**Soil Organic Carbon is Increased in Mixed-species Plantations of *Eucalyptus*
and Nitrogen-fixing *Acacia***

Short title: Soil carbon under mixed-species plantations

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PK designed the long-term experiment and JB and PK conceived the study. DF, CO, AP performed the research. CO developed the fractionation methodology. DF analyzed the data and DF and JB wrote the paper.

ABSTRACT

Soil organic carbon (soil C) sequestration in forests is often higher under nitrogen (N₂)-fixing than under non-N₂-fixing tree species. Here, we examined whether soil C could be increased using mixed-species plantations compared to monocultures, which are less productive above-ground than mixtures. In addition, we compared soil C sequestration under N₂-fixing trees with non-N₂-fixing trees that received N fertilizer. Monocultures of *Eucalyptus globulus* (E) and the N₂-fixing *Acacia mearnsii* (A) and mixtures of these species were planted in a replacement series: 100%E, 75%E + 25%A, 50%E + 50%A, 25%E + 75%A, and 100%A. Soil samples were also collected from fertilized monoculture treatments (100%E_{Fer}) of *E. globulus* (250 kg N ha⁻¹). Total organic C, N and phosphorus (P) were determined at age 8 years at two soil depths (0-10 cm and 10-30 cm) and three density fractions of soil organic matter (SOM) were quantified for 0-5 cm depth. Soil C was highest in the 50%E + 50%A mixed stand and was highly correlated with aboveground biomass, not to the percentage of *A. mearnsii* in mixtures. This was largely due to soil C at 10-30 cm because there were no treatment effects on soil C at 0-10 cm. All density fractions of SOM at 0-5 cm increased with the percentage of *A. mearnsii*. In *E. globulus* monocultures, N fertilization did not increase soil C when compared with unfertilized stands. These results indicate that the inclusion of N₂-fixing trees into eucalypt plantations may increase soil C stocks through increased productivity.

Keywords: soil carbon sequestration; organic matter fractionation; decomposition; nitrogen-fixation

INTRODUCTION

46

47 Forest management is increasingly focusing on maintaining or augmenting soil organic carbon
48 (soil C) levels. The organic carbon in forests soils is not only important to soil fertility and
49 nutrient cycling, but also as a major storage pool in the global C cycle (Canadell and Raupach
50 2008; Schmidt and others 2011; Dungait and others 2012). For these reasons, afforestation of
51 formerly deforested land is regarded as a means to sequester atmospheric C (Canadell and
52 Raupach 2008). Soil C sequestration varies strongly with tree species (Binkley and Giardina
53 1998) and it may be of particular interest to use tree species for afforestation that increase soil C.
54 Several studies in temperate and tropical regions have shown that given similar aboveground
55 productivities, soil C sequestration in forest soils is greater under N₂-fixing than under non-N₂-
56 fixing species (Kaye and others 2000; Johnson and Curtis 2001; Resh and others 2002; Binkley
57 2005; Jeddi and others 2009; Wang and others 2010).

58

59 The mechanisms behind differences in soil C under N₂-fixing species are not well established and
60 are likely to be the net effect of three processes (Figure 1; Binkley 2005). Soil C could increase
61 by (1) increasing detritus inputs, (2) decreasing rates of decomposition of the recent detrital C
62 pool or the stabilized/old C pool, or (3) by increasing the rate at which the recent detrital C is
63 incorporated into the pool of stabilized or slowly turning over soil C. The effects of N₂-fixing
64 species on changes in soil C appear to vary from those of fertilizer N (Gregorich and others
65 2001), although few comparisons are available for the same sites. For example, soil C increased
66 under *F. moluccana* when compared with *E. saligna* plantations in Hawaii (Kaye and others
67 2000; Resh and others 2002), but on nearby sites, large applications of N fertilizer had no
68 significant effect on soil C compared with unfertilized *E. saligna* plantations (Binkley and others

2004). This suggests that nitrogen *per se* may not have the main influence on soil C dynamics, and instead changes in soil biology may influence decomposition (Binkley and others 2004; Binkley 2005).

The potential to increase soil C with N₂-fixing species could be further enhanced by increasing stand productivity and hence the production of litter through mixing with non-N₂-fixing tree species. Many studies have found that mixing *Eucalyptus* species with N₂-fixing species can significantly increase productivity (Forrester and others 2006b), however, few studies have compared soil C in mixtures and monocultures. In these cases, increasing soil C with increasing proportions of the N₂-fixing species (Vogel and Gower 1998; Kaye and others 2000; Mao and others 2010) or no significant changes have been reported (Wang and others 2009; Voigtlaender and others 2011). Both studies that included eucalypts (Kaye and others 2000; Voigtlaender and others 2011) were in the tropics, where changes in soil C following afforestation are often greater than those in temperate regions (Paul and others 2002).

Soil organic matter (SOM) is not homogeneous; it is a continuum from freshly incorporated plant debris to highly decomposed OM. Different physical fractions of particulate organic matter (POM) may provide additional information about OM functions and associated nutrient cycling processes, particularly when a given change in N availability has opposing influences on different POM fractions (Post and Kwon 2000; Neff and others 2002; Russell and others 2004). In addition, rates of OM decomposition can correlate with its chemistry, such as N concentration and the fraction of OM that is not hydrolyzed by strong acid treatments (Taylor and others 1991). Therefore we analyzed in addition to total quantities of C and OM, the acid hydrolyzable fraction

in different physical fractions of particulate organic matter and the concentrations of N and P in these physical fractions.

This study examined soil C in mixtures and monocultures of *Eucalyptus globulus* and *Acacia mearnsii* in temperate, south-eastern Australia. In this experiment the aboveground biomass of 1:1 mixtures was 30% or 86% higher than *A. mearnsii* or *E. globulus* monocultures, respectively, at age 10.7 years. We aimed to determine:

1. Whether the soil C increased with increasing proportions of *A. mearnsii*, as suggested by studies comparing monocultures of N₂-fixing and non-N₂-fixing species, or whether soil C was highest in mixtures, consistent with aboveground biomass and litter production,
2. Whether early rates of OM decomposition increased, whereas later stages decreased, with increasing proportions of *A. mearnsii* by examining the mass of different density fractions of soil organic matter and their N concentrations, and
3. Whether the addition of 250 kg ha⁻¹ of N fertilizer to *E. globulus* monocultures had similar effects on soil C to addition of N₂-fixing species in mixtures.

MATERIALS AND METHODS

Experimental design and site characteristics

The experiment was located 5 km southeast of Cann River in East Gippsland, Victoria, Australia (37°35'S, 149°10'E). Prior to trial establishment the site vegetation was a dry sclerophyll forest dominated by *Eucalyptus sieberi* (L. Johnson) (Costermans 1983). The site is about 100-120 m a.s.l.. Soils are Mottled, Dystrophic, Brown Kurosols (Dy5.21) or Mottled, Mesotrophic, Brown

Dermosols (Gn4.31) (Stace and others 1968; Isbell 1998) and have sandy loam A horizons, sandy clay loam B1 and B21 horizons and a light to medium clay B22 horizon. The pH (H₂O) ranges from 4.6 to 5.7. The average annual rainfall at Cann River is 1009 mm and is evenly distributed throughout the year. The mean maximum temperature is 26°C in January and the mean minimum temperature is 6.2°C in July.

Eucalyptus globulus ssp. *pseudoglobulus* (Naudin ex Maiden) Kirkpatr. and *Acacia mearnsii* De Wild. were planted in a replacement series of monocultures (100E and 100A) and mixtures containing 75, 50 or 25% *E. globulus* (75E:25A, 50E:50A and 25E:75A). Plots were arranged in a randomized block design with four replicate blocks. Trees were planted at a spacing of 3.3 × 3 m (1010 trees ha⁻¹) in plots that were 23 m × 28 m, including a surrounding row of buffer trees. In the mixed-species plots trees were mixed within rows, resulting in a checkerboard arrangement. *E. globulus* seedlings were planted in early July 1992 and *A. mearnsii* in early October 1992. The stands were fertilized with 25 kg P ha⁻¹ in the form of superphosphate, which was applied on the surface of planting mounds, about 25 cm from the tree, three months after establishment.

To compare the effects of symbiotically fixed N with those of fertilizer N on soil C, additional plots were established in monospecific stands of *E. globulus* ssp. *pseudoglobulus* that received N fertilizer (E100Fer). These stands were located in an experiment that was planted at the same time, received the same site preparation treatments and was located on the same site, adjacent to the species replacement series. This treatment received N, P, and K at cumulative rates of 250, 160, and 170 kg ha⁻¹, respectively, by 14 months. Samples were also collected from the

surrounding native forest, which resembles ecosystem conditions prior to clearing and plantation establishment.

Total soil C, nitrogen and phosphorous at 0-30 cm depth

Soil sampling took into account the distinct micro-topography created by site preparation, which involved ripping to 60 cm and mounding of soils over rip lines, after the native vegetation was cleared and the logs had been removed or burned in windrows. Plots were divided into (i) mounds on which the trees were planted, (ii) trenches that were created by plough discs to build the mounds and (iii) flat uncultivated areas between the trenches. Line-transects running perpendicular to the mounds were used to show that the stand area consisted of 21 % , 41 % and 38 % trench, mound and flat areas, respectively. These data were used when converting soil and litter C concentrations to per ha values.

Soil samples were collected in November 2000 at about age 8.1 years. Eight pairs of soil cores (7.5 cm diameter) were collected from each microsite in each treatment. Each pair was combined into a single sample and no surface litter was included. These cores were divided into 0-10 cm and 10-30 cm depths. In monocultures and 50E:50A, soil cores were collected at mid-points between trees. In 75E:25A and 25E:75A, soil cores on the flats were also collected at the center of four trees, but on the mounds and in the trenches the soil cores were collected about 80-90 cm from the more abundant species to be more representative of the plot conditions. Three cores were also collected from each of four locations in the surrounding native forest, and divided into 0-10 cm and 10-30 cm depths. Soil samples were stored cool at 4°C in polyethylene bags until processing in the laboratory.

Soil samples were manually homogenized, air-dried and sieved (< 4.75 mm diameter) to remove roots, rocks, charcoal and other debris. Bulk density was estimated from the sieved soil as the ratio of sieved oven-dry (105°C for 24 hours) soil mass per unit volume of soil core. Total soil C was measured using the combustion furnace technique of an automated C/N analyzer (LECO CHN-2000, St. Joseph, Michigan). Soil N and P concentrations were determined to calculate the sizes of soil N and P pools at 0-30 cm depth. To determine soil N and P concentrations, ground and dried (60°C for 24 hours) soil material was digested in acid (potassium sulphate dissolved in concentrated sulphuric acid) and hydrogen peroxide (modified from (Heffernan 1985). A copper sulphate solution catalyst was added to the soil for 12 h prior to the digestion. Total N and P were determined simultaneously in a continuous flow system (Technicon® TRAACS 800™ or Technicon Auto-Analyser II) using an ascorbic acid/ammonium molybdate method.

The mass of the O horizon was sampled in March 2003. Three forest floor litter samples were collected from the Flat microsite (equidistant from four trees) in each 100E, 50E:50A and 100A plot using 0.25 m² quadrats. Mineral soil that was accidentally collected was removed by sieving (2 mm) before oven drying to constant weight at 50°C. More information about the sampling strategy can be found in Forrester and others (2005).

Fractionation of particulate organic matter fractions and acid hydrolysis

To examine potential differences in decomposition processes, particulate organic matter (POM) was divided into three density fractions, (i) water floatable, (ii) light fraction (density between 1 and 1.23 g cm⁻³) and (iii) heavy fraction (density>1.23 g cm⁻³). Three soil cores (0-5 cm depth)

from the Flat microsite per plot were collected in January 2003 and mixed into a single sample per plot. Samples were collected from 100E, 100A and 50E:50A. The soil was sieved moist with a 2 mm sieve to remove coarse material. The equivalent of 100 g dry weight of soil was washed over a 63 μm sieve. Water floatable POM was recovered by repeated (3-4 times) floatation in water and decanting of material until no more material was recovered. The remaining soil was then suspended in LudoxTM colloidal silica (Du Pont Industries) to obtain the light fraction. Ludox grade TMA was used, which has a specific gravity of 1.23 g cm⁻³, consists of 34% silica as SiO₂ by weight and has a pH range of 4 to 7. This was also repeated about 3-4 times. The heavy fraction POM was material that did not float on Ludox, but sinks much less rapidly than sand. All fractions were washed for several minutes, including the water floatable fraction, for consistency. These samples were dried at 65°C for 48 hours, ground, weighed and analyzed for C and N using the same methods described above.

The percentage of acid-hydrolyzable OM in each density fraction was determined and used as an indicator of decomposability. A 0.1 g sample of OM was hydrolyzed in concentrated (72%) sulfuric acid (for 2 hours), then diluted to 2.5% for 1 hour. The suspension was centrifuged and the supernatant carefully poured off, the sample was re-suspended with 50 ml dH₂O, centrifuged, supernatant poured off, and this was repeated four times before washing the samples and drying at 105°C for 48 hours. Samples were weighed to determine the mass loss. These values were corrected for the ash content, which was determined by combustion at 550°C for four hours.

Aboveground biomass

Diameter at 1.3 m was measured in March 2003 on all trees. Aboveground biomass was estimated using an allometric equation for 10-year-old *A. mearnsii* developed in these stands (Forrester and others 2004), and an equation for *E. globulus* developed by Veiga (2008) using data from a range of ages, site qualities and initial spacing treatments.

Statistical analysis

Regression analysis was used to examine the relationship between soil C and aboveground biomass, and the effect of the percentage of *A. mearnsii* in each treatment on soil C, N and P. A separate t-test was used to compare *E. globulus* monocultures that were unfertilized (100E) and fertilized (100Efert). Differences between microsites were tested using Residual Maximum Likelihood analysis (REML) with replicate/treatment as a random effect because there were no interactions between treatment and microsite for any variable. REML was also used to examine treatment effects on particulate organic matter (with replicate as a random effect), because this was measured in three treatments, preventing the use of regression. Standard errors of difference (SEDs) are provided for comparison of treatment means. All analyses were performed in GenstatTM (VSN Int., Hemel Hemestead, UK).

Results

Soil C was non-linearly related to the percent of *A. mearnsii* in the treatment, peaking in the mixtures, especially in the 50E:50A treatment (Figure 2). Soil C was higher in all treatments that contained *A. mearnsii* compared with the native forest or *E. globulus* monocultures, whether they had been fertilized or not (Figure 2; $P < 0.001$). This treatment effect was attributable to

differences in soil C at 10-30 cm depth; there were no significant differences at 0-10 cm depth. Soil C stocks were highly, positively and linearly correlated with aboveground biomass (Figure 3). Microsites significantly influenced soil C, which was highest in the Mounds and lowest in the Flats (Table 2).

The N and P contents of the top 30 cm of soil were not correlated with the percent of *A. mearnsii* in the treatments ($P>0.05$). Instead, soil N was higher in treatments containing *A. mearnsii* compared with those that did not ($P=0.002$, Table 1). It was highest in acacia monocultures (100A) and lowest in the fertilized *E. globulus* monocultures (Table 1). Comparisons between N in mineral soil, the O horizon and aboveground biomass in 100E compared with 50E:50A or 100A suggested that rates of symbiotic N₂-fixation were 38 and 86 kg ha⁻¹ year⁻¹ in 50E:50A and 100A, respectively, for the first 10 years (Forrester and others 2007). Soil C was not correlated with soil N ($P=0.527$) or P contents ($P=0.310$) (excluding data for 100Efert and the native forest). There was no significant difference in soil N or P between *E. globulus* monocultures that were fertilized (100Efert) or unfertilized (100E) (Table 1).

C/P and N/P ratios were not related to the percent *A. mearnsii*, but C/N ratios at 0-10 cm were negatively and linearly related to the percent *A. mearnsii* ($P=0.03$, Adj. R²=0.03, $y=46.19-0.15x$). C/N ratios were lower at 0-10 cm compared with 10-30 cm, whereas C/P ratios were lower at 10-30 cm compared with 0-10 cm (Table 1).

Particulate organic matter fractions (0-5 cm depth) were highest in 100A, lowest in 100E and intermediate in 50E:50A (Figure 4a). The N concentration of this POM was lower in 100E

compared with stands containing *A. mearnsii* (Figure 4b). There were no treatment effects on P contents of POM. The percent of O horizon mass that was resistant to acid-hydrolysis was higher in 100A compared with 100E or mixtures. A similar trend occurred for the water floatable fraction of POM, although this was not significant.

Soil bulk density in the plantation plots increased with depth ($P < 0.001$) from 1.34 g cm^{-3} at 0-10 cm to 1.60 g cm^{-3} at 10-30 cm. There were no interactions between treatments and microsites. Bulk density in the Flats, Trenches and Mounds were 1.55 , 1.53 and 1.32 g cm^{-3} respectively, but these differences were not significant ($P > 0.05$). There were also no significant treatment effects.

Discussion

Soil C was highest in mixtures and lowest in *E. globulus* monocultures, whether they were fertilized or not. Higher soil C stocks under N_2 -fixing species compared with non- N_2 -fixing species have been reported previously (Kaye and others 2000; Johnson and Curtis 2001; Resh and others 2002; Binkley 2005; Jeddi and others 2009; Wang and others 2010). However, the effects of mixtures on soil C stocks have rarely been examined. Here, C stocks were not linearly related to the proportion of *A. mearnsii*. Soil C was highly positively correlated with above-ground biomass, and because mixtures were significantly more productive than monocultures, soil C was also highest in mixtures.

Higher soil C under mixtures than monocultures

The 50E:50A mixtures had greater C inputs into soil (process 1 in Figure 1) than monocultures in terms of litterfall and total belowground C allocation, although the latter was not significantly

different (Table 4). Increased C inputs were not the only process influencing soil C in these mixed stands. Rates of litter decomposition as well as litter N and P contents and concentrations, all increased with the proportion of *A. mearnsii* (Table 4; Forrester and others 2005). Depending on the fate of C in the O horizon, the more rapid decomposition could indicate that more C was incorporated into the mineral soil (process 3) but might also have had the opposite effect on soil C if more of the O horizon C was lost to the atmosphere as CO₂ (process 2a). The mass of all OM density fractions in the top 0-5 cm (and their N concentrations) increased with increasing proportions of *A. mearnsii*, indicating that a greater proportion of O horizon C was incorporated into mineral soil beneath N-fixing trees to increase soil C at this depth (process 3). Similarly, increases in soil C under *F. moluccana* compared with *E. saligna* resulted from an increase in new tree-derived C and not only reduced decomposition of older C derived from the previous sugar cane cropping system (Kaye and others 2000).

Although early stages of OM decomposition usually proceed faster for litter with higher N concentrations (2a), the later stages of decomposition can be decelerated (2b) (Berg 2000; Neff and others 2002; Prescott 2010) thereby increasing soil C. In this study, the mass of all OM fractions (0-5 cm) increased, and had higher N concentrations, as the proportions of *A. mearnsii* increased. The decomposability, as indicated by the percentage of acid-hydrolyzable OM, also tended to decrease as the proportion of *A. mearnsii* increased. This suggests that OM in stands with the N₂-fixing *A. mearnsii* is more protected or complexed and thus harder for decomposers to break down. Root C may contribute more to soil C stocks because it may be less accessible to decomposers (Post and Kwon 2000; Prescott 2010; Schmidt and others 2011). The significant increases in OM in the 0-5 cm depth might be influenced by aboveground litter inputs as well as

fine-roots. However, root inputs are more likely to dominate soil C in the 10-30 cm soil layer, and the changes in soil C at this depth led to the higher soil C stocks in mixtures compared with monocultures. Thus, inputs from roots (process 1) and/or reductions in their decomposition (process 2b) were probably important mechanisms in these stands. Further support for this assumption is higher soil C in mounds, which likely represent a better rooting substrate than the other microsites, whereas litter-fall would not have been different between microsites in these stands were the canopy closed very rapidly. N concentrations in fine-roots of *A. mearnsii* were rather constant, at around 0.9 mg g^{-1} , whereas those of *E. globulus* increased from 0.2 mg g^{-1} in monocultures to 0.6 mg g^{-1} in the 50E:50A mixture (Bauhus and others 2000). Therefore, the higher soil C of mixtures was likely the net effect of (i) higher C inputs (process 1) resulting from synergistic interactions between species that increased above- and belowground productivity, (ii) reduced rates of decomposition of soil C (process 2b) with increasing proportions of *A. mearnsii* or (iii) a faster rate of conversion of O horizon C to mineral soil C with increasing proportions of *A. mearnsii*.

The application of fertilizer N did not increase soil C. Similarly, application of 600 kg ha^{-1} of fertilizer N over six years did not increase soil C under *E. saligna* plantations (Binkley and others 2004), even though N fixed by *F. molucanna* on the same soils did increase soil C (Kaye and others 2000). Hence, it was concluded that the increase in soil C was not simply the result of a chemical reaction in response to the increased availability of inorganic N (Binkley and others 2004; Binkley 2005). Schmidt and others (2011) propose that organic matter persists because physicochemical and biological influences reduce rates of decomposition and enable OM to persist. That is, the persistence of OM is an ecosystem property more than the result of any

intrinsic property of OM itself. Mixtures also had lower soil moisture due to greater growth and evapotranspiration (Forrester and others 2010), and P pools and fluxes also varied with the proportion of *A. mearnsii* (Forrester and others 2005), and these characteristics might also have modified soil ecological processes in a way that contributed to the soil C dynamics in these stands.

Increasing soil C with increasing proportions of N₂-fixing species

In contrast to this study, where soil C was highest in the mixtures, others have found that soil C increased with the proportion of the N₂-fixing species. Although the same processes in Figure 1 are likely to occur in each study, their relative contributions vary. For example, soil organic C down to a depth of 50 cm was also examined in 17-year-old mixed and monospecific stands of *E. saligna* and *F. moluccana* (= *Albizia falcataria*, an N₂-fixing species) in Hawaii planted on an ex-sugar cane site (Kaye and others 2000). Aboveground biomass and C were highest in mixtures and lowest in monocultures, which is consistent with this study. However in contrast to this study, soil C increased with the proportion of *F. moluccana*. Thus although the same processes are likely to have occurred as in our study (Figure 1), their relative influence must have been different. Higher soil C under *F. moluccana* than *E. saligna* was caused by reduced decomposition of older (sugar cane derived) C as well as an increase in new (tree-derived) C (Kaye and others 2000), which is consistent with our results. The new (tree-derived) C decomposed as fast or faster under *F. moluccana* than *E. saligna* (Resh and others 2002). Litterfall was similar in each monoculture, however, total belowground C allocation was higher for monocultures of *F. moluccana* than *E. saligna* (Binkley and Ryan 1998). The latter contrasts with this study, where both fluxes did not significantly differ between monocultures (Table 4;

Forrester and others 2006a). This is an important difference between the studies because below-ground C inputs might be even more important than aboveground inputs for increasing soil C (Post and Kwon 2000; Prescott 2010; Schmidt and others 2011).

Mixtures of *Pinus banksiana* and *Alnus crispa* also had a greater aboveground biomass and O horizon C compared with *P. banksiana* stands that regenerated without the N₂-fixing *A. crispa*, however, increases in soil C were not significant (Vogel and Gower 1998). Soil C was also not significantly different in *E. grandis* and *A. mangium* mixtures and monocultures (Voigtlaender and others 2011). Stem and coarse-root mass were greatest in *E. grandis* monocultures, intermediate in 50E:50A mixtures and lowest in *A. mangium* monocultures (Lacclau and others 2008). In these mixtures, *A. mangium* was suppressed by *E. grandis*. Thus in contrast to this study there may have been decreasing C inputs (process 1) with increasing proportions of *A. mangium*, which would have worked against a possible increase in soil C.

In conclusion, soil C was linearly correlated with aboveground productivity and not with the proportion of the N₂-fixing species. The soil C stocks were the net effect of several (sometimes opposing) mechanisms that varied in importance between this and previously published experiments, resulting in contrasting mixture effects on soil C. The *A. mearnsii* effect was not due to N *per se*, because the application of fertilizer N did not change soil C. The potential to increase soil C using mixtures warrants more attention, and because changes in soil C were greatest in the 10-30 cm depth, sampling to at least this depth, and preferably deeper would be useful.

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Table 1. Mineral Soil N, P and Soil C/N, C/P and N/P Ratios in the Mixtures (25E:75A, 50E:50A, 75E:25A), Unfertilized Monocultures (100A, 100E), Fertilized Monoculture (100Efert) and Adjacent Native Forest (Nfst), 8.3 Years after Planting

	DEPTH	100A	25E:75A	50E:50A	75E:25A	100E	100Efert	Nfst	SED	F. prob.
Soil N (kg ha ⁻¹)	0-10 cm	1150	798	693	858	712	552		112	<0.001
	10-30 cm	837	780	868	1014	781	619		83	0.001
	0-30 cm	1987	1578	1561	1872	1493	1171	1484	179	0.002
Soil P (kg ha ⁻¹)	0-10 cm	58.1	56.7	53.2	70.8	49.7	62.5		13.3	0.638
	10-30 cm	195.7	154.7	164.2	104.9	156.2	177.7		32.8	0.120
	0-30 cm	253.8	211.4	217.4	175.7	205.9	240.2	186.7	33.9	0.342
C/N (kg kg ⁻¹)	0-10 cm	31.4	35.5	42.0	38.8	42.2	52.5		7.2	0.107
	10-30 cm	55.3	70.6	65.7	51.1	49.5	58.4		7.3	0.034
	0-30 cm	40.8	52.8	55.1	43.8	44.9	54.8	48.1	5.6	0.101
C/P (kg kg ⁻¹)	0-10 cm	651	475	547	483	604	481		65.8	0.046
	10-30 cm	258	382	380	528	292	229		60.9	<0.001
	0-30 cm	346	401	417	475	362	280	372	56.0	0.045
N/P (kg kg ⁻¹)	0-10 cm	21.7	14.4	13.2	14.1	15.1	10.3		2.5	0.003
	10-30 cm	4.6	5.4	6.2	10.7	6.6	3.9		1.3	<0.001
	0-30 cm	8.5	7.8	7.9	11.3	8.2	5.1	8.2	1.2	0.002

SED = standard error of difference. Native forest data were not included in depth analysis. n = 8 except for 100A where n = 6.

Table 2. Soil C, Soil N and Soil P in the Flat, Mound and Trench Microsites (0-30 cm depth)
(excluding Nfst plots) 8.3 Years after Planting

	Flat	Mound	Trench	SED	F prob.
Soil C (Mg ha ⁻¹)	71.8	83.4	66.1	4.6	0.001
Soil N (kg ha ⁻¹)	1516	1689	1555	77.4	0.069
Soil P (kg ha ⁻¹)	206.3	208.8	240.3	19.0	0.141

SED = standard error of difference. n = 46.

Table 3. P Concentration of Particulate Organic Matter (POM), and the Percent of Acid-Hydrolysable Organic Matter in Different Density Fractions of Surface Soil (0-5 cm) at Age 10.5 Years

	100A	50E:50A	100E	SED	F prob.
P concentration of OM (mg g ⁻¹)					
Water floatable	245	235	267	11.5	0.051
Ludox light fraction	214	232	241	13.7	0.257
Ludox heavy fraction	243	226	223	16.6	0.496
Percent of acid-hydrolysis resistant organic matter					
O horizon	60.2	46.6	44.6	4.3	0.025
Water floatable	54.7	42.9	42.1	4.5	0.063
Ludox light fraction	62.2	64.6	62.8	4.2	0.844
Ludox heavy fraction	73.3	62.2	64.5	5.7	0.225

SED = standard error of difference. n = 4, except 100A where n = 3.

Table 4. Aboveground Biomass (age 10.7 years), Litterfall and O Horizon Characteristics (age 9.25 to 11.25 years), Fine-root Biomass (age 6.5 years), Total Belowground C Allocation (during year 11) and N Fixation (to age 10 years)

	100E	75E:25A	50E:50A	25E:75A	100A	100Efert
Above-ground biomass						
(Mg ha ⁻¹)	43.4a	91.8bc	99.1c	91.6c	75.3b	182.4
Litterfall (Mg ha ⁻¹ y ⁻¹) ¹	2.43a	3.56b	3.74b	3.69b	3.29b	3.05
N in litterfall (kg ha ⁻¹ y ⁻¹) ¹	13.7a	31.6b	39.2bc	46.6cd	48.8d	17.6
P in litterfall (kg ha ⁻¹ y ⁻¹) ¹	0.458a	0.685ab	0.800b	0.829b	0.741b	0.838
O horizon (Mg ha ⁻¹) ¹	7.32a		6.27a		3.53b	9.34
O horizon N (kg ha ⁻¹) ¹	50.1a		49.6a		48.1a	
O horizon P (kg ha ⁻¹) ¹	1.164a		0.937b		0.584c	
Rate of litter decomposition						
(y ⁻¹) ¹	0.32a		0.56b		0.85c	0.33
Live fine-root mass (0-30 cm)						
(Mg ha ⁻¹) ²	4.06	4.99	5.19	4.30	4.90	
Total below-ground C allocation						
(Mg ha ⁻¹ year ⁻¹) ³	14.604a		16.310a		15.846a	
N ₂ -fixed (kg ha ⁻¹ year ⁻¹) ⁴			38.3		86.2	

¹Forrester and others (2005); ²Bauhus and others (2000); ³Forrester and others (2006a); ⁴Forrester and others (2007)

For a given variable, means sharing the same letter are not significantly different ($P>0.05$, REML). 100Efert was not included in the analyses.

Figure captions

Figure 1. The pools and fluxes of soil C, modified from Binkley (2005). Soil C could increase if detritus inputs increase (1), rates of decomposition decrease (2a, 2b), or by increasing humus formation (3). Detritus inputs (1) may come from aboveground (for example, leaf litter) or belowground sources (for example, fine-roots). The constraint is that $1 = (2a + 2b + 3) +$ plus any change in mass of the recent detrital C pool.

Figure 2. Soil C at two depths (0-10 cm or 10-30 cm) and their sum (0-30 cm) at age 8.3 years. Error bars are ± 1 se of the mean. For each point $n = 8$ except for 100A where $n = 6$. There were no differences (t-test) between 100E and 100Efert at 0-30 cm ($P=0.172$), 0-10 cm ($P=0.086$) or 10-30 cm ($P = 0.741$).

Figure 3. Relationship between soil C (0-30 cm) at age 8.3 years and aboveground biomass in mixtures and monocultures of *E. globulus* and *A. mearnsii* measured at age 10.7 years. Black points (and line) are soil C without the O horizon, and grey dots are soil C including C in the O horizon.

Figure 4. Density fractions of particulate organic matter (<2 mm) at 0-5 cm depth (a) and N concentrations of that POM (b) at age 10.1 years. Means for a given density fraction sharing the same letter are not significantly different at $P < 0.05$ (REML). $n = 4$ for each bar, except 100A where $n = 3$.

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