0.7
700 μL L^{-1} CO_2 & 20 g N m^{-2}y^{-1}	5.4	2.4	0.8
	Root-derived C		
	g m^{-2}		
700 μL L^{-1} CO_2 & 0 g N m^{-2}y^{-1}	146.1	60.6	21.6
700 μL L^{-1} CO_2 & 20 g N m^{-2}y$^{-)1}$	215.1	52.0	19.2

* Values followed by the same letter are not significantly different at $P = 0.05$.
† Values in parentheses are the standard errors of the mean of three observations.

uted to root-derived C. Approximately 4.5% of the SOC in the surface horizon was derived from the added $^{13}CO_2$. This dropped to 2.6% in the 18–30 cm depth. There was no statistical difference in δ ^{13}C value at 30 to 60 cm showing few root effects at this depth. The proportion of root-derived soil C was not influenced by N fertilization. The root-derived soil C, to a depth of 60 cm (228 g C) represented 44% of the C found in the roots (516 g C) of the elevated CO_2 and zero N treatment. The 286 g of root derived soil C in the elevated CO_2 plus N treatment (Table 4) represented 45% of 632 g C roots in this soil and 31% of the total belowground root plus root-derived-C in this soil.

The δ ^{13}C values for the evolved CO_2 during laboratory incubation of the ambient CO_2 treatments, with added N, changed from −25‰ in the control surface soil at 10 d incubation to −27.7‰ after 187 d (Table 5). Subsurface samples showed greater changes in the ^{13}C signal. The 18- to 30-cm depth at −22.9‰ and the 30- to 60-cm depth at −19.1‰ indicate the possible presence of a small labile fraction with C_4 plant heritage. This was released in the first 38 d of incubation with a continued slow change to −27.3‰ after 187 d. The deep-

est layer changed a total of 9.5‰ during incubation. The nonenriched unfertilized plots evolved CO_2 of −22.45‰ during the first 10 d incubation of the surface sample and −16.6‰ from the 30- to 60-cm depth. The lowest depth changed from −16.6‰ to −27.9‰ at 187 d of incubation (Table 6).

The calculation of CO_2 evolved from the label is based on the $^{13}CO_2$ from elevated CO_2 plots relative to the appropriate plus or minus N treatments without added CO_2 used as controls during the same incubation period. The SOC mineralized during the first 10 d of incubation in the laboratory was −30.9‰ (Table 5) for the fertilized elevated CO_2 chamber, and −30.8‰ (Table 6) for the unfertilized elevated CO_2 chamber. This indicates that 42% of the SOC mineralized from the unfertilized, elevated CO_2 was derived from the label. In the case of the fertilized CO_2 plots, this was 34%. The $^{13}CO_2$ showed that 11 to 14% of the CO_2 evolved from the surface soil and 5% of that from the lowest depth was derived from the label after 187 d of incubation.

Knowledge of the proportion of CO_2, that came from the label, during laboratory incubation made it possible to determine the $^{13}CO_2$ evolution that could be attrib-

Table 5. The δ ^{13}C of CO_2 and percentage evolved CO_2 from the label at 700 μL L^{-1} CO_2 of the N-fertilized chambers.

Depth	Days						
	10	17	38	61	101	146	187
cm	δ ^{13}C						
	‰						
350 μL L^{-1} CO_2 & 20 g N m^{-2}							
0–18	−25.0b*	−25.2bc	−26.0b	−27.0b	−27.5b	−27.5cd	−27.7cd
18–30	−22.9ab	−24.1b	−25.7b	−26.6b	−27.4b	−27.2d	−27.3d
30–60	−19.1a	−21.8a	−24.7b	−26.9b	−29.4b	−28.3bc	−28.6bc
700 μL L^{-1} CO_2 & 20 g N m^{-2}							
0–18	−30.9d	−30.3e	−30.0a	−30.5a	−30.1a	−29.7a	−29.8a
18–30	−28.1cd	−29.0de	−29.5a	−29.9a	−29.7a	−29.7a	−29.4ab
30–60	−25.1bc	−27.0cd	−28.4a	−29.6a	−30.6a	−28.7ab	−29.3ab
	Evolved CO_2 from the label (700 μL L^{-1} $^{13}CO_2$)						
	%						
0–18	34.3	29.7	24.1	23.1	17.7	14.6	14.4
18–30	26.7	26.9	22.7	21.3	15.6	16.6	13.7
30–60	26.1	25.1	21.0	17.3	9.4	2.6	4.8

* Values within a column followed by the same letter are not significantly different at $P = 0.05$.

Table 6. The δ ^{13}C of CO_2 and percentage evolved CO_2 from the label at 700 μL L^{-1} CO_2 without N fertilization.

	Days						
Depth	10	17	38	61	101	146	187
cm				^{13}C ‰			
350 μL L^{-1} CO_2 & 0 g N m^{-2}							
0–18	−22.4c*	−24.6b	−26.1bc	−26.0d	−26.1a	−26.6a	−27.2c
18–30	−20.1b	−24.4b	−25.8cd	−26.7cd	−26.5a	−27.1a	−27.4bc
30–60	−16.6a	−21.9a	−23.7d	−25.7d	−26.5a	−27.0a	−27.9bc
700 μL L^{-1} CO_2 & 0 g N m^{-2}							
0–18	−30.8e	−31.0d	−30.0a	−29.5a	−29.1b	−29.0b	−29.1a
18–30	−27.8d	−29.3cd	−29.1a	−29.0ab	−29.1b	−28.7b	−29.1a
30–60	−23.9c	−27.2c	−28.0ab	−27.8bc	−28.4b	−28.3b	−28.5ab
			Evolved CO_2 from the label (700 μL L^{-1} $^{13}CO_2$)				
			%				
0–18	42.4	36.3	23.8	21.9	18.5	15.2	12.4
18–30	34.9	27.4	20.4	14.9	16.4	10.4	11.3
30–60	28.7	26.2	23.1	12.5	12.3	8.6	4.4

* Values within a column followed by the same letter are not significantly different at $P = 0.05$.

uted to root derived C on a per unit time basis (Fig. 2). We then calculated the dynamics of the soil C derived from the trees in the elevated CO_2 experiments (Table 7). An average of 12% of the labeled SOC of the 0- to 18- and 18- to 30-cm layers is in the C_a pool with a field MRT averaging 45 d. For comparison, the C_a pool of the total soil represented 1% of the SOC with a similar MRT. The 30- to 60-cm depth has only a small amount of labeled SOC but approximately 40% of this is in the C_a pool. The labeled C_s pool comprising the rest of the ^{13}C showed a MRT averaging 10 yr. The C_s of the total C pool was previously shown to have a MRT that ranged from 24 to 67 yr.

Organic Carbon Distribution with Texture before and after Extended Incubation

The preceding analysis provided information on the SOC derived from the labeled and unlabeled pools and fluxes as defined by chemical and biological approaches. Further information can be obtained by investigating the distribution of the label after biophysical fraction-

ation. The LF that floated at a specific gravity of 1.7 g cm^{-3} accounted for 5.5% of the total SOC in the ambient CO_2 site without N and 7.7% with N (Table 8). This increased to 8% in the elevated CO_2 site without N and 11% where both N and an elevated CO_2 atmosphere occur. The LF is composed primarily of plant residues as shown by the C contents of 35 to 39% (Table 8). Twenty percent of the LF was comprised of labeled material attributable to growth in elevated CO_2 without N and slightly more when grown with N.

The POM (>53 μm) that was released from aggregates on dispersion comprised 10 to 12% of the total SOC in all treatments (Table 8). It is not as clearly related to treatment as the LF. Eleven percent of this fraction is label-derived in the CO_2-enriched site without N and 16.7% label derived in the presence of both added CO_2 and N. Three percent of the silt SOC is attributable to the label. The clay is the least active with less than 3% of the label, showing that these pools have long MRTs.

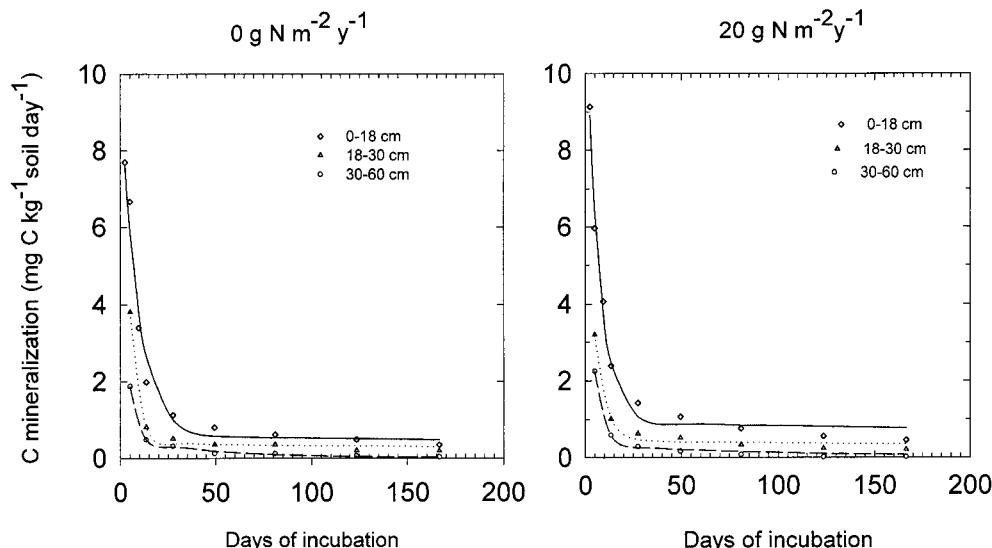Reanalysis of the physical fractions after 187 d laboratory incubation (Table 9) that released approximately

Fig. 2. Dynamics of ^{13}C root derived soil organic carbon in the absence and presence of N, Placerville, CA.

Table 7. Pool size and C mineralization kinetics of the root-derived ^{13}C.

Depth	Total C	Active C			Slow C		
		C_a pool	C_a/LSOC†	Field MRT‡	C_s pool	C_s/LSOC	Field MRT
cm	—— g kg^{-1} ——		%	days	g kg^{-1}	%	yr
700 μL L^{-1} CO$_2$ & 0 g N m^{-2}							
0–18	0.66	0.09	14.3	49	0.56	85.7	10.5
18–30	0.35	0.05	13.7	28	0.30	86.3	9.6
30–60	0.05	0.02	37.1	20	0.03	62.9	0.5
700 μL L^{-1} CO$_2$ & 20 g N m^{-2}							
0–18	1.04	0.09	8.9	41	0.95	91.1	11.6
18–30	0.38	0.04	11.1	41	0.34	88.9	8.5
30–60	0.06	0.03	43.7	9	0.03	56.3	0.3

† LSOC is labeled SOC.
‡ MRT is mean residence times converted to field rates using a Q^{10} of two correction; $(2^{(37-t)/10})$; where t = mean annual temperature.

6% of the SOC showed that the proportion of the LF was slightly reduced after incubation of the ambient soils, but reduced by half in elevated CO$_2$ soils. This corroborated the data obtained from the CO$_2$ during incubation in that its ^{13}C signal became more negative with incubation. The ^{13}C contents of the LF after the laboratory incubation showed that an average of 33% of this fraction was derived from the label. The >53-μm sized organic C released from aggregates (POM) by dispersion was only slightly affected by incubation in both size and δ ^{13}C signal indicating that this material is protected within the aggregates from decomposition. The silt and clay, as expected for an older fraction, are not affected by incubation.

DISCUSSION

This California Ultisols with its moderate C level and an orchard and grassland heritage shows surface horizon C ages of 500 yr not too dissimilar from other soils in California (Trumbore, 1993) and the grassland and Corn Belt (Paul et al., 1997, Follett et al., 1997). It differs in that the SOC MRT does not increase with depth or hydrolysis as much as those of the other soils. The great soil pedogenic age has not affected the MRT of the SOC. This and the lower level of changes in the δ ^{13}C

values on hydrolysis indicate that the California Ultisols used in this experiment have less aromaticity and lower humification levels at all depths than soils from the Great Plains.

The total SOC of the elevated CO$_2$ and fertilized N treatments reflect the negligible input of above ground residues from young ponderosa pines that retained their needles for most of the experiment. The more sensitive, CO$_2$ evolution approach showed SOC responses to both N additions and elevated atmospheric CO$_2$. Cumulative CO$_2$ evolution accounted for 5.5% of the SOC in the control and 6.9% in the N fertilized and elevated atmospheric CO$_2$ treatment. Curve analyses of CO$_2$ evolution showed a very small C_a fraction.

Treatment altered the proportion of the active and slow pool with depth. This is similar to observations by Collins et al. (2000) that deeper layers have C_a and C_s pools with relatively rapid MRTs. This suggests the presence of decomposable materials such as carbohydrates in the root-derived material at depth (Mary et al., 1992). We still know little about roots and root-derived SOC but the rapid turnover of the C_a and C_s with depth indicates that soil studies in global change scenarios must include all depths as well as well conducted bulk density measurements. Paul et al. (2000) characterized C_s as representing the accumulation and

Table 8. Organic C distribution with texture and percentage of the fraction derived from the label for the whole soil of Placerville, CA.

	Wt. fraction	Total C	C distribution	δ ^{13}C	Wt. fraction	Total C	C distribution	δ ^{13}C
	%			‰	%			‰
	350 μL L^{-1} CO$_2$ & 0 g N m^{-2}				350 μL L^{-1} CO$_2$ & 20 g N m^{-2}			
LF	0.3 (0.02)†	37.7 (3.7)	5.5	−25.3 (0.25)	0.4 (0.06)	34.9 (5.6)	7.7	−25.0 (0.37)
POM + sand	27.0 (0.36)	0.7 (0.5)	10.7	−24.8 (0.63)	27.1 (1.48)	0.7 (0.1)	10.0	−25.0 (0.20)
Silt	41.1 (0.63)	1.7 (0.27)	38.8	−25.1 (0.16)	40.7 (0.98)	2.0 (0.12)	44.3	−25.4 (0.14)
Clay	31.7 (0.84)	1.7 (0.09)	29.3	−24.0 (0.11)	31.8 (0.62)	1.7 (0.13)	29.1	−24.0 (0.23)
Whole soil		1.8 (0.27)		−25.0 (0.05)		1.8 (0.45)		−25.1 (0.09)
	700 μL L^{-1} CO$_2$ & 0 g N m^{-2}				700 μL L^{-1} CO$_2$ & 20 g N m^{-2}			
LF	0.4 (0.03)	34.4 (6.6)	8.0	−29.1 (2.37)	0.6 (0.05)	39.0 (5.2)	11.1	−29.2 (0.12)
POM + sand	26.4 (1.48)	0.7 (0.04)	9.9	−27.0 (0.25)	25.3 (0.87)	0.9 (0.12)	12.3	−28.2 (1.13)
Silt	41.8 (0.49)	1.8 (0.15)	43.3	−25.8 (0.20)	42.5 (1.35)	2.1 (0.46)	46.6	−25.9 (0.24)
Clay	31.3 (1.94)	1.6 (0.13)	28.6	−24.5 (0.10)	31.7 (2.26)	1.5 (0.28)	24.4	−24.4 (0.31)
Whole soil		1.8 (0.95)		−25.7 (0.08)		1.9 (1.28)		−26.0 (0.10)
	Derived from the label (700 μL L^{-1} δ^{13}CO$_2$							
	%							
LF			20.2				22.2	
POM + sand			11.1				16.7	
Silt			3.6				2.8	
Clay			2.3				2.4	
Whole soil			3.4				4.9	

† Values in parentheses are the standard errors of the mean of three observations.

Table 9. Organic C distribution with texture and percentage of the fraction derived from the label after 187 d of incubation.

	Wt. fraction	Total C	C distribution	δ ^{13}C	Wt. fraction	Total C	C distribution	δ ^{13}C
		%		‰		%		‰
	350 μL L^{-1} CO$_2$ & 0 g N m^{-2}				350 μL L^{-1} CO$_2$ & 20 g N m^{-2}			
LF	0.2 (0.04)†	36.5 (3.5)	4.7	−25.6 (0.7)	0.3 (0.03)	24.8 (4.0)	5.0	−27.6 (1.8)
POM + sand	29.8 (0.79)	0.7 (0.1)	12.2	−24.6 (0.7)	28.3 (1.1)	0.7 (0.1)	11.4	−25.0 (0.4)
Silt	39.1 (0.56)	1.5 (0.03)	35.6	−24.8 (0.2)	38.9 (1.1)	1.6 (0.1)	37.6	−25.0 (1.3)
Clay	30.9 (0.47)	1.8 (0.2)	33.4	−23.7 (0.1)	32.5 (0.4)	1.7 (0.2)	33.5	−23.8 (0.1)
Whole soil		1.7 (0.1)		−25.0 (0.2)		1.6 (0.3)		−25.2 (0.3)
	700 μL L^{-1} CO$_2$ & 0 g N m^{-2}				700 μL L^{-1} CO$_2$ & 20 g N m^{-2}			
LF	0.3 (0.07)	18.1 (0.8)	3.8	−32.2 (1.1)	0.5 (0.06)	14.5 (3.9)	3.9	−32.7 (0.5)
POM + sand	28.1 (0.6)	0.9 (0.2)	16.5	−26.7 (0.7)	29.2 (0.5)	1.0 (0.01)	16.6	−27.6 (0.5)
Silt	38.3 (1.01)	1.6 (0.4)	41.0	−26.7 (0.2)	38.7 (0.20)	2.0 (0.3)	44.1	−26.0 (0.04)
Clay	33.3 (1.18)	1.6 (0.1)	34.9	−24.3 (0.1)	31.7 (0.17)	1.9 (0.05)	34.6	−24.7 (0.3)
Whole soil		1.5 (0.1)		−25.9 (0.1)		1.7 (0.4)		−26.6 (1.1)
	Derived CO$_2$ from the label (700 μL L^{-1} δ ^{13}CO$_2$							
		%						
LF			35.6					31.1
POM + sand			10.7					13.3
Silt			9.7					5.6
Clay			2.9					4.6
Whole soil			4.7					7.3

† Values in parentheses are the standard errors of the mean of three observations.

turnover of the partially stabilized residue C. This is the pool that controls the long-term fertility of the soil and is the most important in C storage calculations in global change. This is corroborated in this study in that the large C_s pool had much larger MRTs than the active fraction. We conducted this incubation at 37°C rather than the 25°C of our previous studies to obtain soil dynamics data in one year. MacDonald et al. (1995) showed an increase in the measured pool sizes at higher incubation temperatures; however, the proportion of the pool sizes at the end of an extended incubation is not affected by increased incubation temperature (Townsend et al., 1997). This was attributed at least in part to a change in the decomposer population (Zogg et al., 1997). It could also be due to lower microbial growth efficiencies at the higher temperatures.

Our approaches using acid hydrolysis, C dating, and extended incubation with curve fitting have been shown, in this paper, applicable to SOC under trees in a Mediterranean type climate. The acid hydrolysis approach is known to include some recent lignin in the residue fraction if plant residues are not thoroughly removed. It, however, is the most straight forward, analytical approach that provides consistent fractions that are much older than the SOC (Collins et al., 2000).

The ^{13}C label inherent in the fossil-fuel derived CO$_2$ used for elevated atmospheric CO$_2$ study provided the expected useful label. The fact that the seedlings had δ ^{13}C values different from the original soil provided another label. These could be analyzed separately because of the availability of pretreatment soils and the analyses of soils from the CO$_2$ elevated plots from N and non-N treatments. The plots, however, did not contain a tracer control, at normal atmospheric concentration, to measure ^{13}C incorporation in the absence of elevated CO$_2$. This can be supplied in future experiments, by the addition of highly enriched ^{13}CO$_2$ to chambers at ambient CO$_2$. Alternatively, atmospheric CO$_2$ can be scrubbed from the air entering some chambers and replaced with anthropogenic-derived CO$_2$ with its built in

^{13}C signal. In our case, the trees themselves produced a second label that helped in part overcome the lack of an adequate tracer control. Normal turnover would incorporate some labeled C into the SOC. Our estimates, therefore, are probably maximum ones for elevated atmospheric CO$_2$ effects.

The lack of appreciable leaf litter during the experiment made it possible to attribute, by means of the different δ ^{13}C values to partition the SOC sources, 4% of the SOC in the 0- to 18-cm depth to root production during the course of this experiment. Slightly more than 2% of the intermediate depth SOC and less than 1% at the lowest depth was root derived. Root C removed during harvest contained 516 g C m^{-2} in the elevated CO$_2$ treatment without N and 632 g C m^{-2} in the N fertilized treatment. The ^{13}C studies showed an additional 228 of root derived C was left in the harvested soil in the treatment without N and 286 g in the elevated CO$_2$ treatment with N. This showed that approximately 45% of the root C was transformed to soil C in all treatments and indicates that the soil is capable of sequestering significant C.

It is tempting to extrapolate the 45% conversion value to sequestration capacities of more mature pine forests. We have not done this because (i) the 6-yr experiment did not result in appreciable litter and thus is not typical of a more mature coniferous forests, (ii) it is dangerous to extrapolate from only one site, and (iii) the root-to-soil conversion values are a maximum as they include ^{13}C incorporation but not ^{12}C turnover during this time period. The extended laboratory incubations that are independent of tracers give an independent estimate of total C sequestration potential. The 78% increase in production of C by the elevated CO$_2$ and N treatment resulted in a 50% increase in mineralizable C during the 187-d incubation. This is indicative of increases in the size of the slow pool that will with time show up in measurable total C sequestration and can be modeled.

The physical analysis complemented the incubation and hydrolysis-carbon dating. The LF is the most easily

identifiable labile pool. Only 20% of this fraction was labeled after 6 yr of tracer exposure where the majority of ^{13}C inputs occurred during the last few years of exposure. This fraction also was slowly labeled by the ^{13}C signal inherent to the trees without elevated [13]CO_2. This was confirmed by the change in δ ^{13}C of the CO_2 of the ambient treatment changing from -25‰ at Day 10 to -27.7‰ at Day 187 showing that the root-derived material took some time to be incorporated into the LF and then to decay. The light fraction most closely coincided with the C_a pool. The POM that comprised 10.7% of the total SOC in the ambient non fertilized treatment was only slightly raised to 12.3% in the elevated N treatment with 80% more productivity. Its δ ^{13}C values confirms that this fraction is a component of the C_s pool as determined by incubation with a field MRT of 24 to 34 yr depending on treatment.

Calculations of the role of SOC dynamics in sustainable agriculture, forestry management, and global change are best made by modeling techniques. These in turn need to be based on analytically derived SOC pools and fluxes as determined in this paper. We have shown that a combination of chemical (acid hydolysis), physical (LF, POM), and biological approaches (incubation) together with tracers gives the information required for modeling C sequestration both on a site specific and landscape basis. The N fertilization and the elevated CO_2 treatments produced significant responses in tree growth. These were not measurable in the total SOC during the relatively short-term study. The use of the C_a and C_s pool as derived here when incorporated in to the Century Model has been shown to give good estimates of field CO_2 evolution rates (Paul et al., 1999). Further modeling incorporating the data on C dynamics from this site together with data from the Great Plains (Follet et al., 1997) and the Corn Belt (Collins et al., 2000) should provide much greater insight into C sequestration by soils.

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Long-Term Effects of Urea and Green Manure on Rice Yields and Nitrogen Balance

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ABSTRACT

Data from a 14-yr double-crop rice (*Oryza sativa* L.) experiment allowed comparison of the long-term effects of N fertilizer from different sources (urea and in situ grown azolla [*Azolla microphylla* Kaulf.] and sesbania [*Sesbania rostrata* Bremek. & Oberm.]) on N balances, soil N pools (both total and available), and yields. Although data show that plant-available N was maintained over time in both wet (WS) and dry seasons (DS), yields declined significantly, indicating a decline in physiological N use efficiency. The yield declines were generally similar regardless of N source in both seasons. The WS decline averaged 150 kg ha^{-1} yr^{-1} in the three added-N treatments, while the DS decline averaged 185 kg ha^{-1} yr^{-1}. After 27 crops, the cumulative positive N balance was estimated at 1244, 348, 646, and 1039 kg N ha^{-1} in control, urea, sesbania, and azolla treatments, respectively. There was no significant change in soil total N content in the control and urea treatments, whereas it increased to 344 to 541 kg after 27 crops in the sesbania and azolla treatments. Conservation of the soil N status and positive N balance, in spite of the high amounts of N removed through grain and straw, reflect the N contribution (13–46 kg ha^{-1} crop^{-1}) from nonsymbiotic N_2 fixation. In addition, sesbania and azolla were estimated to add ≈57 to 64 kg ha^{-1} crop^{-1} through symbiotic N_2 fixation. These results demonstrate that in rice–rice cropping systems biological N fixation plays a vital role in replenishing the soil N pool. However, continuous application of green manure N (GM-N) did not increase crop N availability, perhaps because of the presence of a recalcitrant soil organic matter fraction. Residual effects on rice grain yield and N uptake were observed only with GM-N sources.

R$^{ICE IS THE WORLD}$'s most important staple food for more than two billion people in Asia and for hundreds of millions in Africa and Latin America. Subsistence rice farming of the pre-chemical era sustained the N status of soils by maintaining an equilibrium between N loss from crop harvest and N gain from biological N fixation (BNF) (Ladha and Peoples, 1995). In today's intensive rice monocropping systems, however, this equilibrium has been disturbed, with inputs from mineral fertilizers now playing a significant role.

The objectives of this study were to examine the long-term effects of urea applications on N balances, soil N pools (both total and available), and yields, and to ascertain the effects when green manure (GM) grown in situ (azolla and sesbania) was substituted for urea. The analysis is based on a 14-yr double-crop rice experiment conducted at the International Rice Research Institute. Long-term experiments are essential for accurately measuring nutrient balances because large year-to-year variation in crop growth can dominate measurements taken during short periods and because the changes must be measured against the large quantities of nutrient usually present in the soil (Greenland, 1994; Powlson, 1994).

Yield trends have been estimated in many other long-term experiments, but it is rare for these experiments to conduct periodic monitoring of changes in N uptake and soil N pools. Such measurements can provide valuable insight into the possible causes of observed yield trends and the implications of these trends for sustainability. In addition, most tropical experiments were designed to study yield trends with mineral N input, while ignoring GM as an alternative source of N. There has been much speculation on the long-term effects of continuous N use, particularly GM-N, but actual data for intensive rice systems are lacking (Bouldin, 1988).

MATERIALS AND METHODS

Experimental Details

A long-term experiment began in March 1985 in an isohyperthermic Andaqueptic Haplaquoll (Maahas silty clay) at the experimental farm of the International Rice Research Institute, Los Baños, Philippines, with the following soil characteristics: pH 6.3 (1:1 w/v water), 12.5 g organic C kg^{-1}, 1.4 g

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Abbreviations: BNF, biological N fixation; DS, dry season; GM, green manure; GM-N, green manure N; WS, wet season.