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DISSERTATION

NITROGEN-FIXING TROPICAL TREES SEQUESTER SOIL CARBON

Submitted by

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In partial fulfillment of the requirements
for the Degree of Doctor of Philosophy
Colorado State University
Fort Collins, Colorado
Fall 1999

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SIGRID CHIMNER RESH ENTITLED NITROGEN-FIXING TROPICAL TREES SEQUESTER SOIL CARBON BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

NITROGEN-FIXING TROPICAL TREES SEQUESTER SOIL CARBON

The objective of my dissertation research was to determine how nitrogen-fixing tree species (N-fixers) increase total soil carbon storage relative to non-N-fixers (*Eucalyptus*) by examining the effects of N-fixers on the decomposition of old soil carbon and the accumulation of new soil carbon. Using stable carbon isotope techniques, I tracked the loss of the old soil organic carbon from the previous C_4 land use (SOC_4) and the gain of new soil organic carbon from the C_3, N-fixer and *Eucalyptus* plantations (SOC_3). I tested four hypotheses. H_1: Increased soil N from N-fixation will increase the decomposition of older SOC_4 under the N-fixers relative to the non-N-fixers. H_2: Greater total soil carbon accretion under N-fixers will result from greater accretion of N-fixer SOC_3 relative to the non-N-fixers. H_3: Greater accretion of N-fixer SOC_3 will result from increased litter inputs rather than increased recalcitrance of N-fixer litter. H_4: Soil carbon pools under mixtures of N-fixers and non-N-fixers will follow linear trends with increasing proportions of the N-fixer.

I found greater retention of the old soil carbon and greater accretion of new soil carbon under the N-fixers relative to *Eucalyptus*. The N-fixers sequestered 0.11 kg m^{-2} yr^{-1} more total soil carbon with 0.06 kg m^{-2} yr^{-1} of that from greater retention of old carbon and 0.05 kg m^{-2} yr^{-1} from greater accretion of new carbon relative to *Eucalyptus*.

The annual differences between the N-fixers and *Eucalyptus* for the total, old, and new soil carbon pools corresponded with nitrogen accretion differences between the N-fixers and *Eucalyptus*.

Eight-month soil incubations of the surface soils from all species at three of the sites supported the hypothesis that greater N-fixer litter inputs are necessary for the greater SOC_3 accretion under the N-fixers compared with the non-N-fixers. The results from the incubations cannot rule out the possibility of greater recalcitrance of some portion of the N-fixer litter inputs.

Mixed species plots of *Albizia* and *Eucalyptus* showed that there is a neutral interaction for species mixtures on soil organic carbon pools. That is, knowing the pure species endpoints of old soil carbon retention and new soil carbon accretion is enough information to extrapolate linearly to mixtures.

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Dedicated to Rod, my incredible husband,

Your love, support, and acceptance of all that I am

makes me strive to become a better person.

And

Dedicated to our children, Lynx and Kootenai,

Your soft fur and happy "wooooos" kept me going.

"All is swell

Life is good"

--Old billboard graffiti on Highway 34 to Estes Park

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Globally, soils store approximately 1500 x 10^{15} g of organic carbon, or twice as much carbon as the atmosphere and three times as much as terrestrial vegetation (Schlesinger 1997). The vulnerability of this large pool of carbon to human disturbance makes soil a relevant topic of study for at least two major reasons. First, soil organic carbon is intricately linked to the cycling of soil nutrients that influence ecosystem productivity (Van Cleve and Powers 1995). Second, this huge pool of carbon has the potential to mitigate or exacerbate the increasing atmospheric CO_2 concentrations that have the potential alter global climate (Schlesinger 1990, 1999, Nadelhoffer *et al.* 1999).

Deforestation may contribute to the loss of soil carbon and, therefore, may currently be increasing atmospheric CO_2 concentrations by changing the balance between biomass production and decomposition. One estimate for tropical deforestation carbon emission is 1.1 to 1.6 x 10^{15} g C yr^{-1} with up to 25% of that coming from soils (Houghton 1994). Intensive cultivation can also decrease soil carbon, contributing to terrestrial net fluxes of carbon to the atmosphere and decreased net primary productivity (Burke *et al.* 1989, Johnson 1992). Some land use practices (such as low-tillage, legume-based, or manure application agriculture) can increase soil carbon storage relative to conventional agricultural systems (Paustian *et al.* 1997 , Drinkwater *et al.* 1998), but reforestation and afforestation are viewed by some to have the greatest potential in terms of land use change for offsetting increasing CO_2 emissions (Vitousek 1991, Brown *et al.* 1992, Moffat 1997, Bruce *et al.* 1999).

While it is clear that reforestation or afforestation of agricultural land increases carbon storage in plant biomass over that of the pasture or crop vegetation, it is less clear whether reforestation or afforestation increases carbon storage in the mineral soil. (Bashkin and Binkley 1998, Binkley and Resh 1999, Richter *et al.* 1999). The degree to which species differ in their effects on carbon storage in the mineral soil and whether there are any general species patterns is also unclear. Tree species differ in biomass production and tissue nutrient concentrations and in their effects on soil properties such as pH, nutrient cycling, and soil biota (Binkley 1996, Binkley and Giardina 1998). Species also differ in soil carbon accretion rates especially when nitrogen-fixing species (N-fixers) are included (Lugo *et al.* 1990). All comparisons of N-fixers and non-N-fixers have found 20 to 100% more soil carbon under N-fixers compared with non-N-fixers (Johnson 1992, Cole *et al.* 1995, Rhoades *et al.* 1998), despite generally greater N-fixer litter decomposition (Binkley and Giardina 1997, but see Cole *et al.* 1995).

This net increase in soil organic carbon under N-fixers can be separated into two potential mechanisms. One mechanism is the possible differences in species impacts on decomposition of soil organic matter. The second mechanism results from possible species differences in the addition of soil organic matter. The objective of my dissertation research was to determine how N-fixers increase total soil carbon storage relative to non-N-fixers by examining these two potential mechanisms. I used a change in land use from C_4 agriculture to C_3 forest plantations and stable carbon isotope techniques to differentiate between losses of older, C_4 soil carbon and gains of newer, C_3

soil carbon (SOC_4 and SOC_3, respectively). I examined the soil carbon dynamics under N-fixers compared with non-N-fixers across four sites in Hawaii and Puerto Rico with differing precipitation regimes and edaphic conditions to assess the general pattern of N-fixers versus non-N-fixers in tropical forests. Within this context, I tested the following four hypotheses:

H_1 Increased soil N from N-fixation will increase the decomposition of older SOC_4 under the N-fixers relative to the non-N-fixers.

H_2 Greater total soil carbon difference under N-fixers will result from greater accretion of N-fixer SOC_3 relative to the non-N-fixers.

H_3 Greater accretion of N-fixer SOC_3 will result from increased litter inputs rather than increased recalcitrance of N-fixer litter.

H_4 Soil carbon pools under mixtures of N-fixers and non-N-fixers will follow linear trends with increasing proportions of the N-fixer.

I first addressed the question of whether N-fixers effect the retention of old soil carbon relative to non-N-fixers (H_1) in Chapter II. Hypothesis 1 was based on the idea that microbial decomposition of soil carbon is nitrogen limited. Several studies have shown a positive effect of nitrogen additions on litter/forest floor decomposition (*cf.* Salonius 1972, Foster *et al.* 1980, Harmon *et al.* 1990, Green *et al.* 1995). Using the isotopically distinct pool of older soil carbon, my goal was to isolate a humified pool of carbon to determine the effect of the N-fixers (and increased nitrogen supply) on the decomposition of more recalcitrant organic carbon. I tested H_1 by comparing the losses in the SOC_4 pools under the N-fixers and non-N-fixers since the plantation establishment.

I then compared the accretion of new soil carbon under N-fixers and non-N-fixers (H_2) in Chapter III. Here my goal was to determine the species differences in the accretion rates of soil organic matter. I tested H_2 by comparing the changes in SOC_3 pools under N-fixers and non-N-fixers since plantation establishment.

In the case of new tree soil carbon, comparing the *in situ* soil carbon accretion rates could not demonstrate the mechanism behind any differential accretion of soil carbon. Unlike the old soil carbon pool where the only mechanism changing that pool size was decomposition, the new tree soil carbon results from both soil organic matter decomposition and production. Greater new carbon accretion under N-fixers could result from greater carbon inputs by the N-fixer, or from slower decomposition of the N-fixer inputs (H_3). Using long-term laboratory incubations, my goal was to compare the loss of new soil carbon relative to initial magnitudes of soil carbon from under the N-fixers and non-N-fixers. In Chapter III, I also examined the patterns of SOC_4 loss and SOC_3 gain under the N-fixers and non-N-fixer through time at one site for a true time course comparison.

My goal in Chapter IV was to determine whether the pure species effects addressed in H_1 and H_2 could be used to predict the effect of N-fixer/non-N-fixer mixtures on soil organic carbon. I used a replacement series of N-fixers and non-N-fixers with four mixtures and pure species endpoints to evaluate the interactive effects of N-fixer/non-N-fixer mixtures on total soil carbon accretion and the components of older carbon retention and newer carbon accretion (H_4).

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CHAPTER II

GREATER RETENTION OF OLD SOIL CARBON UNDER N-FIXING TREES

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ABSTRACT

Anthropogenic increases in nitrogen inputs may enhance terrestrial carbon sequestration through increased plant production. The increase in nitrogen supply may also affect soil carbon sequestration through altered decomposition. We examined soil organic carbon pools under tropical plantations of nitrogen-fixing tree species and *Eucalyptus* that replaced agricultural land use. We found greater retention of the old, agricultural soil carbon under the N-fixers relative to *Eucalyptus*, and the greater retention corresponded with nitrogen accretion. The effect of increased nitrogen supply on soil carbon may be an important mechanism of carbon sequestration that is not currently considered in global change research.

TEXT

Although soils contain the third largest active pool of carbon (1.5×10^{18} g C) behind extractable fossil fuels and the ocean (1), annual soil carbon sequestration is not considered an important part of the global carbon budget (2,3). With accumulation rates commonly ranging from 0.7 to 10 g m^{-2} yr^{-1}, forest mineral soils sequester about 0.4×10^{15} g C yr^{-1} or only 0.7% of terrestrial net primary productivity (NPP) estimates (60×10^{15} g C yr^{-1}) (2). However, human activities are increasing nitrogen inputs to ecosystems with intentional and unintentional effects on ecosystem carbon sequestration. Increased nitrogen supply may enhance terrestrial carbon sequestration through increased NPP (4,5,6). Another effect may be a net change in soil carbon storage due to the effects of nitrogen on decomposition. The objective of this study was to examine the effect of increased nitrogen supply on soil carbon retention beneath nitrogen-fixing forests (N-fixers) and non-N-fixers (*Eucalyptus*). A recent shift in land use from C_4 agriculture to C_3 forest plantations allowed us to use stable carbon isotopes to separate the loss of older soil organic carbon (SOC_4) from the gain of newer soil organic carbon (SOC_3) (7).

Until recently, the dominant paradigm was that nitrogen additions to soils increased microbial decomposition of organic matter (8). Because microbes manufacture enzymes to decompose organic substrates and enzymes require nitrogen, microbes with more nitrogen might produce more enzymes and thus speed decomposition (9). Increased decomposition of litter (10,11) and increased microbial activity (12) following nitrogen additions have been reported.

However, empirical, field-based studies have shown that nitrogen additions actually inhibit decomposition (8,13,14,15,16,17). The majority of these studies consisted of comparisons of litter mass loss from litter quality differences or soil CO_2 evolution that examined short-term effects of nitrogen amendments on decomposition. A recent review of nitrogen deposition and fertilization effects on decomposition concluded that both direct effects of high soil nitrogen supply and indirect effects of high nitrogen concentrations in litter generally increase initial decomposition rates of fresh litter (18,19 but see 16), but inhibits decomposition of humified soil carbon (17).

We measured the soil organic carbon pools at four tropical plantations consisting of replicated monocultures of N-fixers and *Eucalyptus* (20). Two sites were in Hawaii: Kamae (21) (Andisol soil, 4000 mm yr^{-1} precipitation, 15-year-old plantations) and Chinchuck (22) (Andisol soil, 4000 mm yr^{-1} precipitation, 16-year-old plantations). The other two sites were in Puerto Rico: Lajas (23) (Vertisol soil and 1100 mm yr^{-1} precipitation, 16-year-old plantations) and Toa Baja (24) (Entisol soil and 1700 mm yr^{-1} precipitation, 7-year-old plantations). Each site consisted of a completely randomized block design with three to six replications of each tree species. At both Hawaii sites, the tree species were *Albizia falcataria* (N-fixer) and *Eucalyptus saligna* (both C_3 species) and the previous land cover was sugarcane (*Saccharum officinarum*, C_4 species). The Chinchuck site also included mixtures of *Albizia* and *Eucalyptus* planted at a constant density (2500 trees ha^{-1}) as part of the replicated experimental design. We sampled the soils from the 25, 33, 50, and 66% *Albizia* plots (22). At both Puerto Rico sites, the C_3 tree species were *Casuarina equisetifolia* (N-fixer), *Leucaena leucocephala* (N-fixer), and *E. robusta* and the previous land cover was sugarcane (at Lajas) or pasture grass (mostly *Panicum maximum* a C_4 species at Toa Baja).

The influence of recent carbon inputs from current forest plantations created differences in soil $\delta^{13}C$ values of 2.2 to 5.6‰ (25) between tree and cane or pasture in the upper 10 to 20 cm of the soil profile (or throughout the soil profile for the Chinchuck site; Figure 2.1). However, the average soil $\delta^{13}C$ among tree species was similar, ranging from 0.02 to 0.63‰. Differences in total soil organic carbon (SOC_T) (26) were apparent among tree species with N-fixers averaging 1.36 kg m^{-2} more SOC_T than *Eucalyptus* (Figure 2.2).

The small $\delta^{13}C$ differences combined with the larger SOC_T differences resulted in some striking differences between trees species in total SOC_4 pools (27). The N-fixers across the four sites showed a consistent pattern of greater SOC_4 compared to those of *Eucalyptus* (Figure 2.3). The larger SOC_4 pools at the Lajas site likely reflect a greater influence of C_4 vegetation compared with the other three sites. Because SOC_4 inputs essentially ceased (except for minor understory C_4 grasses) when the forest plantations were established, the larger SOC_4 pools under the N-fixers compared with *Eucalyptus* indicated smaller losses of SOC_4. Annual rates of change in SOC_4 (28) revealed the importance of species effects (split-plot analysis of variance (29); $P = 0.02$). The N-fixers lost only 0.06 kg C_4 m^{-2} yr^{-1} compared to a *Eucalyptus* loss of 0.11 kg C_4 m^{-2} yr^{-1}. There were no significant interactions between site and species.

To explain why N-fixers are retaining more old, SOC_4, we examined the relationship between the additional soil nitrogen and the additional SOC_4 under the N-fixers. Soil nitrogen accretion from N-fixation explained 60% of the greater SOC_4 retention under pure N-fixer plots and mixed N-fixer/*Eucalyptus* plots relative to under pure *Eucalyptus* plots (linear regression; $P < 0.01$; Figure 2.4). The pure N-fixer plots accrued 0.01 kg m^{-2} yr^{-1} more soil nitrogen relative to the pure *Eucalyptus* plots.

How might greater nitrogen supply result in greater storage of old SOC_4? Two working hypotheses address nitrogen suppression of later phases of litter and humus decomposition (8,18,30). (1.) Nitrogen additions, especially in the form of ammonium, may give a competitive edge to several cellulose decomposers (e.g., bacteria) over lignocellulose decomposers (e.g., fungi) due to the suppression of lignolytic enzyme synthesis by many species of fungi. This would favor the decomposition of labile soil organic carbon while inhibiting the decomposition of recalcitrant soil organic carbon. (2) Low molecular weight nitrogen compounds (e.g., amino acids, ammonium, and nitrate) may be incorporated into covalent bonds between C molecules (e.g., lignin molecules and phenolics). This would create larger molecules that are resistant to microbial decomposition. Though we did not set out to test the mechanisms behind the N-fixer effects on SOC, there is some evidence in Hawaii for the microbial community shift hypothesis (Hypothesis 1 above). *Albizia* soils had lower active fungal and higher active bacterial biomass compared to *Eucalyptus* soils in a three-site comparison in Hawaii that includes the two Hawaii sites addressed in the present study (31).

Regardless of the exact mechanism, we found a strong pattern of 50 g m^{-2} yr^{-1} greater old soil carbon sequestration under the N-fixers over that of the *Eucalyptus* that corresponded with soil N accretion under the N-fixers. This is fives times greater than the high end estimates of carbon sequestration in forest soil (2). These results suggest that additions of nitrogen to the soil may decrease the turnover rates of soil carbon resulting in less CO_2 cycled to the atmosphere.

What are the global implications for increased nitrogen supply on soil carbon retention? Humans alter the global soil nitrogen supply via three major pathways: planting

N-fixer species, nitrogen fertilization, and nitrogen deposition. Our estimates of the effects of those soil nitrogen additions on soil carbon retention are presented in Table 2.1. Our equation (Figure 2.4) predicts that 4.65 kg of carbon are retained in the soil for every kg of nitrogen retained. Generalizing our forest N-fixer effects to other symbiotic N-fixers, including agricultural legumes and natural ecosystem symbiotic N-fixers, is the most conservative extrapolation. This results in an additional soil carbon retention of 0.223×10^{15} g C yr^{-1} (Table 2.1) or 12 to 15% of the missing terrestrial carbon sink (1.5 to 1.9×10^{15} g C yr^{-1}) (32). Agricultural nitrogen fertilization could result in the additional retention of 0.186×10^{15} g C yr^{-1} or 10 to 12% of the missing sink. Though nitrogen fertilization of forests is not likely to have a global impact on of soil carbon storage, there could be important regional implications for areas of high forest plantation coverage. For example, forest plantations in Brazil cover 7 million ha (33) with nitrogen fertilizer applications ranging from 0.003 to 0.01 kg m^{-2} (34).

Average atmospheric deposition of nitrogen on forests is relatively small in magnitude compared with terrestrial N-fixer and nitrogen fertilizer inputs. Consequently, soil carbon retention from this nitrogen source would be small, only 0.8 to 1% of the missing sink. Recently, nitrogen deposition on forests has been discounted as one of the possibilities for explaining the missing sink (35). Shindler (36) suggests that several smaller sinks most likely will balance the global carbon budget. Greater soil carbon retention as a consequence of soil nitrogen accretion may be one of those small sinks.

The question remains as to whether soil carbon retention is a property of soil nitrogen accretion under N-fixer forests only or would nitrogen accretion from agricultural legumes, fertilization and atmospheric deposition create a similar story of soil carbon retention. Results from other studies indicate that increased nitrogen supply from sources other than N-fixers (8,17,18,29,37) and to ecosystems other than forests like agricultural systems (38,39) and peatlands (40) may similarly suppress decomposition. We need to directly examination changes in soil carbon retention with applications of inorganic nitrogen before we can fully comprehend the significance of our results.

In summary, we found a broad tropical pattern of greater retention of old soil organic carbon under N-fixers. If greater retention of soil carbon results from of greater soil nitrogen supply, nitrogen fertilization and deposition could have major implications for global carbon sequestration. The implications may extend only to other sites with N-fixers or they may also encompass areas of high nitrogen fertilization. Either contingency may need to be factored into assessments of the carbon cycle and global change. Extending our N-fixer results to include nitrogen inputs from other N-fixer species alone accounts for an additional soil carbon sequestration of up to 15% of the annual missing carbon sink.

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7. Plants using the C_3 photosynthetic pathway fix CO_2 via the Rubisco (ribulosebisphosphate oxygenase carboxylase) enzyme. This enzyme discriminates against the heavier carbon isotope (^{13}C) during the carboxylation phase. Plants using the C_4 pathway rely additionally on the PEP (phosphoenolpyruvate) carboxylase enzyme for carboxylation. This enzyme does not discriminate as greatly against ^{13}C. As a result of this differential ^{13}C discrimination, tissues from a C_3 plant have isotopic signatures (referenced to a standard) of -21 to -35 ‰ (per mil) compared to tissues of a C_4 plant with isotopic signatures of -8 to –15‰. When these plant tissues enter the soil carbon pool, they retain their differential signatures allowing the identification of the soil carbon belonging to the C_3 and C_4 plants in a situation where there has been a land use/cover change.
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    Bulk density was determined for each of the soil segments by oven drying a known fresh mass and volume at 105°C to a constant dry mass; rock mass was negligible. Hawaii soil bulk density did not differ between tree species or with neighboring cane fields. However, Lajas soil bulk density differed between the pasture and forest soil samples, and Toa Baja bulk density differed between pre-plantation and current soil samples. To correct for the bulk density differences, Lajas forest soil mass was adjusted to pasture mass and Toa Baja forest and pasture soil masses were adjusted to 1989 masses to reflect equivalent soil mass comparisons from pasture conditions for Lajas or though time for Toa Baja. The bulk density adjustments for Lajas increased the average total soil organic carbon (kg m^{-2}) under the trees by 4%.

    All soils were treated with a 0.2N HCl acid wash [D. Binkley and S.C. Resh, *Soil Sci. Soc. of Am. J.* **63,** 222 (1999)] to remove $CaCO_3$ that would affect $\delta^{13}C$ values [J.R. Ehleringer, in *Carbon isotope techniques*, D.C. Coleman and B. Fry, Eds. (Academic Press, Inc., San Diego, CA 1991) pp. 187-200]. Soil carbon and nitrogen concentrations were determined using dry combustion (LECO 1000, LECO Corporation, St. Joseph, MI, USA). We corrected the carbon and nitrogen concentrations to account for the mass of salt added in the acidification process. The $\delta^{13}C$ values of the soil organic carbon were measured using a combustion elemental analyzer (Fisions NA 1500, Carlo Erba Instrumentazione) coupled with an isotopic ratio mass spectrometer (VG ISOGAS, Middlewich, UK).
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25. $\delta^{13}C$ values are expressed in per mil units (‰) and were calculated with respect to Pee Dee Belemnite using NBS standard 21.
26. Total soil organic carbon (SOC_T) in kg C m^{-2} was calculated as SOC_T (kg C m^{-2}) = ρ_b x [C] x sample depth, where, ρ_b is the average bulk density for that segment of soil (kg L^{-1}), [C] is the concentration of soil C (g C kg^{-1} soil), and sample depth is the segment of the soil profile (m). Total soil organic carbon from each site consists of sugarcane or pasture C_4 carbon and tree C_3 carbon (pre-sugarcane/pasture and contemporary). We assumed no difference between $\delta^{13}C$ values of the pre-

sugarcane/pasture and contemporary C_3 vegetation for the %C_4 calculation below.

27. The proportion of total soil organic carbon coming from grasses or sugarcane (C_4) was calculated using a two-endpoint mixing model: $\%C_4 = [(\delta^{13}C_{sample} - \delta^{13}C_3)/(\delta^{13}C_4 - \delta^{13}C_3)] \times 100$ [V.A. Vitorello, C.C. Cerri, F. Andreux, C. Feller, R.L. Victória, *Soil Science Society of America Journal* **53**, 773 (1989)]. The C_3 and C_4 endpoints were different for each site and tree species. For the Hawaii sites the C_3 and C_4 endpoints (-25.11 and −11.52, respecitively) are taken from elsewhere [M.A. Bashkin and D. Binkley, *Ecology* **79**, 828 (1998)]. For the Lajas site the C_3 endpoints for the *Eucalyptus*, *Casuarina*, *Leucaena*, and pasture were −27.01, -26.30, -26.94, -26.60, respectively, and the C_4 endpoint was −13.80. For the Toa Baja site the C_3 endpoints for the *Eucalyptus*, *Casuarina*, *Leucaena*, and pasture were −28.33, -29.41, -28.64, and −28.78, respectively, and the C_4 endpoint was −14. The C_3 and C_4 endpoints for the Puerto Rico sites were composites of litterfall and roots from each sample plot. Root $\delta^{13}C$ values are from roots collected throughout all 0-40 cm soil cores and composited for each species. The equation for SOC_4 (kg C m^{-2}) was $SOC_4 = (\%C_4/100) * SOC_T$.
28. To determine the annual SOC_4 changes since plantation establishment, SOC_4 values (kg C m^{-2}) from neighboring sugarcane or pasture fields or pre-plantation archived soil samples (i.e., for Toa Baja) were used to approximate the initial conditions. Thus, the annual changes in SOC_4 were estimated by subtracting the initial condition from the recent sample values and dividing by the age of plantation. The annual SOC_4 changes were $\log_{10}$ transformed to equalize variance across the four sites for the split-plot ANOVA.
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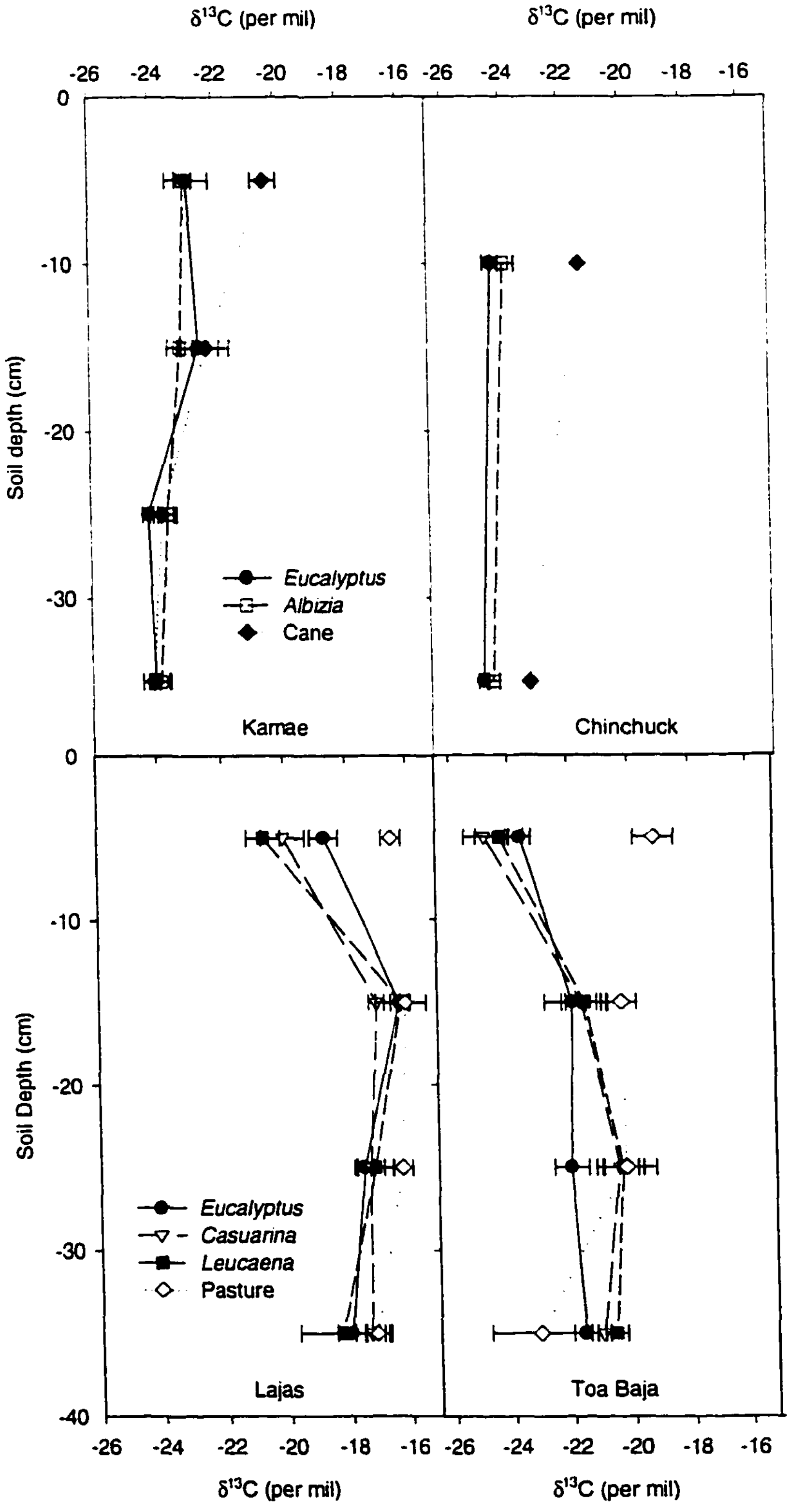
41. We thank Ariel Lugo for permission to sample from the Lajas site; Rod Chimner, Christian Giardina, Xiaoming Zou, Zhigang Liu, Margot Kaye, and Jason Kaye for field assistance; and Wendy Estes, Cindy Shimada, Carol Volk, and Jennifer Clark for laboratory assistance. We also thank J.R. ZumBrunnen of the Statistical Laboratory at Colorado State University for consulting services on statistical analysis. Additional thanks to Jason Kaye for editing help on previous drafts of the manuscript.

Table 2.1. Potential for soil carbon retention from various sources of nitrogen. The fraction of nitrogen retained in the soil is our best guess for symbiotic N-fixers and N fertilization but has been measured for N deposition (35). Soil carbon retention is based on our equation in Figure 2.4.

Nitrogen source	Total Nitrogen Fixed (10^{12} g N yr^{-1})	Fraction of Nitrogen Retained in Soil	Carbon Retained in Soil (col 1 x col 2 x 4.649) (10^{15} g C yr^{-1})
Symbiotic N-fixers (1)	96	0.5	0.223
N fertilization (1)	80	0.5	0.186
N deposition on forests (35)	5.1	0.7	0.017

Figure 2.1. Site and species comparisons of soil $\delta^{13}C$ values by soil depth. Symbols represent mean values for vegetation samples (26) of 10 cm soil segments (or 20 and 30 cm segments for the Chinchuck site) ± s.e.m (when sample size allowed). Replicates for mean values consist of 3, 4, and 3 for *Eucalyptus*, *Albizia*, and cane at Kamae, respectively; 4, 4, and 1 for *Eucalyptus*, *Albizia*, and cane at Chinchuck (M.A. Bashkin, unpublished data), respectively; 6 for all species at Lajas, and 3 for all species at Toa Baja.


δ13C (per mil)
δ13C (per mil)
-26
-24
-22
-20
-18
-16
0
-10
-20
-30
-40
Soil depth (cm)
Soil Depth (cm)
Eucalyptus
Albizia
Cane
Kamae
Chinchuck
Eucalyptus
Casuarina
Leucaena
Pasture
Lajas
Toa Baja

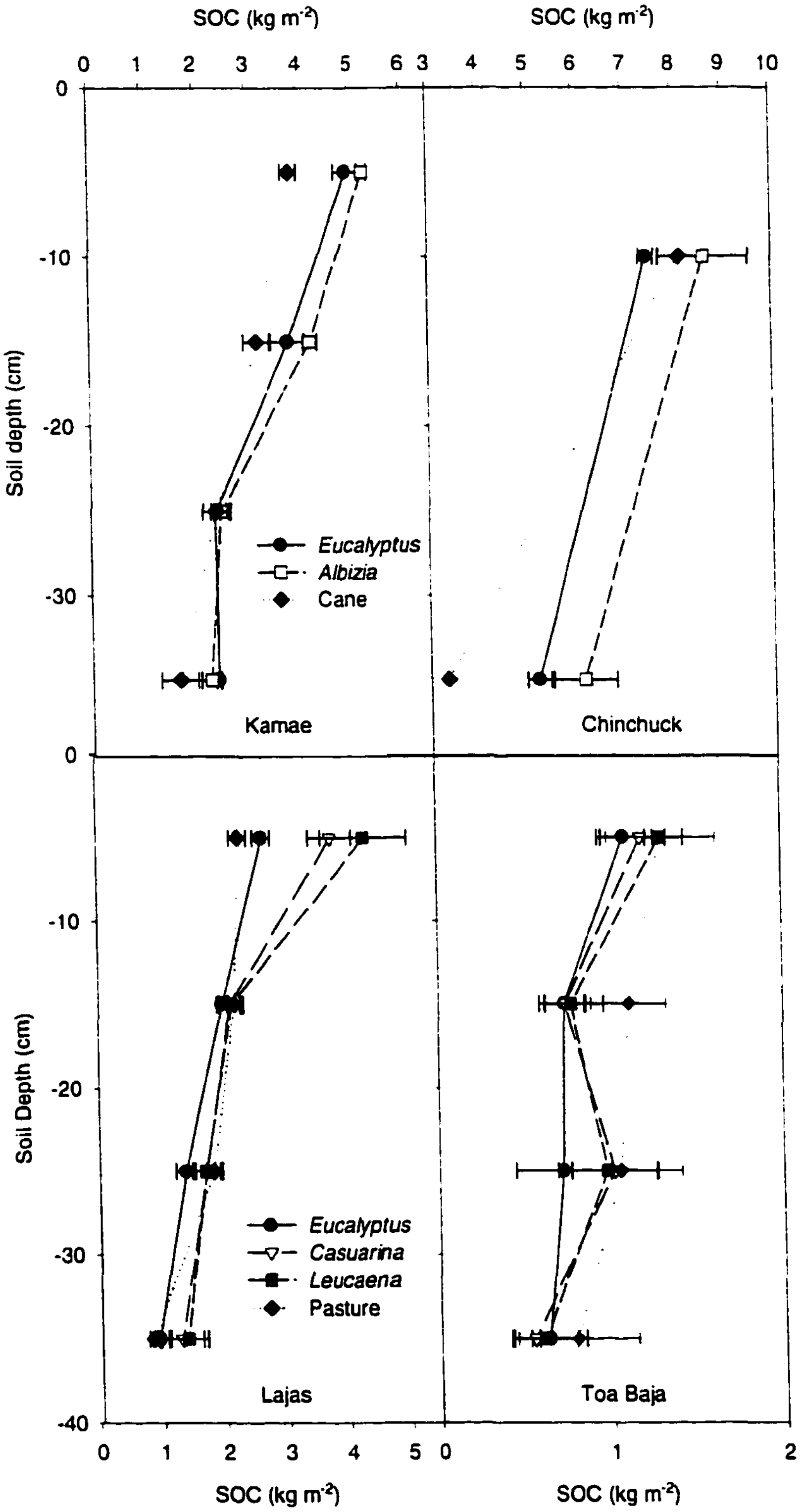


Figure 2.2. Site and species comparisons of total soil organic carbon (SOC_T) values by soil depth. Symbols represent mean values for SOC_T ± s.e.m. Replicates are the same as in Figure 2.1.

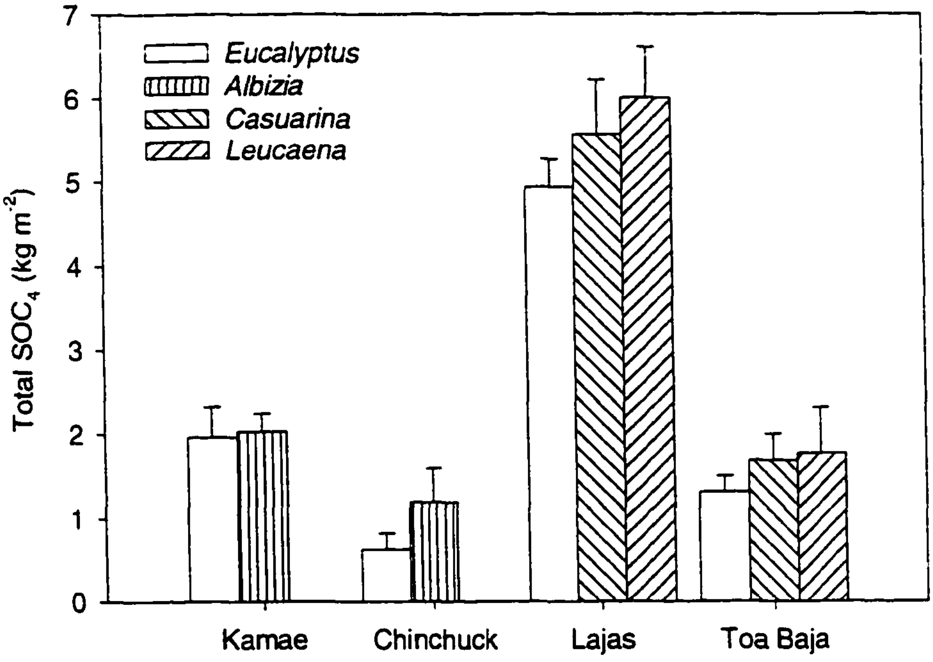


Figure 2.3. Total SOC_4 to 40 cm for each site (to 50 cm for Chinchuck) and tree species (pure species only); means ± s.e.m. are shown. Replicates are the same as in Figure 2.1.

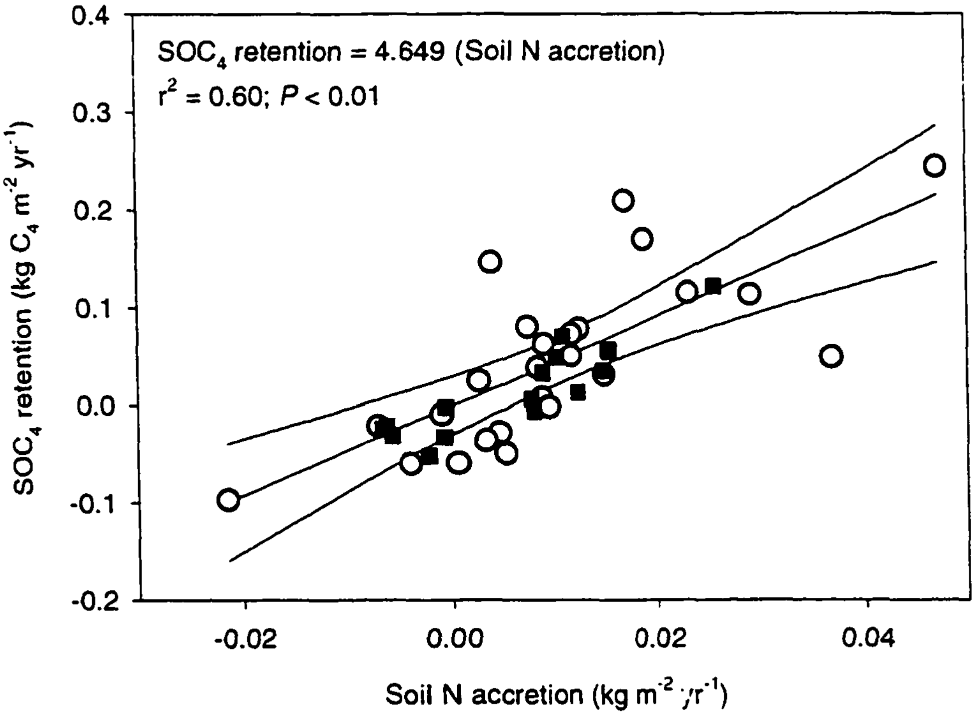


Figure 2.4. Annual N-fixer SOC_4 differences relative to *Eucalyptus* plots (SOC_4 retention) versus annual N-fixer soil nitrogen (N) differences relative to *Eucalyptus* plots (N accretion). The lines are the best-fit curve with 95% confidence interval for pure species plots (plotting symbols are open circles) across all four sites (n = 25) and for *Albizia/Eucalyptus* mixture plots (plotting symbols are filled squares) at the Chinchuck site (n = 16).

CHAPTER III

GREATER SOIL CARBON RETENTION AND ACCRETION UNDER NITROGEN-FIXING TREES COMPARED WITH *EUCALYPTUS*

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ABSTRACT

We compared the soil carbon pools under nitrogen-fixing tree species with *Eucalyptus* at four tropical sites. Using stable carbon isotope techniques, we tracked the loss of the old soil organic carbon from the previous C_4 land use (SOC_4) and the gain of new soil organic carbon from the C_3, N-fixer and *Eucalyptus* plantations (SOC_3). Our objective was to determine tree species effects on old carbon retention and new carbon accretion as a proportion of change in total soil carbon (SOC_T) through time and across multiple sites.

Across all tree species at Toa Baja, we found a significant quadratic time trend for SOC_T, which was dominated by an early loss of the old SOC_4 followed by a delayed, but significant gain of new SOC_3. Species effects were not apparent over the first seven

years of plantation growth; however by the seventh year the N-fixers had clearly higher SOC_T compared with the *Eucalyptus*. Across the four sites, N-fixers accrued 0.11 ± 0.03 kg m^{-2} yr^{-1} (mean ± one standard error) of SOC_T compared with a loss of 0.01 ± 0.03 kg m^{-2} yr^{-1} under the *Eucalyptus*. About half of the greater SOC_T accretion resulted from significantly greater retention of old SOC_4 under the N-fixers, and half from greater accretion of new SOC_3.

Strategies that use reforestation or afforestation to offset carbon emissions may gain from consideration of the differential effects of N-fixers versus non-N-fixers on soil carbon storage. Globally, symbiotic N-fixers may be responsible for up to 0.42×10^{15} g/yr of soil carbon storage.

INTRODUCTION

Symbiotic nitrogen-fixing species (N-fixers) are a component of most ecosystems from the tundra to the tropics (Werner and Muller 1990). Ecosystems with substantial presence of N-fixers differ fundamentally from ecosystems without N-fixers. Nitrogen-fixers increase nutrient cycling and soil N supply, increase or decrease soil P supply, increase or decrease soil pH, change understory vegetation, and increase N leaching losses (van Miegroet and Cole 1984, Hibbs *et al.* 1994, MacDicken 1994, Binkley and Giardina 1997).

N-fixing forests also tend to have larger soil organic carbon pools than non-N-fixing forests. Soils under temperate and tropical N-fixer forests contain 20-100% (0.05-0.12 kg m^{-2} yr^{-1}) more total soil organic carbon (SOC_T) over that of non-N-fixer forests (Johnson 1992, Tarrant and Miller 1963, Binkley *et al.* 1982, Binkley 1983, Binkley and Sollins 1990, Cole *et al.* 1995, Rhoades *et al.* 1998). This difference in SOC_T is generally attributed to greater accretion of new carbon from N-fixer litter (Cole *et al.* 1995). However, the differential retention of older SOC is also an important contribution to the greater SOC_T under N-fixers compared with non-N-fixers (Resh *et al.* 1999). The mechanisms leading to the ubiquity of larger SOC_T pools under N-fixers compared with non-N-fixers merits further investigation, especially in light of the possibility of using afforestation to mitigate increasing global atmospheric CO_2 concentrations (UNFCCC 1997).

Our primary objective was to compare the soil carbon pools under N-fixer and non-N-fixer tree species to determine the relative importance of old carbon retention and new carbon accretion to the difference in SOC_T pools between N-fixers and non-N-fixers.

We used the isotopic signatures of the soil organic carbon to track the loss of old carbon from C_4 plants (SOC_4) and the gain of new carbon from C_3 trees (SOC_3) under N-fixers and non-N-fixers. We addressed this primary objective by tracking the changes in the soil organic carbon pools through time within a single site and across four sites containing replicated, pure-species stands of N-fixers and non-N-fixers. Because greater new SOC_3 accretion under N-fixers can result from greater litter inputs or greater retention of those inputs, our secondary objective was to determine which mechanism was responsible. We conducted long-term laboratory incubations of the surface soils from the N-fixer and non-N-fixer stands from three of our study sites.

METHODS

Site Descriptions

We sampled soils from two sites in Hawaii and two sites in Puerto Rico, which represent a range of precipitation levels and soil types (Table 3.1). The sites consisted of seven- to 16-yr-old plantations of N-fixers and species of *Eucalyptus* (non-N-fixers) with three to six replicated plots of each species at each site. At the Hawaii sites, the plantation species were *Albizia falcataria* (L.) Fosberg (also *Paraserianthes falcataria* (L.) Nielson) and *Eucalyptus saligna* (Sm.). At the Puerto Rico sites, the plantation species were *Casuarina equisetifolia* (L.), *Leucaena leucocephala* (Lam.) de Wit variety, and *Eucalyptus robusta* J.E. Smith. *Albizia* and *Leucaena* belong to the family Fabaceae and subfamily Mimosoideae, and *Casuarina* belongs to the family Casuarinaceae.

Albizia is nodulated by the *Rhizobia* and *Bradyrhizobia* bacteria and also hosts vesicular-arbuscular mycorrhizae (VAM; Turk and Keyser 1992). *Albizia* is native to the eastern islands of the Indonesian archipelago and is widely grown in tropical plantations for fuel, pulp, and light construction (Binkley and Giardina 1997). *Albizia* trees grow very rapidly to 25-30 m tall and up to 80 cm in diameter at rates of 6-18 Mg ha^{-1} yr^{-1} of stem increment with N-fixing rates of 65-140 kg N ha^{-1} yr^{-1} (Little and Skolmen 1989, Binkley and Giardina 1997).

Leucaena is nodulated by *Rhizobia* bacteria and hosts VAM (Binkley and Giardina 1997). *Leucaena* is native to Central America, and *Leucaena leucocephala* is planted extensively in tropical plantations for fuel, roundwood, pulp and paper, and soil improvement (Parrotta 1992a). *Leucaena* trees are rapidly growing small trees of up to 20 m in height and 50 cm in diameter with aboveground biomass production of 5-55 Mg ha^{-1} yr^{-1} (Parrotta 1992a) and N-fixation rates of 100-150 kg N ha^{-1} yr^{-1} (Little and Skolmen 1989, Binkley and Giardina 1997).

Casuarina is nodulated by *Frankia* actinomycetes and also hosts VAM and ectomycorrhizae (Binkley and Giardina 1997). *Casuarina* is native to Australia, Malaysia, and Polynesia (Subbarao and Rodríguez-Barrueco 1995) and is grown for land reclamation, sand dune stabilization, and wind breaks as well as timber throughout the tropics (Parrotta 1993, Binkley and Giardina 1997). *Casuarina* grows 30-40 m tall and 20-50 cm in diameter with wood increments on good sites of 10-15 Mg ha^{-1} yr^{-1} and N-fixation rates of 60-95 kg N ha^{-1} yr^{-1} in pure stands (Parrotta 1993, Parrotta *et al.* 1994, Subbarao and Rodríguez-Barrueco 1995, Binkley and Giardina 1997).

The two species of *Eucalyptus* are native to southeastern Australia. *E. robusta* grows to 48 m in height and 0.9-1.2 m in diameter. *E. saligna* reaches heights of 40-60+ m with 0.6-1.2 m diameters. *E. robusta* is well adapted to Puerto Rico and was planted extensively in Hawaii until the 1960s when it was replaced by *E. saligna* and *E. grandis*. *Eucalyptus* wood is used for housing, pulp, fuel, charcoal, and pallets (Little and Skolmen 1989).

The Hawaii study sites are located on the big island of Hawaii on the northeast coast (Table 1). These sites are described in greater detail as "Upper Kamae" and "Chinchuck" in Binkley (1997) and Garcia-Montiel and Binkley (1998). In the 1920s, both sites were converted from native forest (C_3 species) to sugarcane (*Saccharum officinarum*; C_4 species). Sugarcane production at the Kamae site was abandoned in 1960 due to low production. Sugarcane production at the Chinchuck site continued for more than 50 years until 1980. In 1981 for both sites, old vegetation was cleared, the site was plowed, and the new growth was treated with herbicide. In 1982, pure species trials of *Albizia* and *Eucalyptus* were planted in a randomized complete block design, with four blocks (8 plots total). The trees were planted at a spacing of 1.5 by 1.5 m in 12 by 18 m plots for both *Albizia* and *Eucalyptus* at the Kamae site and at a spacing of 2 by 2 m in 15 by 15 m plots for *Albizia* and 30 by 30 m plots for *Eucalyptus* at the Chinchuck site, with no buffers between plots for either site. Both sites now have a rich understory of *Guava*, tree fern, and other C_3 and C_4 vegetation with no apparent differences between treatments. Although these two Hawaii sites are in close proximity to each other (within 1 km) on similar soils, tree biomass and total soil N differed substantially between the two sites for the same tree species (Garcia-Montiel and Binkley1998). Based on these differences, we treated the sites as independent units for statistical purposes.

Two sites were used in Puerto Rico (Table 3.1). The first site is located in the Lajas Valley in southwest Puerto Rico (Lugo *et al.* 1990, Wang *et al.* 1991). Before planting the site was plowed and treated with herbicide. In February 1980, seedlings of six taxa were planted in pure species plots—we selected three of these for this study. Weeding and fertilization were used for the first six months, and irrigation was applied when necessary to ensure initial survival.

Pure plots of *Casuarina*, *Leucaena*, and *Eucalyptus* were planted in a complete block design with six blocks. The trees were planted at an original spacing of 1 x 1 m that was thinned to 2 by 2 m by age 1.5 years. Plot size was 10 by 10 m with a 3 m buffer between plots and a 5 m buffer between blocks. The site was fallow sugarcane at the time of plantation establishment. When we sampled the plantation in December 1996, the area consisted of a mixture of C_4 grass (*Panicum maximum*) and some herbaceous C_3 species. Tree species have never dominanted this site prior to these plantations (A. Lugo, personal communication).

The second site is located on the northern coast of Puerto Rico at the University of Puerto Rico's Toa Baja experimental farm (Parrotta *et al.* 1996, Parrotta 1999). The site has had many disturbances that include leveling of the original forested dunes, sand extraction, and cultivation. Pasture grasses, mostly *Panicum maximum*, were the dominant vegetation at plantation establishment. Site preparation consisted of disking to approximately 20 cm. The control plots, which still consisted of pasture grass, were also disked.

Pure and mixed plots of *Casuarina*, *Leucaena*, and *Eucalyptus* were established in a randomized complete block design with three blocks. For this current study, only the pure species plots were used. Trees were planted at a spacing of 1 by 1 m in 16 by 16 m plots. There was a 5 m buffer between plots and a 10 m buffer between blocks. Recent samples (December 1996) and archived soils (November 1989, July 1991, July 1992, and March 1993) were analyzed from this site.

Sample Collection and Processing

We collected soil samples in January 1997 from Kamae, in January 1998 from Chinchuck, and in December 1996 from the Puerto Rico sites. In each of the eight plots at the Kamae site, we collected one soil sample to a depth of 40 cm and 2 samples to a depth of 20 cm near the plot center in a triangular pattern 1 m apart. Three 40 cm cores were collected from neighboring sugarcane fields to be analyzed as a proxy for pre-forest plantation conditions. In each of the eight plots at the Chinchuck site, we collected two soil samples to a depth of 50 cm. Data for neighboring sugarcane soil carbon is from M. Bashkin (unpublished data). In each of the 18 plots at the Lajas site, we collected one 40 cm soil sample near each plot center. Control plots were located at the end of each of the six blocks and a 40 cm soil sample was collected at least 5 m from the plantation edge to use as a proxy for pre-plantation conditions for this site. The same sampling design used at Kamae was used for the Toa Baja plantation in Puerto Rico. However, at this site, control plots of pre-plantation vegetation were included in the experimental design and were sampled in the same way as the forest soils; one 40 cm and two 20 cm samples from each of the three control plots. We used a 6 cm diameter soil core lined with a butyrate plastic liner for each soil sample at the Hawaii and Lajas sites. For the Toa Baja site, we used a 5.1 cm diameter soil corer.

Upon collection, we immediately processed the Kamae, Chinchuck, and Toa Baja soils at a field lab. Lajas soils were stored in the butyrate soil core liners for less than five days for transport back to Colorado State University (CSU) before processing. In the lab, we divided all soils into 10 cm segments, with the exception of the Chinchuck soils, which we divided into 0-20 and 20-50 cm segments. Where we had more than one sample per plot, we composited all soil samples into one sample per plot. Bulk density was determined for each of the soil segments by oven drying a known fresh weight at 105°C to a constant dry weight; rock mass was negligible. For the both Hawaii sites, we used bulk densities from the Chinchuck site.

We treated the soils from Kamae and the two Puerto Rico sites with a 0.2N HCl acid wash to remove $CaCO_3$ that would affect $\delta^{13}C$ values (Ehleringer 1991). There was no evidence of $CaCO_3$ in the Chinchuck soils when 0.2N HCl solution was applied to 10 subsamples. We measured 5 g of oven-dried soil in a scintillation vial, added 20 ml of the weak acid solution, and placed the vials on a shaker overnight. We then tested the soil-acid solutions with litmus paper to determine if the solution had been neutralized by the carbonates. If the solution was neutralized, the liquid was evaporated, and the process was repeated. If the soil-acid solution was not neutralized, the acidification process was considered complete. To complete the process, we neutralized the soil-acid solutions to between pH 6.0-7.9 with 2M NaOH. We recorded the mass of HCl and NaOH added to each sample. We oven-dried the soils and ground them to flour texture for mass spectrometer and total C and N analysis.

Soil organic C and N concentrations were determined by dry combustion (LECO-1000, LECO Corporation, St. Joseph, Michigan, USA). We corrected the C and N concentrations to account for the added salt mass from the acidification process. The $\delta^{13}C$ values of the SOC were measured using a combustion elemental analyzer (Fisions

NA 1500, Carlo Erba Instrumentazione) coupled with an isotopic ratio mass spectrometer (VG ISOGAS, Middlewich, UK). The mass spectrometer is operated in automatic continuous flow mode using helium as the carrier gas after combustion. The $\delta^{13}C$ values, measured in ‰ (parts per thousand), are calculated as:

$$\delta^{13}C = [(R_{sample}/R_{PDB}) - 1] \times 10^3,$$

where $R = {}^{13}C/{}^{12}C$ and PDB = Pee Dee Belemnite standard for carbon.

We used archived soils from the Toa Baja site to investigate the time course of SOC_4 decomposition and of SOC_3 accumulation. We collected soil samples in November 1989, July 1991, July 1992, and March 1993. With the exception of the 1989 sampling, samples were collected from all pure tree species plots and from the established control plots in the neighboring pasture to a depth of 40 cm. In 1989, samples were collected only from the tree plantation plots to a depth of 20 cm. All archived soils were divided into 10-cm increments, air dried and stored in plastic containers. We assumed that no SOC_T changes occurred in the 20-40 cm depth between 1989 and 1991. We used the 1989 C and ^{13}C isotopic concentrations for the 0-20 cm and the 1991 concentrations for the 20-40 cm to estimate total soil profile concentrations to 40 cm in 1989. These 1989/1991 concentrations were then used as pre-plantation estimates for each tree species. The archived soil samples were analyzed following the above analysis procedures for fresh soil samples.

Soil incubations

Four- and eight-month soil incubations were conducted to determine whether SOC_3 derived from N-fixing trees decomposed more rapidly than SOC_3 derived from

Eucalyptus. Surface soils (0-10 cm) from each tree plot from Kamae, Lajas, and Toa Baja were coarsely sieved (6 mm) to aerate the soil. We incubated fresh soil samples in the quantity of the equivalence of 15 g of oven-dried soil. Each soil sample was brought to 0.01 MPa water potential using a pressure plate, the samples were weighed and checked twice weekly and brought back to the initial 0.01 MPa weight. Soils were incubated at 35°C inside loosely covered 1 L mason jars, which also contained a vessel of water to maintain a high humidity environment. The jars were opened to ambient atmosphere twice weekly to ensure adequate availability of oxygen for decomposition.

To determine relative loss of SOC_3, pre- and post-incubation SOC concentration and $\delta^{13}C$ values were measured for each sample, and Equations 1-4 (see below) were used to determine the $\%C_3$ and mass of the pre- and post-incubation SOC_3. The SOC_3 change over time was determined by subtracting the post-incubation values from the pre-incubation values and dividing by the pre-incubation values to get a relative C_3 loss comparison (mg C_3 loss/g initial C_3). We analyzed for the effect of species (i.e., N-fixer versus *Eucalyptus*) on the relative loss of SOC_3 using the split-plot statistical design described below in the *Statistical analysis* section.

Corrections for bulk density

Hawaii bulk densities did not differ between soils under tree species or neighboring cane fields. Bulk density did differ for soils under the pasture and forest at Lajas and over time at Toa Baja. This means that a 0-10 cm sample taken at one place or time represented a different mass of soil at another place or time. To correct for the bulk density differences, Lajas forest soil mass was adjusted to an equivalent pasture mass and

Toa Baja 1991 through 1996 soil masses were adjusted to 1989 equivalent masses to reflect equivalent soil mass comparisons. Concentrations of C and N and $\delta^{13}C$ values were all corrected for the depth adjustments. For simplicity, depths will be addressed as 0-10, 10-20, 20-30, and 30-40 cm. The bulk density adjustments for Lajas increased the average SOC_T (kg/m^2) under the trees by 4%.

Calculations

The percent of total SOC coming from grasses or sugarcane (C_4) was calculated using the equation:

$$\% \; C_4 = [(\delta^{13}C_{sample} - \delta^{13}C_3)/(\delta^{13}C_4 - \delta^{13}C_3)] \times 100 \text{ (Cerri } et\ al. \text{ 1985)}, \qquad (1)$$

where, $\delta^{13}C_3$ is the $\delta^{13}C$ of a composite sample of pure forest litterfall and roots, and $\delta^{13}C_4$ is the $\delta^{13}C$ of a composite of pure C_4 grass or sugarcane litter, leaves, and roots. The C_3 and C_4 endpoints used were different for each site and for each tree species within the Toa Baja and Lajas site (Table 3.2). $\delta^{13}C$ values for Hawaii are from Bashkin and Binkley (1998). Root $\delta^{13}C$ values are from roots collected throughout all 0-40 cm soil cores and composited for each species.

Total SOC in kg C/m^2 is calculated using the equation:

$$SOC_T = \rho_b \times [C] \times \text{sample depth} \qquad (2)$$

where, ρ_b is the average bulk density for that segment of soil (kg/L), [C] is the concentration of soil C (g C/kg dry soil), and sample depth is the segment of the soil profile (m). The equation for SOC_4 (kg C/m^2) is:

$$SOC_4 = (\%C_4/100) * SOC_T \qquad (3)$$

And, SOC_3 (kg C/m^2) is calculated by difference:

$$SOC_3 = SOC_T - SOC_4 \quad (4)$$

Total (kg m^{-2} plantation age^{-1}) and annual (kg m^{-2} yr^{-1}) changes in SOC_T, SOC_4, and SOC_3 since plantation establishment were estimated by subtracting the initial condition from the recent sample values and dividing by the plantation age in years (for annual changes). For example:

$$SOC_3 \text{ change} = \text{current } SOC_3 - \text{initial } SOC_3/\text{plantation age} \quad (5)$$

Neighboring pasture or sugarcane fields or pre-plantation archived soil samples (*i.e.*, at Toa Baja) were used to approximate the initial conditions.

Separating pre-sugarcane/pasture SOC_3 from contemporary SOC_3

The SOC_3 pools for each site included both pre-sugarcane/pasture and contemporary C_3 components. We assumed that changes in pre-sugarcane/pasture SOC_3 were minimal since the establishment of the present forest plantations. Thus, any changes in SOC_3 (equation 5 above) since forest plantation establishment was attributed to additions of contemporary SOC_3. We used the same C_3 endpoints for original C_3 vegetation and contemporary forests (equation 1 above), assuming little difference between $\delta^{13}C$ values. For the Hawaii sites, Bashkin and Binkley (1998) tested the validity of this assumption and found the soil $\delta^{13}C$ values of wildland forest and *Eucalyptus* with no prior sugarcane cultivation to be statistically similar. We had no avenue for testing this assumption for the Puerto Rico sites. A sensitivity analysis using -29‰ (instead of –26.94 ‰) for the *Leucaena* endpoint and 15 ‰ (instead of –13.80‰) for the pasture endpoint at the Lajas site increased the estimated %C_4 by 19%. Changing only the C_4 endpoint to -15 ‰ increased the %C_4 by 11%.

Statistical analysis

All analysis were conducted using SYSTAT version 7.0 (SYSTAT 1997). All $P < 0.10$ are reported; $P > 0.10$ were not considered to be statistically significant. For each analysis, residual plots of the raw data were visually inspected for non-normality and heteroscedasticity violations of analysis of variance (ANOVA) assumptions; where necessary the data were $\log_{10}$ transformed (i.e., SOC_4 change across sites).

Species effects on SOC pools through time. For the Toa Baja multiple year data, we tested for linear and quadratic trends of the SOC pools through time and differences between species using multiple regression techniques. The linear model included species and block as class variables along with year and a year-by-species interaction as covariates. The quadratic model was the same as the linear model but with additional covariates, year^2 and year^2-by-species interaction. The year and year^2 covariates determined the shape of the time trend, and the year-by-species and year^2-by-species covariate interactions tested for species differences across this time trend.

Species effects on SOC pools and changes within site. We used an ANOVA for a general two-factor completely randomized block design with 2-3 levels of species and 3-6 levels of block (the number of species and block depends on site). To test for the tree species effects on the SOC pools and soil N within a given site, we used species as the main effect with species by block interaction as the error term. For the Puerto Rico sites with more than two tree species, we used Tukey's multiple comparison for determination of significant differences.

Species effects on changes in SOC pools across the four sites. To test for N-fixer versus non-N-fixer differences across sites, annual SOC changes were used (e.g., equation 5 above). For the Lajas and Toa Baja sites where two N-fixer species were sampled, the species were averaged together into one N-fixer category. For each site, the research plantations used a completely randomized block design. However, combining the data into a single analysis is no longer a completely randomized block design due to the species-within-blocks restriction. An ANOVA (see Appendix for ANOVA table) was used with site as the main effect, blocks as plot effects, and species-within-blocks as the split-plot effects. With this model, site is treated as a fixed effect, and species and block are random effects. The error terms were plot-within-site sum of squares for the main effect and species by plot-within-site sums of squares for the species effect and the site by species interaction effect (Binkley 1997).

RESULTS AND DISCUSSION

SOC pools through time at the Toa Baja site

From plantation establishment in 1989 to the most recent 1996 sampling at the Toa Baja site, the $\delta^{13}C$ values of the surface soils under the tree species have shifted gradually toward the tree litter $\delta^{13}C$ values away from the sugarcane/pasture influence (Figure 3.1). *Eucalyptus* soil $\delta^{13}C$ values showed the earliest shift, with marked differences compared with the 1989 soil $\delta^{13}C$ values developing by 1992. This early shift occurred throughout the soil profile. The two N-fixers showed the greatest shifts in mean soil $\delta^{13}C$ values since the 1989 soil $\delta^{13}C$ values; however their effects became most apparent only by the 1996 sampling date and only in the top 10 cm of soil.

The N-fixers did not differ from the *Eucalyptus* in their effects on total SOC_T, SOC_4, and SOC_3 pools through time. However, soil carbon pools for the tree species as a whole showed significant time trends (Figure 3.2). The SOC_T trend through time was best described by a quadratic model ($P < 0.02$). The SOC_4 and SOC_3 trends were linear ($P < 0.01$ for both), with losses of old SOC_4 dominating the decrease in SOC_T for the first four years followed by gains of new SOC_3 dominating the increase in SOC_T for last two years. Parrotta (1992b) reported a similar pattern of soil carbon change consisting of an initial loss followed by an increase after conversion of pasture to *Albizia* in a neighboring site.

Despite the lack of difference between N-fixer and *Eucalyptus* for any of the SOC pools through time, a clear trend of greater SOC_T under the N-fixers compared with *Eucalyptus* was established by age 7 yr (discussed in detail below). Given our variance and the size of the effect, we would have needed 14 to 18 replicates to detect any SOC pool differences between the tree species. Perhaps this site is too young for any detectable species differences to manifest themselves in the soil carbon pools. Neill *et al.* (1996) examined a chronosequence of forest-to-pasture conversion in Brazil on soils of similarly low carbon and the same grass species as this Toa Baja site. They found that after nine years the pasture total and forest-derived carbon levels, taking into account standard deviations, (3.98 kg m^{-2} ± 0.85 and 2.71 kg m^{-2} ± 0.46, respectively) were still similar to the original forest levels (3.23 kg m^{-2} ± 0.72). However, after 81 years, the pasture total and forest-derived carbon levels had changed markedly (5.00 kg m^{-2} ± 0.88 and 1.85 kg C_3 m^{-2} ± 0.24, respectively).

It appears that soil variability may not permit early detection of soil carbon changes resulting from species effects. However, over this seven-year time series, SOC storage did show a significant quadratic trend encompassing an initial decrease followed by a gradual increase for the forest plantation as a whole.

SOC pools across four sites

The rest of this paper examines the N-fixer effects on SOC pools across several sites and after longer periods of plantation growth (i.e., up to 16 years). The N-fixers had greater SOC_T pools relative to the *Eucalyptus* (Figure 3.3). At Kamae and Chinchuck, *Albizia* had 0.7 and 2.1 kg/m^2, respectively, more SOC_T than *Eucalyptus*. For the Puerto Rico sites that had two N-fixers, *Leucaena* consistently had the highest SOC_T. At Lajas, *Leucaena* had 2.6 kg/m^2 more SOC_T than did *Eucalyptus* ($P = 0.07$). For Toa Baja, *Leucaena* had 0.5 kg/m^2 more SOC_T than *Eucalyptus.*

Greater SOC_T under the N-fixers resulted from a combination of both larger SOC_4 and SOC_3 pools (Figure 3.3). The differences in SOC_4 pools under the N-fixers compared with the Eucalyptus ranged from 0.07 kg/m^2 higher under the *Albizia* in Hawaii to 1.07 kg/m^2 higher under *Leucaena* at Lajas. The N-fixers, with one exception, also had greater SOC_3 pools than *Eucalyptus*, ranging from 0.04 kg/m^2 higher under *Leucaena* at Toa Baja to 1.5 kg/m^2 higher under *Leucaena* at Lajas and 1.5 kg/m^2 higher under *Albizia* at Chinchuck (Figure 3.3).

Generally, the N-fixers lost less SOC_4 and gained more SOC_3 compared to *Eucalyptus* since plantation establishment (Figure 3.4). There where no statistical differences within sites for these pool changes. However, across the four sites (see Appendix for ANOVA table), changes in SOC_T pools were significantly greater under the

N-fixers (0.11 ± 0.03 kg m^{-2} yr^{-1}; mean ± one standard error) relative to under the *Eucalyptus* (0.01 ± 0.03 kg m^{-2} yr^{-1}; $P < 0.01$). This SOC_T difference between soils under the N-fixers compared with *Eucalyptus* combines a relatively small loss of old SOC_4 (0.05 ± 0.02 kg C_4 m^{-2} yr^{-1}) with a large gain of new SOC_3 (0.16 ± 0.02 kg C_3 m^{-2} yr^{-1}). In comparison, the *Eucalyptus* lost significantly more old SOC_4 (0.11 ± 0.02 kg C_4 m^{-2} yr^{-1}; $P < 0.03$) and accumulated significantly less new SOC_3 (0.12 ± 0.03 kg C_3 m^{-2} yr^{-1}; $P < 0.04$). There were no interactions between site and species.

The sandy soil of the Toa Baja site had the smallest total soil carbon pools (Figure3.3) and the largest annual losses of old carbon (Figure 3.4) compared with the other three sites with smaller particle size (i.e., Andisols in Hawaii and Vertisol clays at Lajas). That the sandy soil had the lowest carbon content and retained the least amount of carbon is consistent with other studies showing that soil organic carbon increases with increased clay content and carbon losses are lowest in clay soils (Tiessen *et al.* 1982, Burke *et al.* 1989). Because we did not replicate the study sites within soil orders, we can only comment on the pattern.

Why greater SOC_4 retention under N-fixers?

Greater old carbon retention under N-fixers compared with non-N-fixers has been identified in at least two other sites. Rhoades *et al.* (1998) found 0.1 kg/m^2 greater old pasture carbon to 15 cm under individual stems and patches of a N-fixer compared with that under a non-N-fixer growing within pastures on the west slope of the Equadorian Andes. Kaye *et al.* (1999) found a positive correlation ($r^2 = 0.61$; $P = 0.07$) between old soil carbon pools and increasing proportions of *Albizia* mixed with *Eucalyptus* in a replicated replacement series design in Hawaii.

SOC_4 retention and nitrogen accretion under the N-fixers relative to the *Eucalyptus* were correlated positively ($r^2 = 0.55$; $P < 0.01$; Figure 3.5 top panel) indicating that increased soil N accretion under the N-fixers may be responsible for greater retention of SOC_4 under the N-fixers compared with *Eucalyptus* (Resh *et al.* 1999). Increased soil N supply may inhibit decomposition of old soil carbon through changes in soil biota communities (Fog 1988, Zou 1993, Berg and Matzner 1997), decreased microbial activity and biomass (Arnebrant *et al.* 1996) or through the provision of bonding sites for the formation of complex, more recalcitrant SOC (Stevenson 1994).

Across the four sites, about half of the SOC_T difference between the N-fixer soils and *Eucalyptus* soils came from greater SOC_4 retention under the N-fixers. However, this proportion differed by site, ranging from 10% at Kamae to 90% at Toa Baja with the remainder of the proportion of the SOC_T difference between the N-fixers and *Eucalyptus* consisting of greater SOC_3 accretion under the N-fixers. Concomitant with this juxtaposition in dominance from SOC_4 retention to SOC_3 accretion is a site difference in average soil N content, ranging from 0.87 kg N/m^2 at Kamae to 0.38 kg N/m^2 at Toa Baja. Thus, there is a general trend across these four sites that as soil N content increases, the accretion of new carbon becomes the more dominant mechanism for the greater total soil carbon difference between N-fixers and *Eucalyptus*.

Why greater SOC_3 accretion under N-fixers?

Across the four sites, greater new N-fixer carbon accretion accounts for the other half the SOC_T difference between N-fixer soils and *Eucalyptus* soils. In the same study addressed in the above section, Rhoades *et al.* (1998) found that over 90% of the SOC_T difference between the N-fixer and non-N-fixer came from new N-fixer carbon accretion. As with SOC_4 retention, SOC_3 accretion was correlated positively with soil N accretion ($r^2 = 0.43$; $P < 0.01$; Figure 3.5 middle panel).

Greater SOC_3 accretion under the N-fixers may result from greater N-fixer carbon inputs to the soil or from slower decomposition of N-fixer SOC_3 litter or some combination of both. We used laboratory soil incubations to try to determine the SOC_3 accretion mechanism by examining the differential loss of SOC_3 between species with no new inputs of SOC_3. Equal or greater loss of N-fixer SOC_3 compared with *Eucalyptus* SOC_3 would reflect the greater N-fixer input scenario. Conversely, greater retention of the N-fixer SOC_3 would indicate the slower decomposition scenario.

The N-fixer soil showed greater SOC_3 loss compared with *Eucalyptus* soil, indicating that greater SOC_3 accretion under the N-fixers relative to the *Eucalyptus* is most likely the result, at least in part, of greater N-fixer inputs (Figure 3.6). However, the difference in SOC_3 loss between the N-fixers and *Eucalyptus* was only significant for the first four months of incubation (88 mg SOC_3 loss/g initial SOC_3; $P < 0.05$). Between four and eight months, the loss of SOC_3 slowed for both the N-fixer and *Eucalyptus* soil and became more variable resulting in an overall greater loss of SOC_3 from the N-fixer soil, though no longer statistically significant (98 mg C_3 loss/g C_3 initial). Although the N-fixers may stabilize a portion of their own litter through the same mechanism by which they appear to stabilize the old SOC_4, our incubations suggest that increased litter inputs are a necessary mechanism for the greater accretion of new SOC_3 under the N-fixers compared with the non-N-fixers.

There is evidence in the literature from temperate N-fixer studies for both slower long-term decomposition of N-fixer litter (Cole *et al.* 1995, Camiré *et al.* 1991) and greater N-fixer inputs (Cole *et al.* 1995). Binkley and Ryan (1998) found higher belowground production under *Albizia* (1.54 kg m^{-2} yr^{-1}) than under *Eucalyptus* (1.18 kg m^{-2} yr^{-1}) in a three site comparison in Hawaii of which this site is one. Aboveground production was lower under *Albizia* than under *Eucalyptus* (2.35 and 2.96 kg m^{-2} yr^{-1}).

Greater SOC_T sequestration under N-fixers—the bigger picture

The greater SOC_T difference under N-fixers compared with *Eucalyptus* resulted from the greater retention of old SOC_4 and the greater accretion of new SOC_3 by the N-fixers. The accretion of soil N explained 78% of the variability in the SOC_T difference across plots ($P < 0.01$; Figure 3.5 bottom panel). This study includes sites from a broad geographical area in the tropics with *Eucalyptus* as a proxy for non-N-fixers. The resulting SOC_T difference between the N-fixers and *Eucalyptus* is in agreement with temperate forest N-fixer/non-N-fixer comparisons (Johnson 1992, Cole *et al.* 1995) and other tropical sites (Rhoades *et al.* 1998, Kaye *et al.* 1999). It seems likely that these results apply to all N-fixer forests and perhaps to crops of symbiotic N-fixers (Drinkwater *et al.*, 1998).

This extensive evidence for N-fixer soil carbon sequestration may have strong implications for global carbon sequestration that have not been previously considered. For example, symbiotic N-fixation accounts for 96 x 10^{12} g N/yr (Schlesinger 1997). If only 50% of this enters the soil, then based on our equation in Figure 3.5 (bottom panel), 0.44 x 10^{15} g C/yr could accumulate in the soil due to symbiotic N-fixation. Even if we assume that only the greater old carbon retention portion applies (i.e., about 0.22 x 10^{15} g C/yr), this is 12 to 15% of the annual missing terrestrial carbon sink i.e., the discrepancy in the global carbon budget between estimated atmospheric carbon increase and net carbon flux from terrestrial pools due to fossil fuel emission and vegetation loss partially offset by ocean uptake (1.5 to 1.9 x 10^{15} g C/yr; Schimel *et al.* 1996).

While reforestation and afforestation are being considered as mechanisms to reduce carbon emission penalties (UNFCCC 1997), it is important to recognize that, from a soil carbon sequestration standpoint, tree species differ in their effects. N-fixers sequester 0.05 to 0.12 kg m^{-2} yr^{-1} more carbon in their soils compared with non-N-fixers. Pure and mixed species forests that include N-fixers appear to be a consistent option for long-term soil carbon sequestration (Johnson 1992, Cole *et al.* 1995, Kaye *et al.* 1999). These findings may be relevant to countries interested in planting forests for carbon sequestration purposes.

CONCLUSIONS

Soil carbon pool comparisons between N-fixer and *Eucalyptus* plantations show several significant findings with implications for forest soil carbon sequestration:

1. N-fixing forests are an important exception to the ambiguous findings that afforestation does not lead to SOC accretion. In this study, N-fixer plantations sequestered an additional 0.11 kg m^{-2} yr^{-1} SOC_T over that of *Eucalyptus* plantations—58% of this SOC_T difference resulted from greater retention of old SOC_4 and 42% is from greater accumulation of new SOC_3.
2. The SOC_4 retention and SOC_3 accretion differences between the N-fixer and

Eucalyptus plantations are correlated positively with N-fixer soil N accretion.

3. Greater SOC_3 accretion under N-fixers relative to *Eucalyptus* resulted, at least in part, from greater litter inputs under N-fixers as revealed by long-term soil incubations.
4. Because of the time it takes for soil organic carbon pools to significantly reflect tree species effects, small areal planting of N-fixers should not be considered for significant short-term carbon sequestration. However, at longer time scales and across greater spatial scales, the N-fixer forests significantly increase the storage of soil carbon over that of non-N-fixers.

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Table 3.1. Description of the four sites. See text for primary references for each site.

Characteristic	Kamae, HI 20°N, 155°W	Chinchuck, HI 20°N, 155°W	Lajas, PR 18°N, 76°W	Toa Baja, PR 18°N, 66°W
Plantation establishment	September 1982	September 1982	February 1980	September 1989
Age at sampling	15 years	16 years	16 years	7 years
Landuse at time of planting	fallow sugarcane	fallow sugarcane	fallow sugarcane	pasture
Species				
•N-fixer(s)	*Albizia*	*Albizia*	*Casuarina* *Leucaena*	*Casuarina* *Leucaena*
•non-N-fixer	*Eucalyptus saligna*	*Eucalyptus saligna*	*Eucalyptus robusta*	*Eucalyptus robusta*
Climate				
•precipitation	4000 mm yr^{-1}	4000 mm yr^{-1}	1100 mm yr^{-1}	1600 mm yr^{-1}
•temperature	21°C	21°C	25°C	27°C
Soils				
•classification	Typic Hydrudands	Typic Hydrudands	Typic Haplusterts	Typic Troposamments
•soil N (kg m^{-2}) to 40 cm (to 50 cm for the Chinchuck, HI site)	*Albizia*: 0.92 *Eucalyptus*: 0.82 Sugarcane: 0.69	*Albizia*: 0.92 *Eucalyptus*: 0.69 Sugarcane: n/a	*Casuarina*: 0.63 *Leucaena*: 0.78 *Eucalyptus*: 0.55 Pasture: 0.54	*Casuarina*: 0.40 *Leucaena*: 0.42 *Eucalyptus*: 0.33 Pasture: 0.45
•surface soil pH ($CaCl_2$)	4.6[a]	5.0[a]	5.6	7.0
Fertilization regimes	11 and 5 g/m^2 N and P at planting	4, 1.8, and 3.3 g/m^2 N, P, and K, respectively, at planting, 4 and 8 months (and at 12, 18, 24, and 36 months for *Eucalyptus* seedlings)	11.2 g/m^2 N before planting and 6-month intervals for initial 2 years	1.0 g/m^2 N at 6-month intervals from March 1990 to 1992

[a] Garcia-Montiel and Binkley 1998.

Table 3.2. Site and species C_3 and C_4 $\delta^{13}C$ endpoints used for equation (1). Endpoints are composites of litterfall and roots from each species at each site (soil for Hawaii plantation species).

Site	Species	C_3 endpoint[a]	C_4 endpoint
Hawaii sites[b]	*Eucalyptus*, *Albizia*, cane	-25.11	-11.52
Lajas (n = 6)	*Eucalyptus*	-27.01±0.53	
	Casuarina[c]	-26.30±0.17	
	Leucaena	-26.94±0.50	
	Pasture	-26.60±1.13	-13.80±0.38
Toa Baja (n = 3)	*Eucalyptus*	-28.33±0.10	
	Casuarina[c]	-29.41±0.59	
	Leucaena	-28.64±0.27	
	Pasture	-28.78[d]	-14.52±0.31

[a] ± 1 standard deviation where applicable.

[b] Bashkin and Binkley 1998.

[c] *Casuarina* differed significantly from both *Eucalyptus* and *Leucaena* ($P < 0.1$ for both sites).

[d] average of the 3 tree species endpoints.

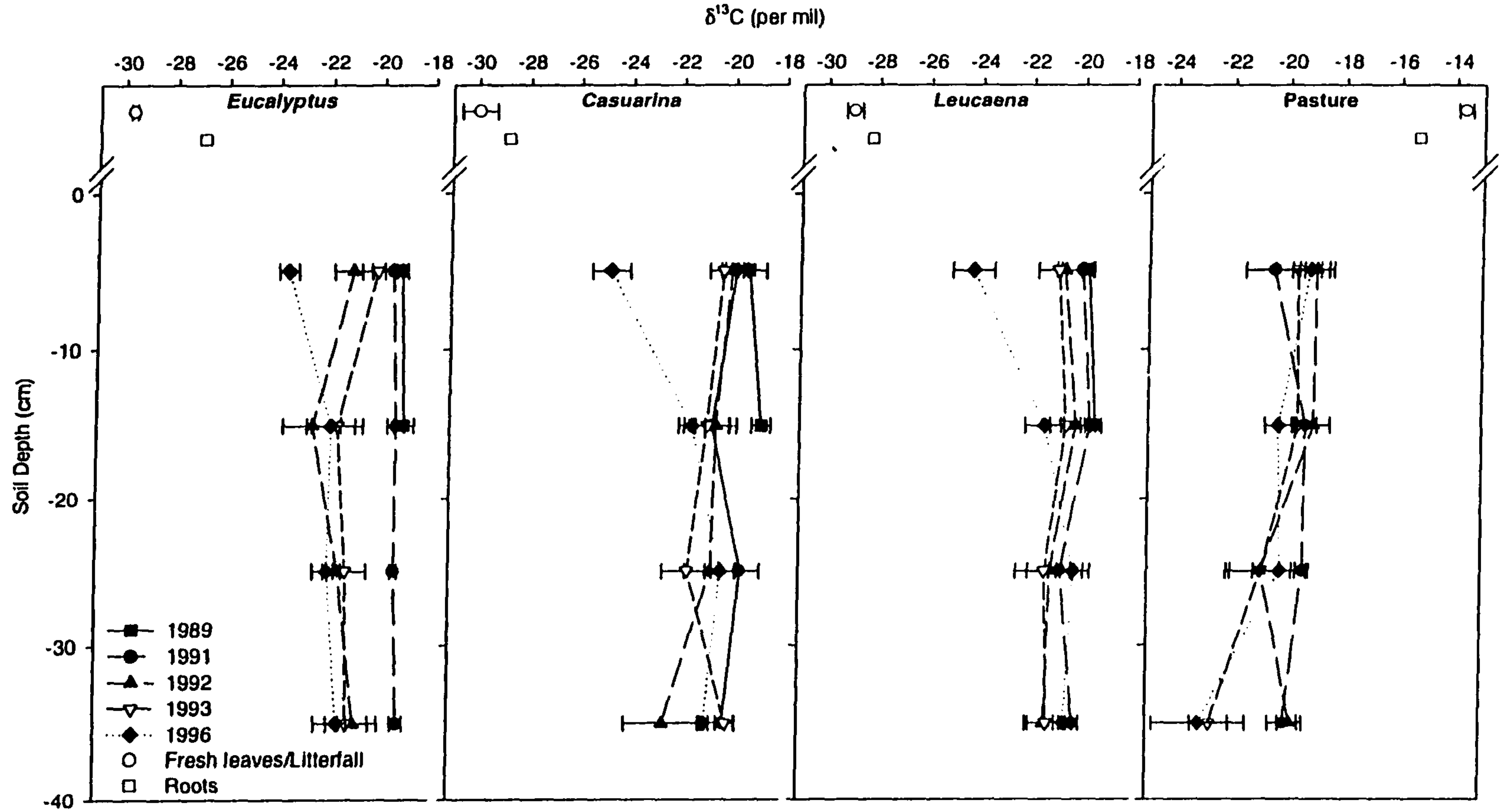

Figure 3.1. Soil $\delta^{13}C$ changes with time through soil profile for each species at the Toa Baja site. The error bars are mean standard errors (n=3).

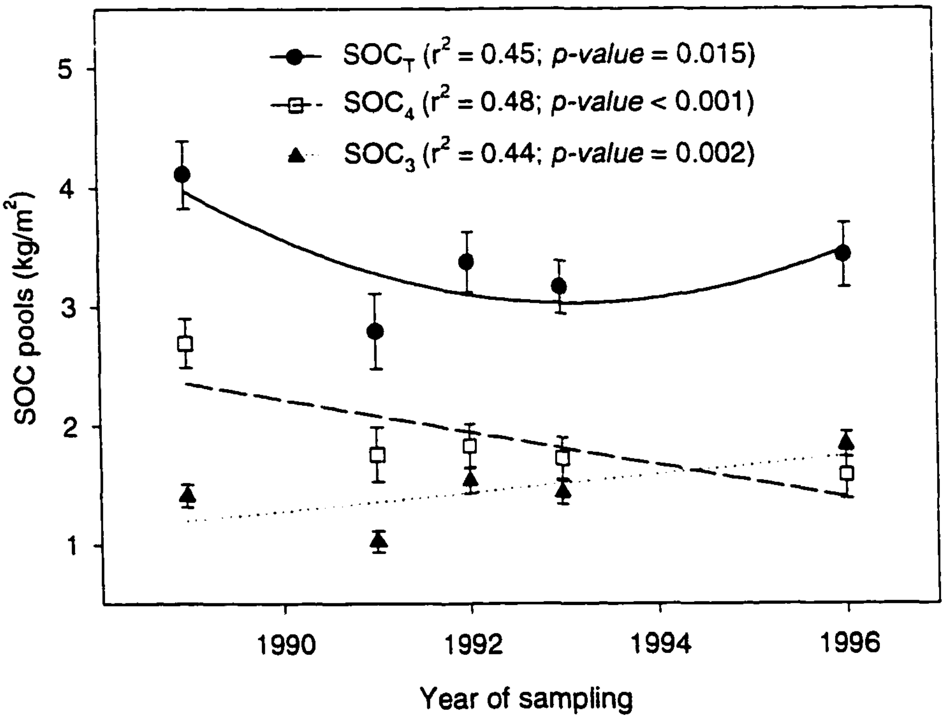

Figure 3.2. Toa Baja tree SOC$_T$, SOC$_4$, and SOC$_3$ trends through time. The error bars are mean standard errors for the each tree species (n = 3).

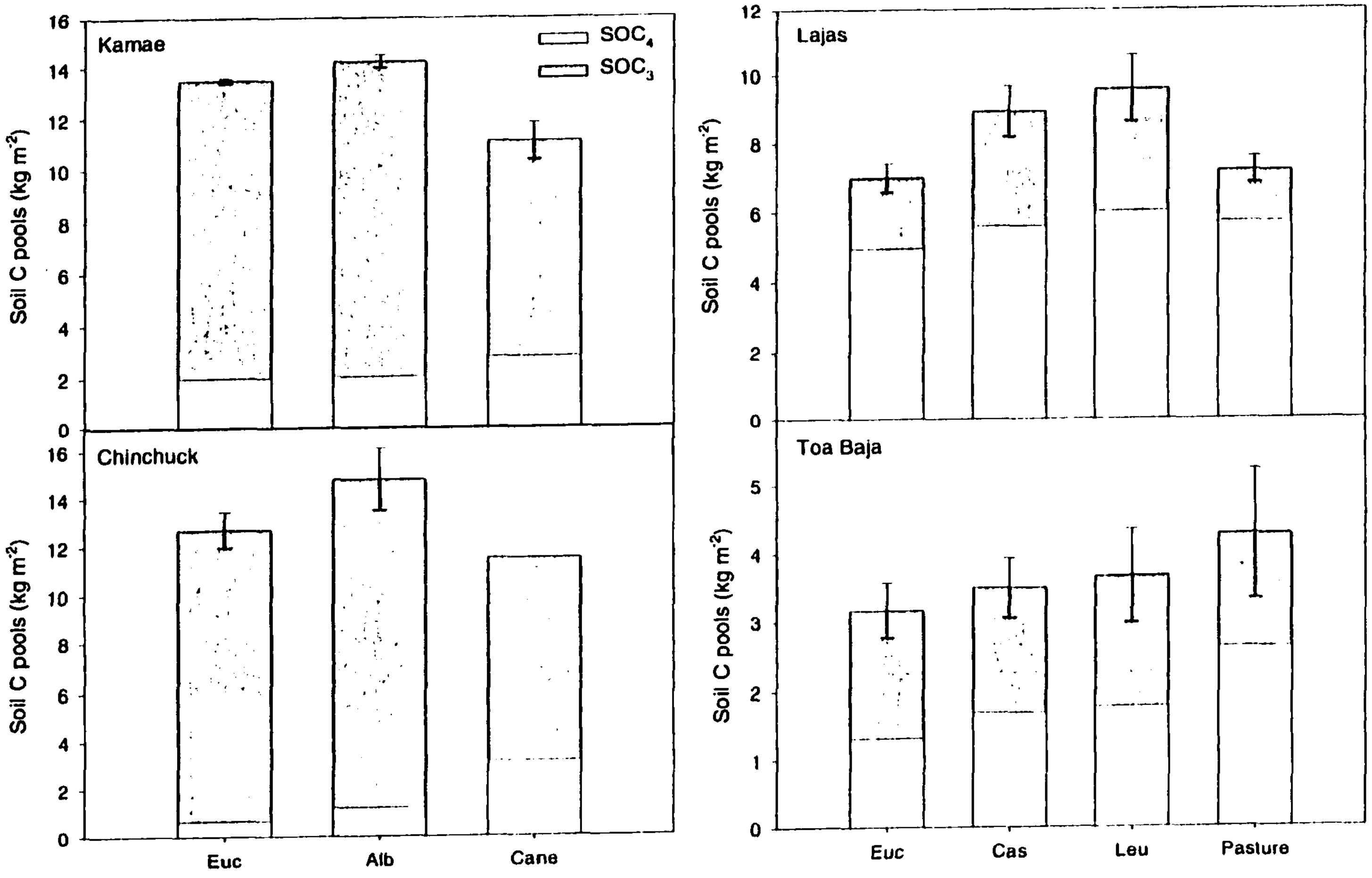

Figure 3.3. SOC pools (to 40 cm/50 cm for Chinchuck) for each species at (top left panel) the Kamae site (n=3 for *Eucalyptus* and cane, n=4 for *Albizia*), (bottom left panel) the Chinchuck site (n=4), (top right panel) the Lajas site (n=6), and (bottom right panel) the Toa Baja site (n=3). The error bars are mean standard errors. The cane data for the Chinchuck site are from M.A. Bashkin (unpublished data). Note the change in y-axis scale across sites.

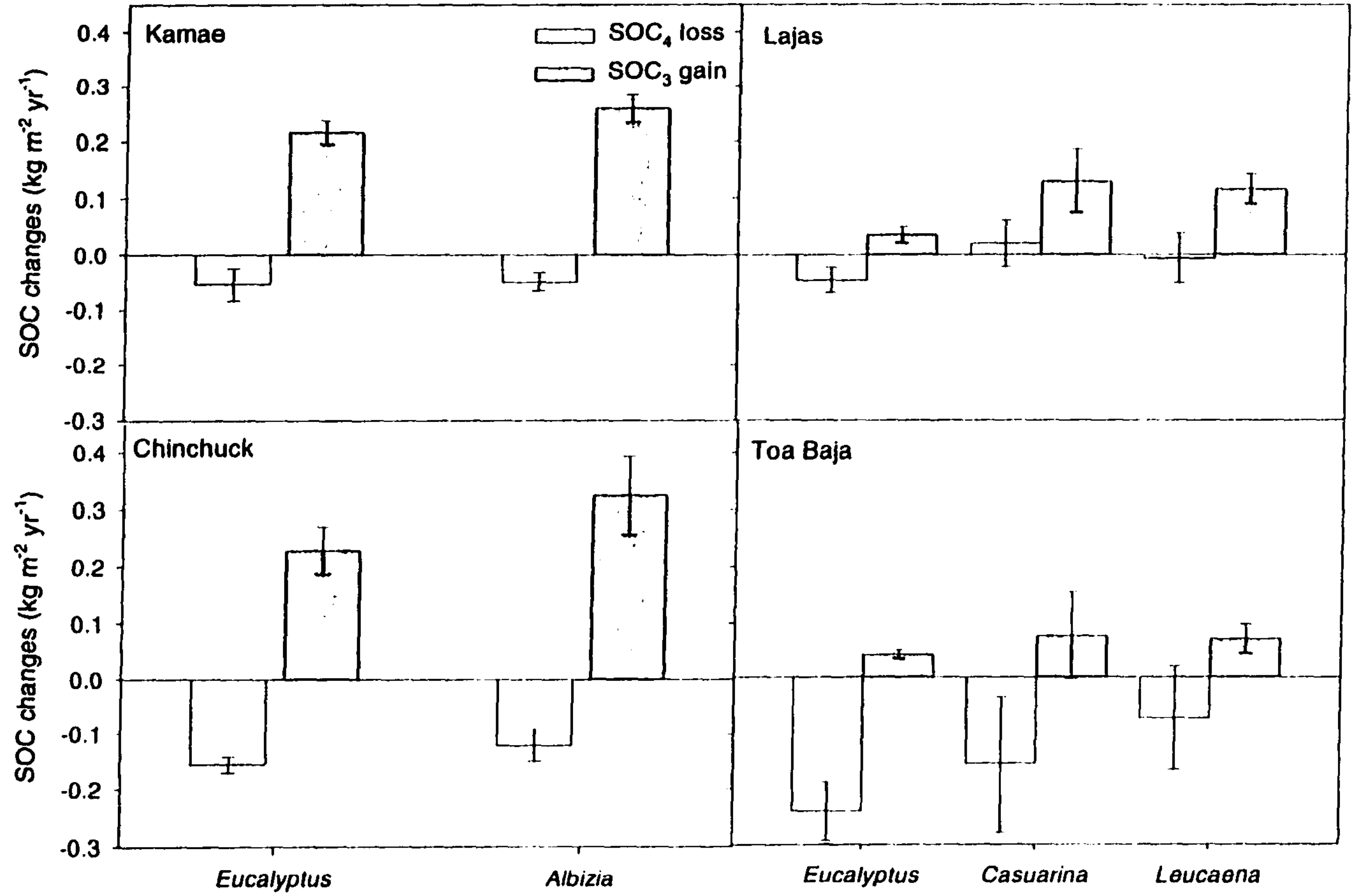

Figure 3.4. Annual changes in SOC$_4$ and SOC$_3$ pools (to 40 cm/50 cm for Chinchuck) since plantation establishment at (top left panel) the Kamae site (n=3 for *Eucalyptus* and cane, n=4 for *Albizia*), (bottom left panel) the Chinchuck site (n=4), (top right panel) the Lajas site (n=6), and (bottom right panel) the Toa Baja site (n=3). The error bars are mean standard errors.

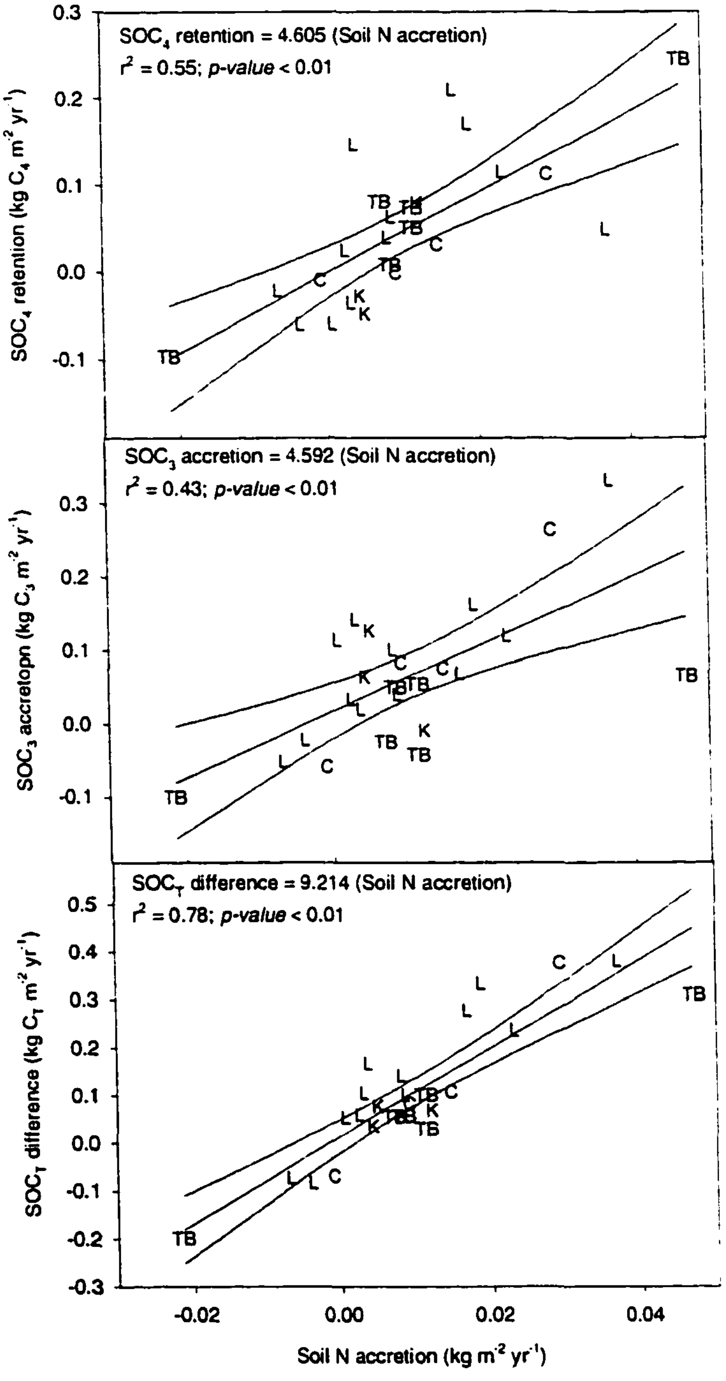

Figure 3.5. Soil nitrogen accretion versus (top panel) SOC$_4$ retention, (middle panel) SOC$_3$ accretion, and (bottom panel) SOC$_T$ difference under the N-fixers (calculated as the difference between N-fixer and *Eucalyptus* soil N and SOC$_4$, SOC$_3$ or SOC$_T$). Each datum is a N-fixer species within a block within a site (n=25). Plotting symbols are K = Kamae site, C = Chinchuck site, L = Lajas site, and TB = Toa Baja site. Linear regressions with 95% confidence intervals are shown.

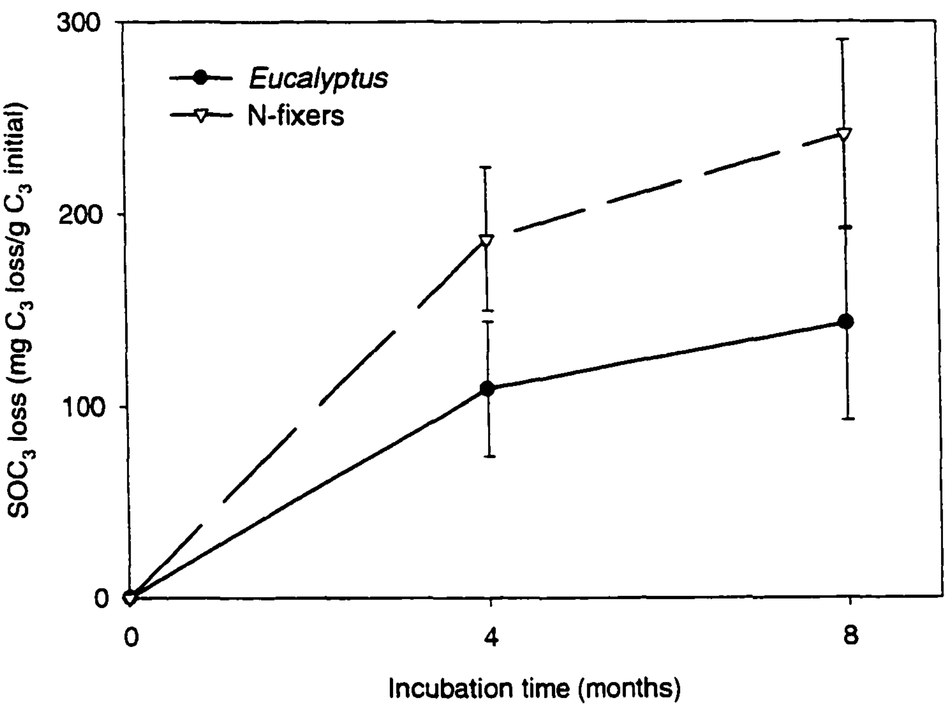

Figure 3.6. Cumulative loss of SOC$_3$ (mg SOC$_3$ loss/g initial SOC$_3$) from N-fixer and *Eucalyptus* soil during four-month (n = 13) and eight-month (n = 12) laboratory incubations. Four-month SOC$_3$ loss differed between N-fixers and *Eucalyptus* ($P < 0.05$). The error bars are mean standard errors.

CHAPTER IV

SOIL CARBON ACCRETION IN A REPLACEMENT SERIES OF *EUCALYPTUS* AND *ALBIZIA* TREES

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ABSTRACT

Soil carbon storage under pure stands of nitrogen-fixing trees (N-fixers) is greater than under pure stands of non-N-fixers, in part from greater retention of soil carbon from previous land use. We measured the soil carbon stocks under a replacement series of *Eucalyptus* (non-N-fixer) and *Albizia* (N-fixer) to determine the interaction effects of mixtures of N-fixers with non-N-fixers on soil carbon dynamics. The forest plantation (C_3 photosynthetic pathway) replaced sugarcane agriculture (C_4 photosynthetic pathway), allowing the use of stable carbon isotopes to track the loss of old C_4 carbon (SOC_4) and the gain of new C_3 (SOC_3) carbon under the mixtures and pure plot of the tree species.

Soil N, SOC_T, SOC_4 and SOC_3 all increased linearly with increasing proportions of *Albizia*. The accretion of SOC_T and SOC_3 and the retention of SOC_4 (in relation to the pure plots of *Eucalyptus*) were all positively, linearly correlated with soil N accretion.

Pure N-fixer plots accrued 0.12 kg m^{-2} yr^{-1} more SOC_T compared to pure *Eucalyptus* with 30% of that resulting from greater SOC_4 retention and 70% resulting from greater SOC_3 accretion. These linear effects indicate neutral interactions rather than threshold or synergistic relationships in species composition for soil carbon dynamics.

INTRODUCTION

Ecosystems with nitrogen-fixing species (N-fixers) increase soil organic carbon storage by 20-100% (0.05-0.13 kg m^{-2} yr^{-1}) over those without N-fixers (Johnson 1992, Tarrant and Miller 1963, Binkley *et al.* 1982, Binkley 1983, Binkley and Sollins 1990, Cole *et al.* 1995, Rhoades *et al.* 1998, Resh *et al.* 1999a). This increased total soil carbon results from greater accretion of N-fixer carbon (Cole *et al.* 1995, Rhoades *et al.* 1998, Resh *et al.* 1999a) as well as from increased retention of carbon from land use prior to N-fixer establishment (i.e., "older" carbon; Rhoades *et al.* 1998, Resh *et al.* 1999b). Though mixed species forests of N-fixers and non-N-fixers have also shown increased soil carbon over pure non-N-fixer forests (Binkley *et al.* 1982 and Binkley and Sollins 1990), no research has been conducted to evaluate the interactive effects of N-fixer/non-N-fixer mixtures on total soil carbon accretion and the components of older carbon retention and newer carbon accretion.

The objective of this study was to determine the response curve of soil N accretion, carbon retention, and carbon accretion across a gradient of N-fixer proportions. Our goal was to measure soil carbon pools under a replicated replacement series with four mixtures and pure species stands of *Albizia* (N-fixer) and *Eucalyptus* (non-N-fixer). The tree plantations (C_3 photosynthetic pathway) were planted on abandoned sugarcane fields (C_4 photosynthetic pathway), allowing us to use stable carbon isotopes to compare the changes in the older soil organic carbon pool (SOC_4) and the newer soil organic carbon pool (SOC_3) between tree species mixtures. We hypothesized that the greater total soil carbon difference and greater SOC_4 retention and SOC_3 accretion under the *Albizia*/*Eucalyptus* mixtures compared with pure *Eucalyptus* would correspond linearly to greater soil N accretion resulting from an increasing proportion of *Albizia* intermixed with *Eucalyptus*.

METHODS

The plantation was located on the northeast coast of the island of Hawaii (19°30'N, 155°15'W) on gentle slopes (< 15%) at an elevation of 480m. Rainfall (>4 m/yr) is evenly distributed throughout the year and mean annual temperature is ~21°C. The soils are in the Kaiwiki series of thixotropic isomesic Typic Hydrudands. After >50 years of sugarcane cultivation, the last cane crop was harvested in 1980. In 1981, the site was plowed and herbicides were used to limit regeneration. In January 1982, *Eucalyptus saligna* (Sm.) and *Albizia falcataria* (L.) Fosberg seedlings were planted at a constant total density (2500 trees/ha) with variable proportions of *Albizia* (0, 25, 34, 50, 66, and 100 %). The stands were planted in a completely randomized block design with 4 blocks (DeBell *et al.* 1989). As of January 1998, the 66% *Albizia* treatment had the highest tree biomass with about 21.5 kg/m^2 (18.0 of that was *Eucalyptus* biomass and 3.5 was *Albizia*) with pure *Eucalyptus* and *Albizia* biomass at about 11.0 kg/m^2 (Kaye *et al.* 1999).

In January 1998, we cored (0.06 m in diameter) soil from the center of every plot to a depth of 0.50 m. The soil was stored in pvc soil core liners in coolers for < 5 days, then weighed, homogenized by hand, and subsampled for gravimetric water content (105°C for 48 hr), total N and C, and $\delta^{13}C$ analyses. Bulk density was determined from the radius and length of the core, the fresh weight of the core, and the gravimetric water content of the subsample. Rock volume was negligible. Soil C and N concentrations were determined by dry combustion (LECO-1000, LECO Corporation, St. Joseph, Michigan, USA) and converted to an areal basis using the bulk density and depth of the core. The carbon isotope ratio was determined on a VG isochrom-NA stable isotope ratio mass spectrometer (VG, Middlewich, UK). There was no evidence of inorganic soil carbon (which might confound the ^{13}C:^{12}C ratio) in the samples when 0.2 M HCl was applied to 10 subsamples. The soil carbon pools derived from sugarcane or trees (pre-sugarcane and contemporary) were calculated as in Binkley and Resh (1999). Briefly, the $\delta^{13}C$ (difference in ^{13}C:^{12}C between the sample and the PeeDee belemnite standard) for sugarcane was determined from sugarcane tissue (-11.5 ‰) and the $\delta^{13}C$ for soil unaffected by sugarcane was determined from soil (-25.1 ‰) in a nearby wildland forest (Bashkin and Binkley 1998). These endpoints were used in a two point mixing model (Vitorello *et al.* 1989) to determine the proportion of soil carbon derived from sugarcane or trees. We call all soil carbon derived from sugarcane "old" carbon.

The SOC_3 pools for each site included both pre-sugarcane and contemporary C_3 components. We assumed that changes in pre-sugarcane SOC_3 were minimal since the establishment of the present forest plantations. Thus, any changes in SOC_3 since forest plantation establishment was attributed to additions of new C_3 plantations. Net soil carbon change (kg m^{-2} yr^{-1}) for old, new, or total soil carbon pools since plantation establishment was calculated as the difference between those pools measured from the tree plantations minus respective pools from neighboring sugarcane fields used to approximate the pre-plantation conditions.

In January 17, 1998 five ion exchange resin bags were placed in each plot along a diagonal transect centered in the middle of the plot. The resin bags were constructed by putting 14 mL of anion exchange resin (Sybron IONAC ASB-IPOH, Sybron International, Milwaukee, WI) and 14 mL of cation exchange resin (Sybron IONAC c-251 H^+) in a nylon stocking and sealing the end of the stocking with a glue gun. The resin bags were buried 0.05 m below the soil surface, 1.5 m apart along a transect. They were left in place until April 13, 1998 and were retrieved, air dried, and extracted with 100 mL of 2*M* KCl. The extracts were analyzed colorimetrically for NH_4^+-N and NO_3^--N (Alpkem continuous flow autoanalyzer). The average total N (NH_4^+-N plus NO_3^--N) content of the 5 bags in each plot was used as the plot value.

All data were analyzed using simple regression with soil N accretion or soil N availability (i.e., exchangeable N) as the independent variable. To determine whether a linear or quadratic model fit the data better, we decomposed the regression error into linear model error, quadratic model error, and lack-of-fit error. When the quadratic model significantly ($p < 0.10$) reduced the lack of fit error relative to the linear model alone, we used the quadratic model (J. ZumBrunnen, Center for Applied Statistical Expertise, Colorado State University, personal communication). All statistical analyses were conducted with SYSTAT 7.0 (SYSTAT 1997).

RESULTS

Soil N increased linearly with increasing proportions of *Albizia*, ranging from 0.69 kg/m^2 under pure *Eucalyptus* to 0.92 kg/m^2 under pure *Albizia* (Figure 4.1). SOC_T ranged from 12.7 kg/m^2 under the *Eucalyptus* to 14.8 under the pure *Albizia*. Thus, the pure N-fixer plots accrued 0.12 kg m^{-2} yr^{-1} more SOC_T over the pure *Eucalyptus* plots. Both component pools of soil carbon also increased linearly with increasing proportions of *Albizia* (Figure 4.2). About 30% of the accrued soil carbon resulted from increasing SOC_4 pools and 70% from increasing SOC_3 pools. The SOC_4 pools contributed a small but increasing component of SOC_T under higher proportions of *Albizia*, with 0.6 kg/m^2 under the pure *Eucalyptus* and 1.2 kg/m^2 under pure *Albizia*. The SOC_3 pools ranged from 12.1 kg/m^2 under the *Eucalyptus* to 13.6 kg/m^2 under the pure *Albizia*.

The mixtures of *Albizia* provided a gradient of soil N and SOC pools with which to examine the effects of soil N accretion on total SOC (SOC_T) accretion, SOC_4 retention, and SOC_3 accretion. SOC_T accretion ($r^2 = 0.85$), SOC_4 retention ($r^2 = 0.77$), and SOC_3 accretion ($r^2 = 0.80$) corresponded linearly with soil N accretion (Figure 4.3).

Soil N availability, assessed by ion exchange resin bags, increased with increasing proportions of *Albizia* (Kaye *et al.* 1999). However, increased N availability over that of the pure *Eucalyptus* (data not shown) explained only a small proportion of the SOC_4 retention ($r^2 = 0.17$; $P = 0.07$) and was not correlated at all with SOC_3 accretion ($r^2 = 0.02$; $P = 0.56$). Thus, N availability using this technique does not provide any information pertinent to the question of soil carbon accretion.

DISCUSSION

Greater retention of old carbon and greater accretion of new carbon (relative to pure *Eucalyptus*) increased linearly with percent of intermixed *Albizia* in direct correlation with the accretion of soil N. Resh *et al.* (1999a) combined this site with three other tropical sites with pure plots of N-fixers compared with *Eucalyptus* to show a broad pattern of greater retention of old soil carbon under N-fixers relative to *Eucalyptus* ($r^2 = 0.60$; $P < 0.01$). Additionally, greater accretion of new soil carbon and greater total soil carbon difference correlated well with soil N accretion at three other sites (in addition to the site in this study) containing pure plantations of N-fixers and *Eucalyptus* ($r^2 = 0.43$ and 0.78, respectively; Resh *et al.* 1999b). Thus, patterns of greater old carbon retention and greater new carbon accretion resulting in a greater total soil carbon difference between N-fixers and *Eucalyptus* are consistent across a range of sites and across a range of N-fixers proportions within one site.

This replicated replacement series allowed us to explicitly examine the relationship between varying proportions of N-fixers mixed with non-N-fixers on old carbon retention and new carbon accretion. One might have assumed a linear response of soil carbon accretion with soil nitrogen accretion under increasing proportions of N-fixers; however, based on other research, this assumption may have missed an important interaction effect. Zou *et al.* (1995) found a synergistic effect of unreplicated, N-fixer/non-N-fixer mixtures on labile phosphorus pools and phosphorus solubilization rates, though these effects were not consistent for both study sites. Kaye *et al.* (1999) found synergistic effects of N-fixer/non-N-fixer mixtures on aboveground tree carbon and phosphorus contents.

Vegetation communities with varying proportions of N-fixers are a natural component of the landscape (Zavitkovski and Newton 1968, Walker 1993). This study allows us to predict the impact of these N-fixer mixtures on soil carbon dynamics with greater assurance. In this case, the retention of old soil carbon and the accretion of new soil carbon responded linearly to soil nitrogen accretion under increasing proportions of N-fixers. These linear effects indicate neutral interactions rather than threshold or synergistic relationships in species composition for soil carbon dynamics.

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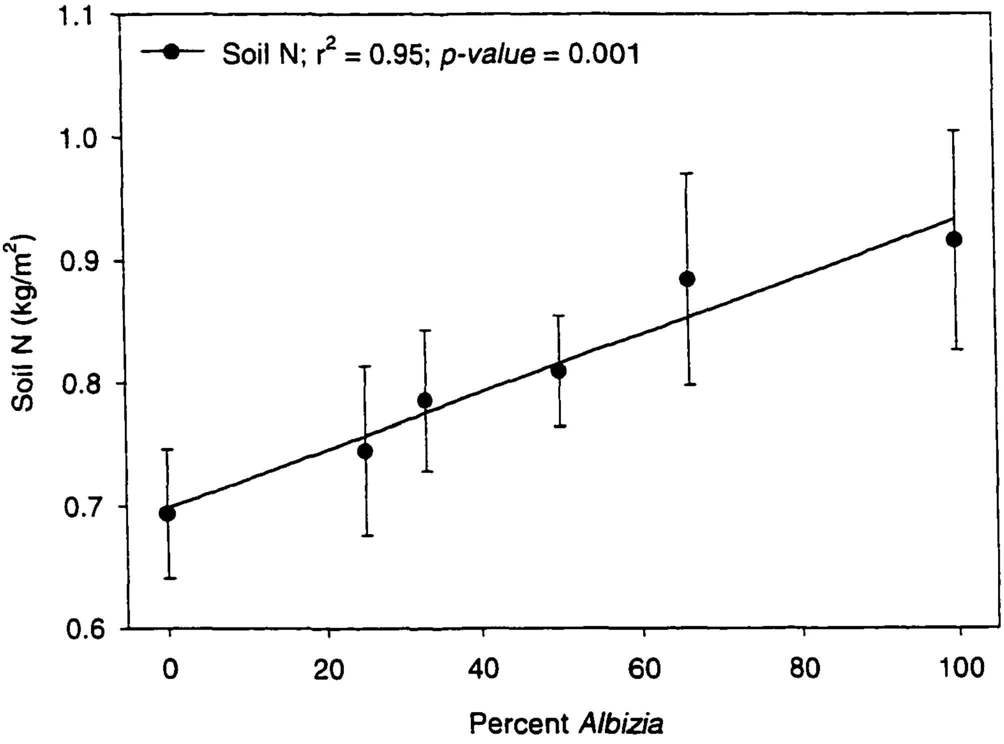

Figure 4.1. Soil nitrogen (N) accumulation (0 to 50cm depth) in a 17-year-old tropical plantation with monocultures of *Eucalyptus* (0% *Albizia*) and *Albizia* (100% *Albizia*) and 4 mixed stands. The x-axis is the percentage of *Albizia* trees in the plantation when the stand was planted. Line is the best-fit curve.

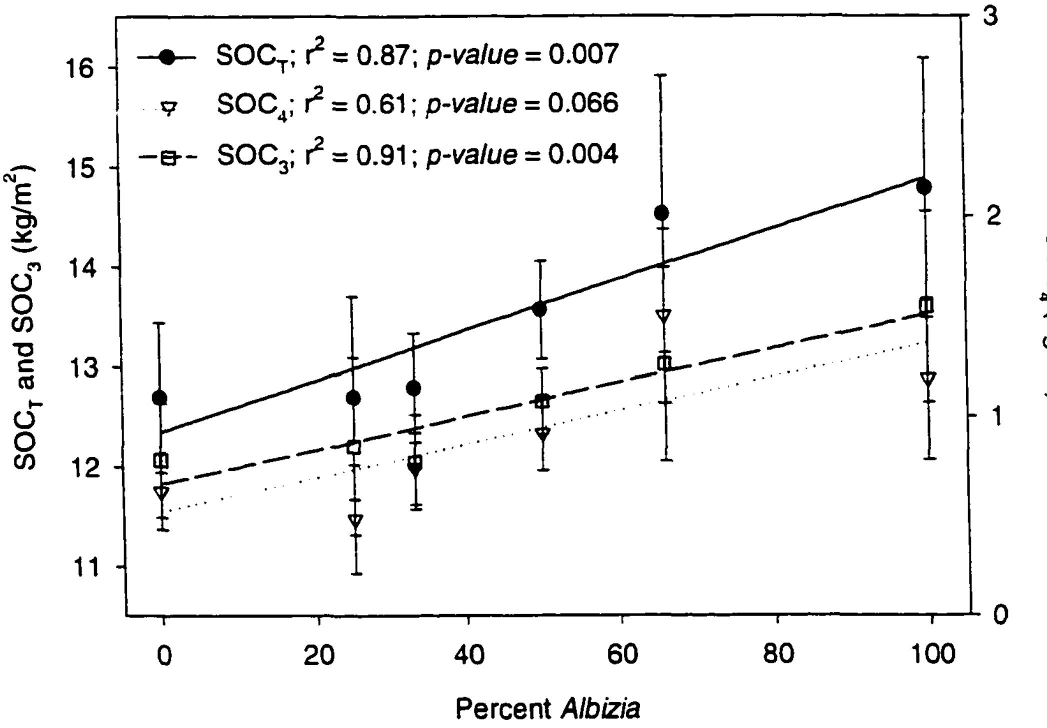

Figure 4.2. Soil organic carbon (SOC) accumulation (to 50 cm) in a 17-year-old tropical plantation with monocultures of *Eucalyptus* (0% *Albizia*) and *Albizia* (100% *Albizia*) and 4 mixed stands. The x-axis is the percentage of *Albizia* trees in the plantation when the stand was planted. The SOC$_3$ carbon is derived from trees, both contemporary and old, while all SOC$_4$ carbon is derived from sugarcane cultivation prior to plantation establishment. Lines are the best-fit curve.

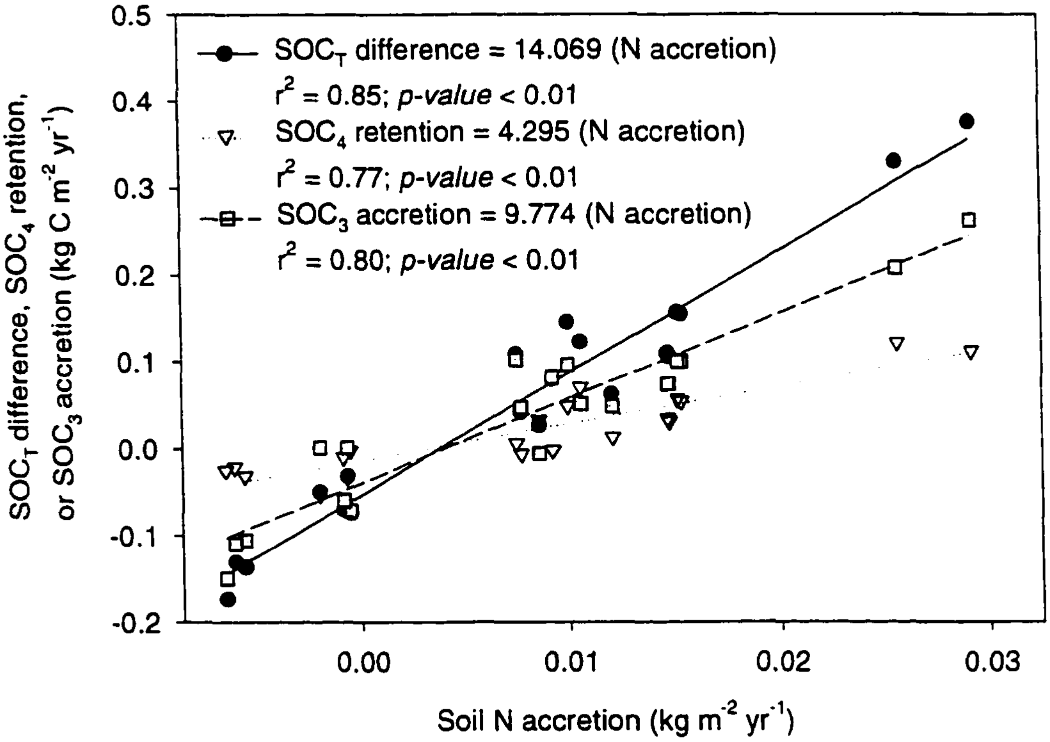

Figure 4.3. Total soil organic carbon (SOC$_T$) difference, SOC$_4$ retention, and SOC$_3$ accretion versus soil N accretion all with respect to pure *Eucalyptus*. Data points are plot averages for each N-fixer mixture (n = 20). Lines are the best-fit curve.

CHAPTER V

SUMMARY

That tree species can alter ecosystem properties in different ways is not a new concept. Common garden experiments comparing tree species growth rates led to the management of more productive forests. Species differences in their effects on soil fertility resulted in the widespread use of N-fixing species in intercropping systems. Continued research on species effects, specifically N-fixer species, has revealed a secondary characteristic—soil carbon sequestration.

My objective of this research was to determine how N-fixers increase soil carbon storage relative to non-N-fixers. I compared the effects of three N-fixer species and two *Eucalyptus* species (non-N-fixers) on the decomposition of old soil carbon (SOC_4; H_1) and the accretion of new soil carbon (SOC_3; H_2 and H_3) with some interesting surprises. I also examine the interaction effects of the N-fixers and *Eucalyptus* mixtures on soil carbon dynamics (H_4).

Old soil carbon retention

I found greater retention of old, SOC_4 under all N-fixer species compared with *Eucalyptus* across four sites (Chapter II). This is the opposite of my initial expectation and refutes H_1 that microbial decomposition of humified carbon is nitrogen limited. Berg and Matzner (1997) conducted a recent review of nitrogen deposition/fertilization effects on fresh litter and later stages of decomposition. They conclude that both direct effects of high soil nitrogen supply and indirect effects of high nitrogen concentrations in litter generally increase initial decomposition rates of fresh litter (see also Melillo *et al.* 1989) but that nitrogen inhibits decomposition in later stages. My findings is consistent with this generalization.

The retention of old carbon may be attributed to the soil nitrogen accretion from N-fixation. Several researchers have addressed possible mechanisms for nitrogen suppression of decomposition (Berg 1986, Fog 1988, Berg and Matzner 1997). As with any hypothesis, it is possible that some other, unexamined factor is responsible for the observed N-fixer effects. In this case, other factors that could suppress decomposition may include N-fixer effects on the availability of other nutrients like phosphorus, calcium, or manganese (Berg and Matzner 1997) or perhaps litter chemistry differences like polyphenol contents (Fox *et al.* 1990). Litter chemistry differences seems more likely to affect newer rather than older soil carbon decomposition. The possibility of another nutrient being the cause of the species effects on soil carbon would require a consistent relationship between that nutrient and N-fixers (e.g., soil phosphorus always being lower under N-fixers versus non-N-fixers) and an acting mechanism by which that nutrient could effect soil carbon (e.g., microbial activity limited by phosphorus).

New soil carbon accretion

In addition to greater SOC_4 retention under the N-fixers, I found greater N-fixer SOC_3 accretion compared with the *Eucalyptus* (Chapter III); therefore, H_2 was supported. Laboratory incubations of soils from N-fixer and *Eucalyptus* plots from three sites suggest that this greater SOC_3 accretion is the result of greater litter inputs from the N-fixers (H_3). After eight-months of laboratory incubations, the N-fixers lost more of their own SOC_3 than did the *Eucalyptus*; though the greater relative loss of SOC_3 from the N-fixer soil was only significant for the first four months of incubation. For greater litter inputs by the N-fixers to be a necessary component to greater SOC_3 accretion, the incubations only needed to show equal decomposition of the new SOC_3. Therefore, H_3 is supported.

The incubations do not rule out the possibility that a portion of the greater SOC_3 accretion under N-fixers is the result of greater recalcitrance of N-fixer litter. While higher litter nitrogen concentrations may increase the decomposition rate of litter (Berg and Matzner 1997), the greater nitrogen supply could suppress the decomposition of some portion of the N-fixer litter. This more recalcitrant portion of the litter could also be a part of the greater SOC_3 accretion. Others have reported higher belowground net primary productivity under N-fixers compared with non-N-fixers (Cole *et al.* 1995, Binkley and Ryan 1999) and slower decomposition of N-fixer litter relative to non-N-fixers (Cole *et al.* 1995).

Mixed species interactions

Using a replacement series of *Albizia* (N-fixer) and *Eucalyptus* at one site, I showed that there is a neutral interaction for species mixtures on soil organic carbon pools (Chapter IV). That is, knowing the pure species endpoints of old soil carbon retention and new soil carbon accretion is enough information to extrapolate to mixtures. H_4 was supported.

Confidence in the data

Before being comfortable with generalizing these results, one should question the accuracy of the measurements leading up to the ecosystem level estimates. I address the credibility of my findings with following three lines of evidence: confidence in the accuracy of my carbon and $\delta^{13}C$ measurements, replicated consistency in results, choice of vegetation endpoints for mixing equation.

First, potential errors for these soil carbon data are bounded by the total carbon present in the soil and the isotope ratios, both of which can be measured with high accuracy. Soil carbon concentrations are measured with an instrument standard deviation of ± 0.05 %. Isotope ratios are measured with an instrument standard deviation $\delta^{13}C$ of 0.2 ‰. The mean differences between tree species %C and $\delta^{13}C$ were 0.44 % and 0.65 ‰, respectively. There is no debate about the N-fixers having a higher soil carbon concentration; however the overall species difference in $\delta^{13}C$ was worrisome initially. Based on visual examination of the $\delta^{13}C$ value depth distributions across tree species, I noticed that in the surface soil (above 10 cm) the soil $\delta^{13}C$ values were most influenced by new tree carbon, but below 10 cm (subsurface) the N-fixers have $\delta^{13}C$ values tending more toward the sugarcane or pasture values than the *Eucalyptus*. Averaging the surface values with the subsurface values would cancel out any tree species difference, because of the opposite effects of tree carbon accretion and sugarcane/pasture carbon retention on the $\delta^{13}C$ values. Based on this observation I conducted a split-plot design analysis across the four sites used in Chapter II to determine if there were any species differences in the surface and subsurface soils (Figure 5.1). N-fixer $\delta^{13}C$ values were significantly different from the *Eucalyptus* in both the surface and subsurface soil ($P < 0.1$). In the surface soil, the $\delta^{13}C$ values were more negative under the N-fixers due to the greater influence of tree SOC_3 relative to *Eucalyptus*. In the subsurface soil, the $\delta^{13}C$ values were less negative under the N-fixers due to the influence of sugarcane/pasture. These species differences reassured me that the SOC_4 retention and SOC_3 accretion under the N-fixers was not an artifact of extrapolation.

A second line of evidence lending credibility to my data is the consistent pattern of SOC_4 retention and SOC_3 accretion across four sites. The accuracy of this methodology must be smaller than the difference between sites.

Finally, a potential source of error may derive from the vegetation $\delta^{13}C$ values I used as endpoints for the mixing models used to estimate SOC_4 and SOC_3. Due to the change in vegetation $\delta^{13}C$ values with initial decomposition (Balesdent *et al.* 1993 and von Fisher and Tieszen 1995) and changes in SOC $\delta^{13}C$ values with depth (Vitorello *et al.* 1989; Martin *et al.* 1990; Balesdent *et al.* 1993; von Fisher and Tieszen 1995), the best endpoints to use are those SOC $\delta^{13}C$ values from the corresponding depths of interest. However, in systems where there are no pure C_4 or C_3 sites from which to obtain the appropriate values, the vegetation $\delta^{13}C$ values are the only alternative. If the C_3 endpoint was enriched by 2 ‰ (a reasonable enrichment using Balesdent *et al.* 1993), the resulting error is a possible overestimation of SOC_4 by 13% in the 30-40 cm depth. This error would affect all species SOC_4 and SOC_3 estimates equally; the species effects would not be biased.

CONCLUSIONS

This dissertation provides a body of evidence showing that greater total carbon sequestration under N-fixers relative to non-N-fixers is the result of greater old carbon retention and greater new carbon accretion. Delving into the mechanisms of that are responsible for greater retention of old carbon may reveal important implications for the effects of other nitrogen sources such as other N-fixing species, nitrogen fertilization, and nitrogen deposition on global carbon sequestration.

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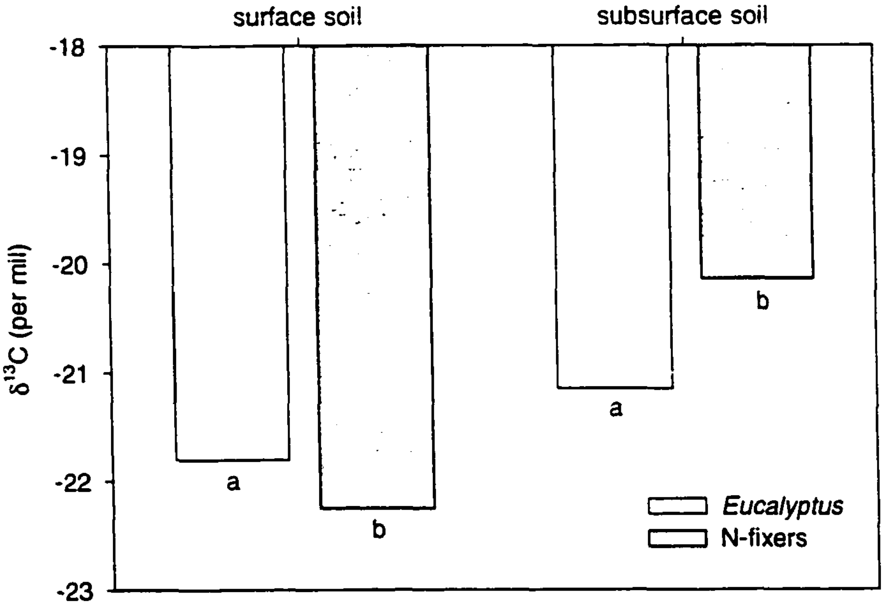

Figure 5.1. Averaged $\delta^{13}C$ values for N-fixers and *Eucalyptus* for surface and subsurface soil across four sites (i.e., Kamae, Chinchuck, Lajas, and Toa Baja). Surface soil is 0-10 (0-20 cm for Chinchuck). Subsurface soil is 10-40 cm (20-50 cm for Chinchuck). Different letters below the paired bars indicates a significant difference at a $P < 0.01$ using a split-plot statistical analysis as described in the methods of Chapter III.

APPENDIX

Appendix A.1. Split-plot design ANOVA table for the site, species, and site-by-species effects for the variables SOC_T, SOC_4 and SOC_3 changes.

		SOC_T change (kg m^{-2} yr^{-1})			$\log_{10}$ SOC_4 change (kg m^{-2} yr^{-1})			SOC_3 change (kg m^{-2} yr^{-1})		
Source	df	ms	F	*P*	ms	F	*P*	ms	F	*P*
Site	3	0.140	17.621	<0.001	0.014	16.854	<0.001	0.092	31.587	<0.001
Block (Site)	12	0.008	—	—	0.001	—	—	0.003	—	—
Species	1	0.113	10.336	0.007	0.006	6.605	0.025	0.023	5.549	0.036
Species x Site	3	0.005	0.461	0.714	0.001	1.578	0.246	0.002	0.449	0.723
Species x Block (Site)	12	0.011	—	—	0.001	—	—	0.004	—	—
Total	31	0.026	—	—	0.002	—	—	0.015	—	—