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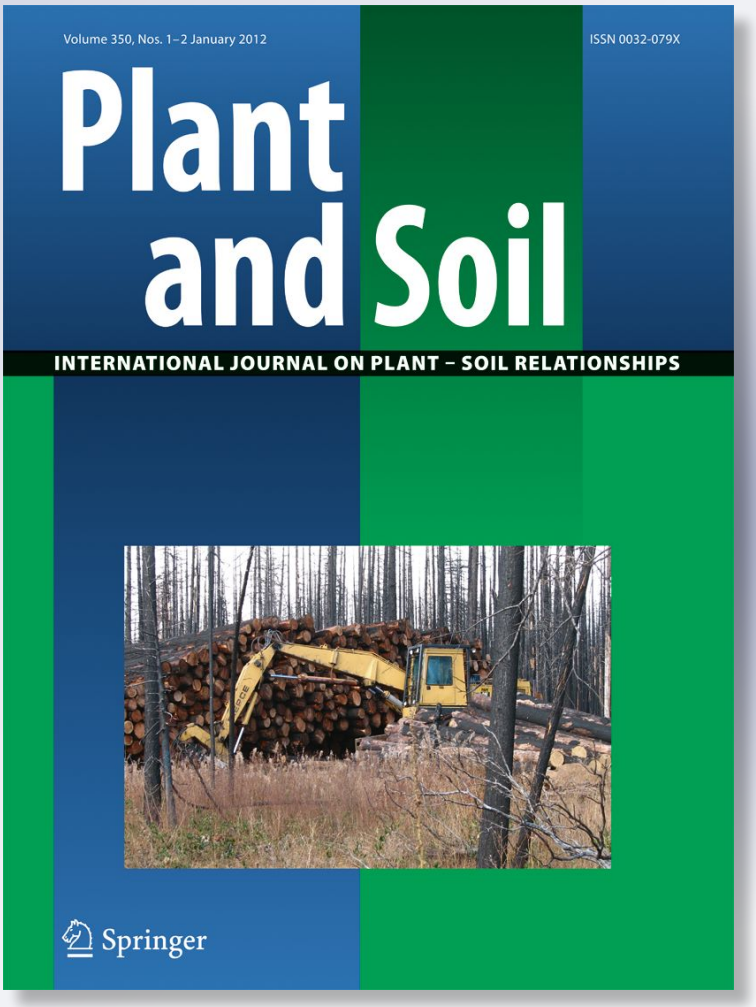

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REGULAR ARTICLE

Introducing *Acacia mangium* trees in *Eucalyptus grandis* plantations: consequences for soil organic matter stocks and nitrogen mineralization

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Abstract
Background and aims Eucalyptus plantations cover 20 million hectares on highly weathered soils. Large amounts of nitrogen (N) exported during harvesting lead to concerns about their sustainability. Our goal was to assess the potential of introducing *A. mangium* trees in highly productive *Eucalyptus* plantations to enhance soil organic matter stocks and N availability.
Methods A randomized block design was set up in a Brazilian Ferralsol soil to assess the effects of mono-specific *Eucalyptus grandis* (100E) and *Acacia mangium* (100A) stands and mixed plantations (50A:50E) on soil organic matter stocks and net N mineralization.
Results A 6-year rotation of mono-specific *A. mangium* plantations led to carbon (C) and N stocks in the forest floor that were 44% lower and 86% higher than in pure *E. grandis* stands, respectively. Carbon and N stocks were not significantly different between the three treatments in the 0–15 cm soil layer. Field incubations conducted every 4 weeks for the two last years of the rotation estimated net soil N mineralization in 100A and 100E at 124 and 64 kg ha^{-1} yr^{-1}, respectively. Nitrogen inputs to soil with litterfall were of the same order as net N mineralization.
Conclusions Acacia mangium trees largely increased the turnover rate of N in the topsoil. Introducing *A. mangium* trees might improve mineral N availability in soils where commercial *Eucalyptus* plantations have been managed for a long time.

Keywords Carbon · Brazil · N_2 fixation · Eucalypt · Acacia · Plantation · Forest · Soil · Fertility · Ecological intensification

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Introduction

Plantation forests are meeting an increasing proportion of global wood demand, with an expansion of their area at an annual rate of 2.0–2.5 million ha over recent decades (FAO 2006). Most of this expansion has been based on exotic species managed in short rotations. *Eucalyptus* is the hardwood genus most planted in tropical regions and covers about 20 million hectares (Iglesias-Trabado and Wilstermann 2008), of which 4.5 million are in Brazil (ABRAF 2011). The majority of tropical *Eucalyptus* plantations are established on nutrient-poor highly weathered soils. Large amounts of nitrogen exported during harvesting every 6–7 years lead to concerns about their economic sustainability.

Harvesting methods and fertilization regimes strongly influence yields in commercial *Eucalyptus* plantations under a large range of tropical soils and climates. While the greatest growth response is usually observed for potassium and phosphorus fertilizer application (Gonçalves et al. 2008; Laclau et al. 2009; du Toit et al. 2010), tree growth is generally more dependent on nitrogen (N) addition in Australia (Smethurst et al. 2004). Nutrient budgets established over a full rotation, as well as modelling approaches, have shown that current silvicultural practices lead to higher N outputs than N inputs in most commercial plantations (Corbeels et al. 2005; Laclau et al. 2005). However, the amounts of N in the soil inherited from the previous vegetation are sufficient to supply tree requirements over the first rotations after afforestation in many tropical *Eucalyptus* plantations (Gonçalves et al. 2008). At present, the need for increasing N fertilizer applications to maintain plantation yields only appears to be necessary in soils that are poor in organic matter (Gonçalves et al. 2004; Smethurst et al. 2004).

Introducing fast-growing legume trees in *Eucalyptus* plantations might be an attractive option for sustaining high yields, combining ecological processes of facilitation between N_2-fixing tree species (NFT) and non-N_2-fixing tree species (non-NFT) with large N inputs resulting from biological fixation of atmospheric N_2 (Binkley et al. 2003). Multi-species forests with annual or perennial N_2-fixing species may lead to greater productivity than monocultures (Binkley et al. 2003; Forrester et al. 2006). However, some studies have shown no impact, or even a negative effect of mixtures on overall stand production (Forrester et al. 2006; Firn et al. 2007). Even when mixed-species plantations that include an NFT component do not increase total biomass production, they are likely to provide other benefits, such as increased organic matter storage and mineral N availability in the upper soil layers, reduced risk of pest damage, and an increased range of products (Resh et al. 2002; Forrester et al. 2004; Garay et al. 2004; Siddique et al. 2008).

Acacia mangium Willd is an NFT species native to Australia and Papua New Guinea. This fast-growing tree species has been largely used to recover degraded tropical lands (Macedo et al. 2008; Wang et al. 2010), and has been planted on about 2 million ha in Southeast Asia, mainly for pulpwood production (Yamashita and Hardjono 2008). Mixed-species plantations combining *Eucalyptus grandis* W. Hill ex Maiden and *Acacia mangium* could be planted on a large scale to supply pulpwood and firewood in the tropics if a clear benefit in soil fertility and/or biomass production relative to mono-specific plantations could be demonstrated. Most studies comparing soil properties and nitrogen mineralization under NFT and non-NFT have been carried out after the afforestation of land covered by C_4 plants. However, consequences of planting NFT on soils that have sustained tropical non-NFT for several decades have been little documented. We put forward the hypothesis that introducing NFT in highly weathered soil managed for several decades in short rotation *Eucalyptus* plantations will increase soil carbon (C) and N stocks as well as mineral N production. The objective of our study was to assess the potential of *A. mangium* plantations to enhance soil organic matter stocks and nitrogen availability in a Brazilian Ferralsol soil representative of large areas of highly productive *Eucalyptus* plantations.

Material and methods

Study site

The study was carried out at the Itatinga Experimental Station (University of São Paulo) located at latitude 23°02′S and longitude 48°38′W. The mean annual rainfall over the study period (from February 2007 to January 2009) was 1,262 mm with a cold season from

June to September (Fig. 1). It was slightly lower than the long term average rainfall of 1,390 mm (from 1990 to 2010). The average annual temperature was 19°C with minimum temperature values below 5°C for a few days each year.

The experiment was located on the top of a hill (slope <3%) at 860 m above sea level. The soils are Ferralsols according to the FAO classification, developed on Cretaceous sandstone, Marília formation, Bauru group. Textural uniformity was high below a depth of 1 m (clay content around 13% in the A_1 horizon and ranging from 20% to 25% between 1 m and 6 m depth). The effective cation exchange capacity ranged from 2 to 20 $mmol_c\ kg^{-1}$ in the upper 3 m of soil and the amounts of exchangeable 'bases' were <2 $mmol_c\ kg^{-1}$ beyond a depth of 5 cm (Table 1).

The experiment was set up in a former *Eucalyptus saligna* Sm. plantation managed as a coppice without fertilizer application from 1940 to 1998. The stumps were devitalized by glyphosate application and *E. grandis* seedlings were planted in 1998 with low fertilizer inputs (300 kg ha^{-1} NPK 10:20:10). High levels of nutrient exports with the boles and the lack of fertilization from 1940 to 1998 made this a suitable area for expecting a response of *Eucalyptus* trees to N inputs.

Experimental layout

Only the boles of the *E. grandis* stand were harvested in December 2002 with harvest residues spread uniformly across the site. A complete randomized block design was established in May 2003 with seven treatments and four blocks in order to assess the influence of *A. mangium* trees on the growth of *Eucalyptus grandis* seedlings (mono progeny from the Suzano Bahia Sul Company). Each plot had an area of 30 m×30 m and an inner plot of 18 m× 18 m with two buffer rows. A complete description of the experimental layout can be found in Laclau et al. (2008). Our study was carried out in three of the original seven treatments planted at a stand density of 1,111 trees ha^{-1} (3×3 m spacing) without N fertilization:

- 100A, mono-specific *A. mangium* stand;
- 100E, mono-specific *E. grandis* stand;
- 50A:50E, mixture in a proportion of 1:1 between *E. grandis* and *A. mangium* (555 trees per hectare of each species).

Seedlings were planted between the rows of the previous plantation after soil cultivation with a ripping tyne to 40 cm depth. *Acacia mangium* seedlings were inoculated with *Rhizobium* strains selected by EMBRAPA for their N_2 fixation capacities and they exhibited high levels of nodulation in the nursery. In the 50A:50E treatment, the two species were planted alternately in the row, and between adjacent rows (Fig. 2). Two tons per hectare of dolomitic limestone was applied at planting and 40 g P $plant^{-1}$ was buried 20 cm from the plants, as

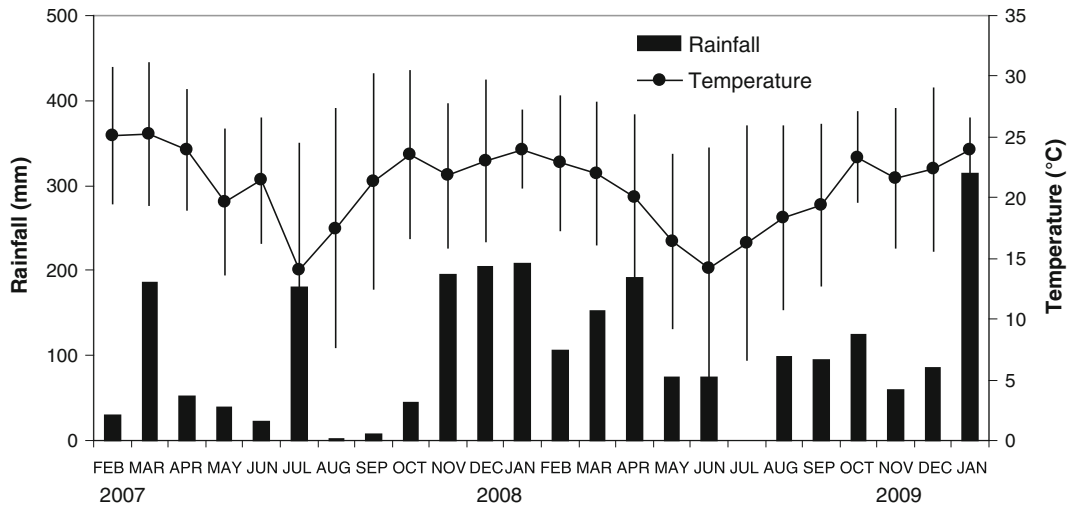

Fig. 1 Monthly rainfall and mean air temperature at the study site over the study period. Ranges of monthly temperature are indicated as *error bars*

Table 1 Main soil characteristics in the experiment

Soil layer (cm)	Particle size distribution (%)			pH (H_2O)	Total C	Total N	P-resin	Exchangeable cations ($cmol_c\ kg^{-1}$)					
	Clay	Silt	Sand		$g\ kg^{-1}$			Ca	Mg	K	Na	Al	CEC
0–5	11.4 (0.7)	4.1 (0.7)	84.5 (0.9)	5.5 (0.2)	17.6 (3.8)	0.9 (0.2)	4.0 (0.4)	0.464 (0.096)	0.424 (0.035)	0.017 (0.004)	0.015 (0.011)	0.581 (0.133)	1.758 (0.274)
5–15	12.8 (0.7)	3.1 (0.3)	84.1 (0.7)	5.5 (0.1)	6.4 (0.7)	0.3 (0.2)	2.5 (0.2)	0.018 (0.002)	0.063 (0.003)	0.006 (0.001)	0.005 (0.005)	0.643 (0.018)	0.947 (0.023)
15–50	14 (0.7)	3.2 (0.4)	83.7 (0.4)	5.4 (0.1)	5.0 (0.4)	0.4 (0.0)	1.9 (0.2)	0.003 (0.001)	0.018 (0.003)	0.005 (0.002)	0.003 (0.002)	0.554 (0.021)	0.751 (0.025)
50–100	16.1 (0.0)	3.4 (0.2)	80.5 (0.2)	5.8 (0.3)	3.5 (0.1)	0.2 (0.0)	1.3 (0.2)	0.002 (0.001)	0.005 (0.000)	0.001 (0.000)	0.000 (0.000)	0.405 (0.017)	0.584 (0.012)
100–200	18.1 (0.0)	4.1 (0.2)	77.8 (0.2)	5.9 (0.0)	2.6 (0.1)	0.2 (0.0)	1.2 (0.0)	0.002 (0.001)	0.008 (0.001)	0.002 (0.000)	0.001 (0.001)	0.299 (0.030)	0.436 (0.029)
200–300	16.1 (0.0)	4.4 (0.8)	79.5 (0.8)	5.7 (0.1)	2.1 (0.1)	0.1 (0.0)	2.0 (0.2)	0.003 (0.003)	0.006 (0.001)	0.000 (0.000)	0.002 (0.001)	0.136 (0.011)	0.211 (0.037)

Standard errors are given in brackets ($n=3$)

N.B. Soils were sampled down to a depth of 3 m, 3 years after treatment establishment, in one pit in each treatment (100A, 100E and 50A:50E) in block 1

well as 9 g $plant^{-1}$ K, 3 g $plant^{-1}$ B, 6 g $plant^{-1}$ Fe, 3 g $plant^{-1}$ Zn, and 1 g $plant^{-1}$ Mn. Litter leachates collected weekly over 2 years in treatments 100A and 100E were acidic (unpublished data) and limestone application 6 years before soil sampling was unlikely to largely increase soil C stocks. Treatment comparisons were not affected by liming soil since the same amount of lime was added in all the treatments. Fertilizer was applied at a rate of 25 kg ha^{-1} K in all treatments, at 6, 12 and 18 months after planting. Another treatment with a total application of 120 kg ha^{-1} of N (ammonitrate fertilizer) in monospecific *E. grandis* stands in this experiment showed that N fertilization significantly enhanced stem biomass in the first 2 years after planting, but tree height was no longer significantly affected by N fertilizer addition at age 3 years (Laclau et al. 2008).

Soil temperature at 8 cm depth, as well as soil moisture between 0 and 6 cm, were monitored every 30 min over the study period in one plot (50A:50E in block 1). We used four replicates of home-made thermocouples and 6 ECH2O-10 soil moisture sensors (Decagon Devices, Inc., Pullman WA, USA) inserted into the soil at an inclination of about 65°, and connected to a CR1000 datalogger (Campbell Scientific, Shepshed, England, UK). The replicates were set up to sample different distances from the trees. Complementary measurements of soil water content using 3 TDR probes (Trase Soilmoisture, Santa Barbara, CA, USA) spaced out evenly in the plots at a depth of 15 cm showed that soil water content dynamics in the topsoil can be considered similar in the three treatments over the study period (data not shown).

Litterfall

Litterfall was collected every 28 days over the study period. Leaf and fruit litterfall were collected in five traps (52 cm×52 cm) per plot installed at various distances from the trees in the 100A and 100E treatment and in ten traps per plot in the 50A:50E treatment (replicated in three blocks). Dead branches and bark were collected in an area of 9 m^2 delimited between four trees in each plot (replicated in three blocks for the three treatments). Litter was separated by species and component of litter, and then dried at 65°C for 72 h before weighing. The replicates in the three blocks were

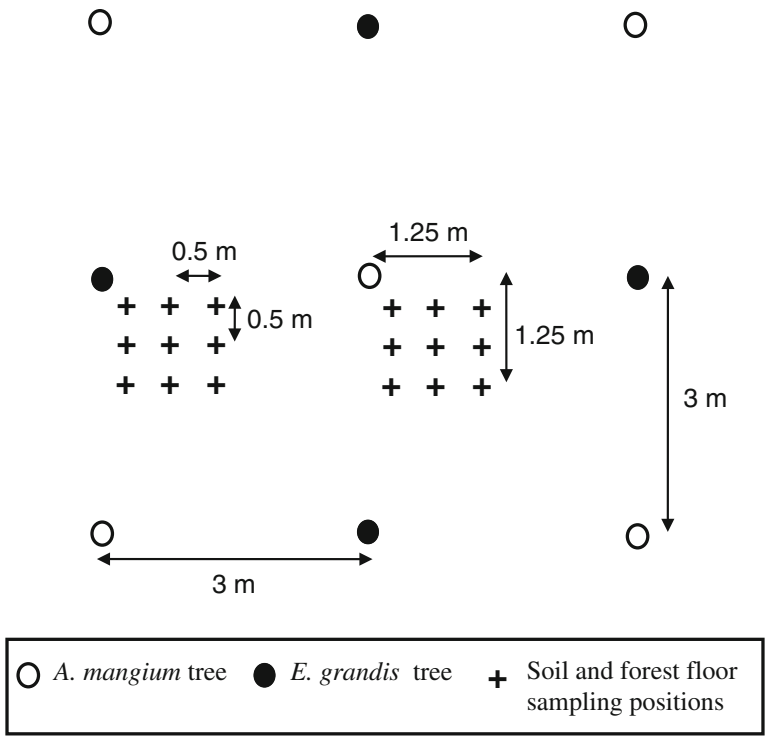

Fig. 2 Sampling positions in the 50A:50E treatment. In each plot, 9 positions were sampled close to 9 different *A. mangium* trees (*empty circles*) and 9 positions were sampled close to 9 different *E. grandis* trees (*full circles*). The same design was used in treatments 100A and 100E with 9 positions per plot (distributed close to 9 different trees)

mixed into one sample of each component of litterfall for each 28-day period and ground for chemical analysis.

Nitrogen mineralization

Nitrogen mineralization in the 0–20 cm soil layer was studied over the study period in the 100A and 100E treatments using the in situ coring technique (Raison et al. 1987). Soil incubations were conducted in eight plots (two treatments × four blocks). At the onset of each sampling period, three pairs of cores (70 mm in diameter) were driven 20 cm into the soil with a hammer. The pairs of soil cores were located 35 cm, 105 cm, and 175 cm from the nearest tree for a representative sample of the interrow. One soil core from each pair was withdrawn immediately. In each of the eight plots, three soil cores (covered with plastic caps to eliminate mineral N leaching) were incubated for 4 weeks under field conditions. Samples were transported in cooled insulated containers and extractions were initiated on the same day for one composite sample in each plot.

Net ammonification and nitrification were estimated by the difference between post- and pre-incubation concentrations of NH_4^+-N and NO_3^--N, respectively. Negative values of monthly net nitrogen mineralization were rare over the study period. They were set to 0 before summing across months to get an annual total. Before extraction, soil samples were homogenized manually and roots were removed (no gravel in this soil). A subsample was collected for determining the water content (at 105°C). Mineral N was extracted by shaking 10 g of soil with 50 ml of 2 M KCl and extracts were analysed for NH_4^+-N and NO_3^--N by an automated flow injection system (Ruzicka and Hansen 1975). Net N mineralization was obtained for each sampling period by summing net ammonification and net nitrification.

Forest floor and mineral soil

The forest floor and the upper soil layers were sampled in three blocks at the end of the study period (March 2009). Nine locations in each plot were sampled for treatments 100A and 100E and 18 positions were sampled in each plot for the 50A:50E treatment. Each position was located close to a different tree in each plot (Fig. 2). The forest floor

Table 2 Mean concentrations of total C and N, $\delta^{15}N$ values and C:N ratios in the components of the forest floor and in the upper soil layers 6 years after planting

	100A	50A:50E	100E
Total C (g kg^{-1})			
Oi	521±2 b	487±3 a	485±3 a
Oe	505±5 a	498±8 a	499±3 a
Oa	474±9 a	455±4 a	455±7 a
0–5 cm	15±4 a	14±1 a	16±1 a
5–15 cm	8±1 a	9±1 a	8±1 a
Total N (g kg^{-1})			
Oi	14±0 b	3±0 a	2±0 a
Oe	18±1 c	10±1 b	7±1 a
Oa	21±0 b	13±1 a	12±1 a
0–5 cm	0.7±0 a	0.7±0 a	0.8±0 a
5–15 cm	0.5±0 a	0.5±0 a	0.4±0 a
$\delta^{15}N$ (‰)			
Oi	0.5±0.2 a	−1.3±0.8 a	−0.5±0.7 a
Oe	0.9±0.2 a	1.0±0.3 a	1.0±0.2 a
Oa	1.5±0.5 a	1.8±0.2 a	1.4±0.2 a
0–5 cm	4.4±0.3 a	4.8±0.1 a	4.5±0.3 a
5–15 cm	6.7±0.3 a	6.8±0.1 a	7.1±0.1 a
C/N			
Oi	37.1±0.9 c	171.4±12.2 b	219.4±33.8 a
Oe	27.5±1.2 c	51.7±4.3 b	75.4±6.2 a
Oa	23.1±0.4 c	34.4±2.5 b	38.6±3.7 a
0–5 cm	18.9±1.1 c	19.7±0.4 b	21.0±1.2 a
5–15 cm	16.3±0.6 b	17.7±0.3 a	17.5±0.3 a

Standard errors between blocks are indicated (n=3). Different letters indicate significant differences between treatments (P<0.05)

was sampled with a 15 cm radius circular frame at each position and divided into three components: Oi (non-fragmented material), Oe (coarse fragments), and Oa (finely fragmented material). The nine samples per component collected in each plot were manually homogenized and one composite sample per plot in mono-specific stands (two samples per plot in 50A:50E, one sample close to each tree species) was ground to pass through a 2 mm mesh stainless steel screen. The ash content of the forest floor samples was determined by combustion for 4 h at 450°C. Values for the forest floor samples were then corrected to eliminate the effect of remaining soil particles.

Mineral soil samples were collected using 5- and 10 cm-long metal cylinders (5 cm in diameter) and were inserted into the upper 0–5 cm and 5–15 cm soil layers after collection of the forest floor (at the same positions). All soil samples were air-dried, weighed and the water content was determined from a subsample (dried at 105°C). Bulk density for the 216 samples collected was calculated for each soil layer, as the ratio between oven-dried soil mass and volume of soil core. All soil samples were air-dried, the roots were removed and the samples were passed through a 2-mm sieve. Analyses for $\delta^{15}N$, C and N were undertaken on 9–11 and 5–7 samples per plot collected in the 0–5 cm and 5–15 cm layer, respectively (for a total of 138 soil samples analysed in the experiment for the two layers).

Isotope and elemental analyses

Carbon and nitrogen concentrations in litter and soil samples were determined by continuous flow isotope ratio mass spectrometry, using a Thermo Delta Plus mass spectrometer (Bremem, Germany) coupled to a Carlo Erba CHN 1110 elemental analyser, (Milan, Italy). In brief, organic matter was converted to gases by full dry combustion, generating N_2 and CO_2, which were then purified in the elemental analyser through chromatographic separation in an ultrapure helium carrier and sequentially admitted to the mass spectrometer by means of an interface (Thermo, Conflo II). The ^{15}N:^{14}N isotope ratios were evaluated after separation of molecules according to isotope mass, and finally compared to the calibrated gas ratios using atmospheric N_2 as a reference. $\delta^{15}N$ values in plant components are usually lower for N_2-fixing species than for non-N_2-fixing species but great variability has been reported for the same species depending on the characteristics of soil organic matter (Boddey et al. 2000).

Data analyses

Differences between treatments and blocks in C and N concentrations in each soil layer were tested with SAS 9.1 using two-way ANOVA. Soil bulk density was similar in the three treatments (means ± standard errors between the three blocks were 1.39±0.02, 1.41±0.02 and 1.43±0.01 in the 0–

15 cm soil layer for treatments 100A, 100E and 50A:50E, respectively). Therefore, C and N stocks in each soil layer were not corrected for differences in bulk density (Maquère et al. 2008). Homogeneity of variances was tested by Levene's test and original values were log-transformed when variances were unequal. The probability level used to determine significance was $P<0.05$. When significant differences between treatment levels were detected, the Student-Newman-Keuls multiple range test was used to compare treatment means. Pearson correlation coefficients between N concentrations in the 0–5 and 5–15 cm soil layers and the distance from the nearest trunk of each species were calculated for each treatment.

Results

Carbon and nitrogen concentrations and stocks

While C concentrations were similar for the three treatments in all the layers (except in Oi), N concentrations were significantly higher in the three components of the forest floor in 100A than in 100E (Table 2). Nitrogen concentrations in the forest floor of treatment 50A:50E were intermediate, and only significantly higher than in 100E for the Oe layer. Carbon and N concentrations in the 0–5 and 5–15 cm soil layers were not significantly correlated with the distance from the nearest *Acacia* or *Eucalyptus* tree, regardless of treatment.

The C:N ratios within each soil layer were more sensitive than $\delta^{15}N$ values for detecting qualitative changes in soil organic matter 6 years after treatment establishment (Table 2). $\delta^{15}N$ values in the forest floor and the upper soil layers did not differ significantly between treatments. In contrast, C:N ratios were significantly lower in 100A than in 100E in all the layers sampled down to a depth of 15 cm. They were intermediate in the 50A:50E treatment and significantly different from values in the mono-specific stands down to a depth of 5 cm.

Carbon and N stocks in the soil down to a depth of 15 cm (including the forest floor) were 36% lower and 44% higher in 100A than in 100E, respectively (Table 3). These large differences were mainly a result of low C contents in the Oi layer and large N contents in the Oe layer for the 100A treatment in comparison with the 100E treatment. Carbon stocks in 50A:50E down to a soil depth of 15 cm were intermediate between those in 100A and 100E. Soil N stocks down to a depth of 15 cm in 50A:50E and 100E were similar, and significantly lower than in 100A. Carbon and N stocks were not significantly different between treatments in the Oa, 0–5 cm and 5–15 cm layers.

Table 3 Carbon and N stocks in the forest floor and in the upper soil layers 6 years after planting in the 100A, 100E and 50A:50E treatments

	100A	50A:50E	100E
Carbon (kg ha^{-1})			
Oi	445±35 a	3065±243 b	4376±303 c
Oe	3412±186 a	3762±177 a	3334±115 a
Oa	1269±192 a	974±115 a	1419±138 a
0–5 cm	870±162 a	875±36 a	1004±82 a
5–15 cm	1257±110 a	1409±132 a	1182±70 a
Forest floor	5126±188 a	7712±233 b	9129±448 c
0–15 cm	2127±271 a	2284±163 a	2187±127 a
TOTAL	7252±404 a	9996±393 b	11315±363 c
Nitrogen (kg ha^{-1})			
Oi	12±1 a	18±3 a	21±4 a
Oe	125±11 b	71±21 a	45±5 a
Oa	55±3 b	28±2 a	38±6 ab
0–5 cm	45±6 a	45±1 a	48±4 a
5–15 cm	77±4 a	80±6 a	67±3 a
Forest floor	192±12 b	96±23 a	104±11 a
0–15 cm	121±10 a	125±7 a	115±7 a
TOTAL	313±18 b	220±30 a	219±4 a

Standard errors between blocks are indicated ($n=3$). Different letters indicate significant differences between treatments ($P<0.05$)

Nitrogen mineralization

Mineral N pools in the non-incubated soils sampled within the 0–20 cm soil layer ranged from 1 to 14 kg ha^{-1} in 100A and <1 to 7 kg ha^{-1} in 100E over the study period (Fig. 3). Inter-month variability was high throughout the 2 years of monitoring (coefficients of variation of about 50% in the two treatments). The lowest amounts of mineral N in the 0–20 cm soil layer were found during the cold and dry seasons, and the highest amounts were found during the rainy and hot season. Mean mineral N pools in non-incubated soils over

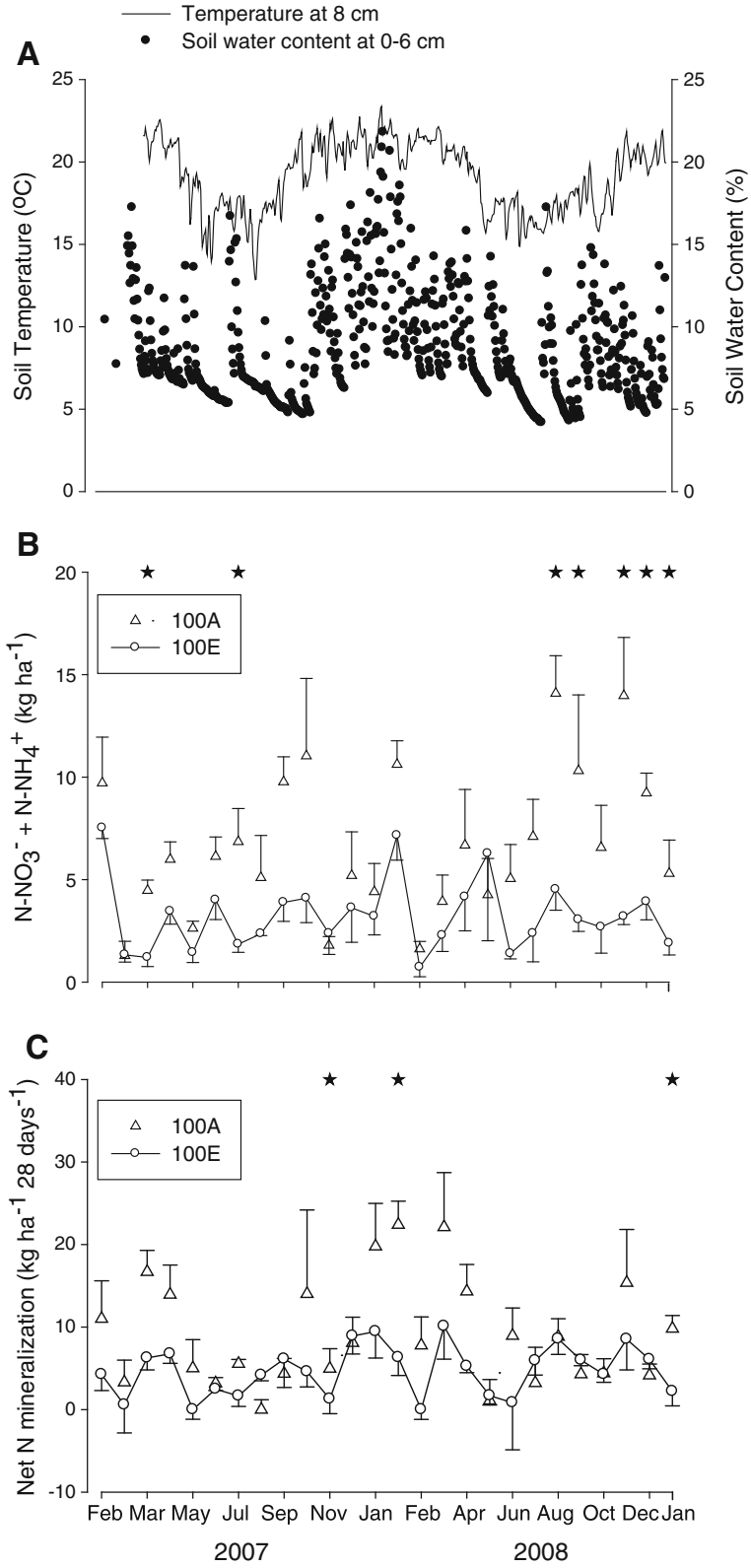

Fig. 3 Dynamics of volumetric soil water contents in the 0–6 cm layer and soil temperature at the depth of 8 cm in the 50A:50E treatment over the study period (**a**), amounts of mineral nitrogen in the 0–20 cm soil layer at each sampling date (**b**), and net nitrogen mineralization in the upper 20 cm of soil over the study period (**c**). Standard errors are indicated by *vertical bars* (n=4) and * indicate significant differences between treatments (P<0.05)

the study period were about twice as high in 100A as in 100E (6.6 vs 3.0 kg ha^{-1}, respectively).

Mean net N mineralization was significantly different between the two, pure species treatments, and amounted to 64 kg ha^{-1} yr^{-1} in 100E and 124 kg ha^{-1} yr^{-1} in 100A over the study period (Fig. 4b). Most of the low N mineralization rates were observed during periods with low soil temperatures (<18°C), but they also occurred when the soil water content was low (<8%), irrespective of soil temperature (Fig. 3a,c). Differences between the pure species treatments were only significant for three incubation periods due to large inter-block variability. Mean net N mineralization rates during the 28 days of in situ incubation averaged 14 and 6 kg ha^{-1} during the hot season (from November to April) and 7 and 4 kg ha^{-1} during the other part of the year in 100A and 100E respectively. Net nitrification comprised about 60% of total net N mineralization in 100A and 100E (Fig. 5).

Litterfall

Cumulative litterfall dry matter from 4 to 6 years after planting was 27% and 13% lower in 100A and 50A:50E than in 100E, respectively, (Fig. 4a). Nitrogen inputs to soil with litterfall were about twice as high in 100A as in 100E (Fig. 4b). They amounted on average to 98, 63 and 49 kg ha^{-1} yr^{-1} in 100A, 50A:50E and 100E respectively. While *A. mangium* components comprised only 14% of the litterfall dry matter in 50A:50E, they amounted to 38% of N contents in the litterfall for this treatment. The amount of N returning to the soil with litterfall

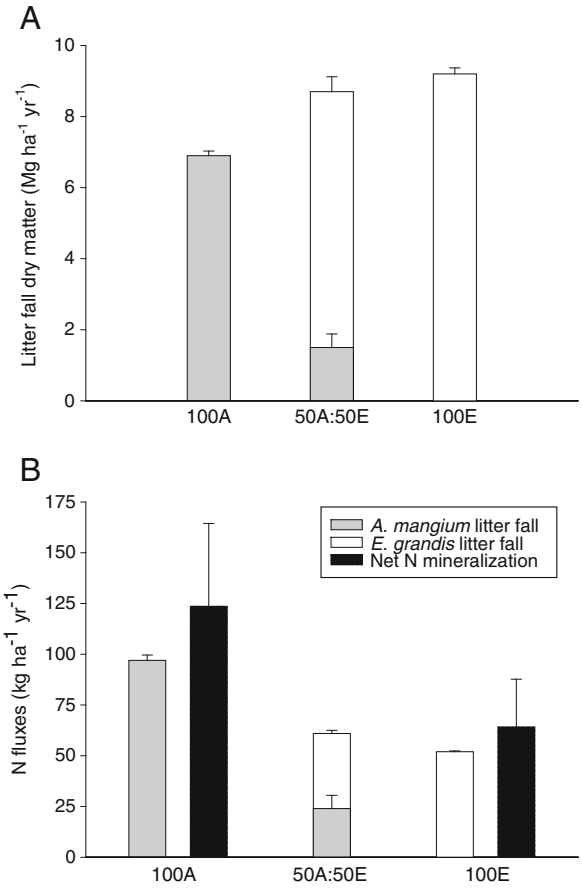

Fig. 4 Mean annual litter fall dry matter (**a**) and nitrogen fluxes (**b**) in the 100A, 50A:50E and 100E treatments. Standard errors between the blocks are indicated by *vertical bars* (n=3 for litterfall and n=4 for net N mineralization)

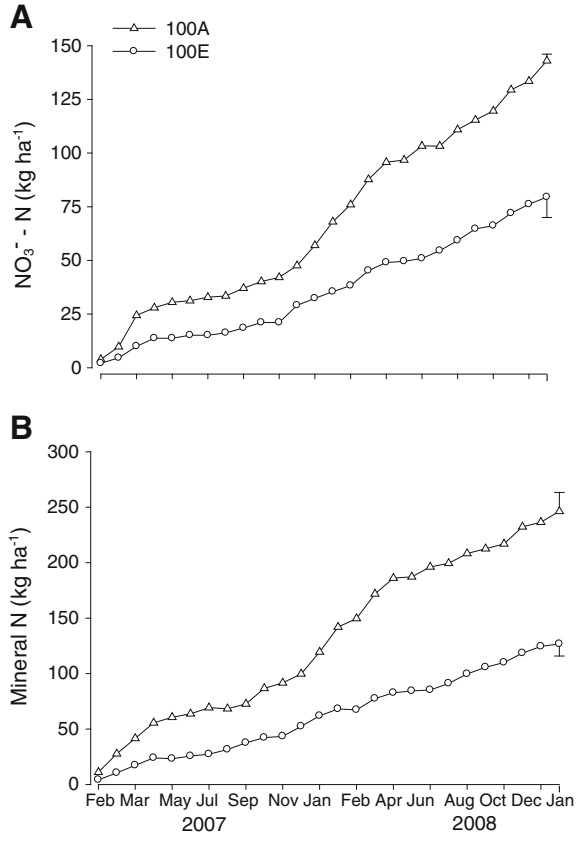

Fig. 5 Cumulative net production of NO_3^--N (**a**), and total mineral N (**b**) over 2 years, in the 100A and 100E treatments. Standard errors between the blocks at the end of the study period are indicated by *vertical bars* (n=4)

was of the same order of magnitude as net N mineralization in each treatment.

Discussion

Soil carbon and nitrogen stocks

Although the potential of NFT to enhance soil C contents has been reported over a broad geographical area (Kaye et al. 2000; Resh et al. 2002; Garay et al. 2004; Mitchell and Ruess 2009), the amount of soil C in the soil at 0–15 cm depth was not significantly different between the three treatments in our experiment. Soil C stocks after 6 years were significantly lower under *A. mangium* than under *E. grandis* trees when organic forest floor layers and the 0–15 cm layer were included in the analysis. Large amounts of dead branches fell from *Eucalyptus* trees from age 3 years and led to a large increase in C content within the Oi component of the forest floor (data not shown). An increase in soil C stocks in the 0–16 cm soil layer under NFT relative to *Eucalyptus* plantations was reported within 7 years after planting *A. mangium* trees on an Ultisol soil in Brazil (Garay et al. 2004), as well as in the 0–40 cm soil layer under *Casuarina* and *Leucaena* species on a Typic Troposamments soil in Puerto Rico (Resh et al. 2002). However, differences in the 0–40 cm layer were not significant at the latter site. Most of the studies showing a significant increment in soil C stocks under NFT in tropical soils were carried out >10 years after afforestation, in areas cultivated previously with grasses (Kaye et al. 2000; Resh et al. 2002; Macedo et al. 2008). Although $\delta^{15}N$ values in the forest floor 6 years after planting were not significantly different in 100A and in 100E in our experiment, the lack of C accumulation under *A. mangium* trees when compared with *E. grandis* stands was unlikely to result from low inputs of N_2 by biological fixation. Nitrogen accumulation in the standing biomass was about 50% higher at age 30 months in 100A than in 100E and the application of labelled ^{15}N fertilizer showed that the percentage of N derived from atmospheric fixation was about 60% at 30 months of age for *A. mangium* trees planted in a mixture with *E. grandis* trees (Bouillet et al. 2008).

Soil N stocks are generally higher under NFT than under adjacent non-NFT (Resh et al. 2002; Garay et al. 2004; Forrester et al. 2005; Macedo et al. 2008; Inagaki et al. 2010). Our results were consistent with this overall trend, with an amount of soil N within organic forest floor layers that was 85% higher in 100A than in 100E. However, the minimal detectable difference (based on the variances among the replicate plots) needed to show N enrichment in the 0–15 cm soil layer was 43 kg ha^{-1} and a 6-year rotation might have been too short to significantly modify N pools in the mineral soil. Long-term laboratory aerobic incubations showed that *Eucalyptus globulus* Labill. leaf litter addition had different effects on microbial activity and organic matter mineralization rates in soils from pasture and forest (Aggangan et al. 1999). *Eucalyptus* leaf litter is rich in lignin, tannins and polyphenols (Chapuis-Lardy et al. 2002) and the effects of N inputs on organic matter decomposition may depend on substrate lignin content (Hobbie 2000). Our results suggest that the impact of NFT on C and N stocks in soils that have been cultivated for several decades with *Eucalyptus* plantations might be different from the common pattern reported for afforestation in soils with a large proportion of organic matter produced by grasses.

Nitrogen cycling

Planting a mono-specific *A. mangium* stand in an area where *Eucalyptus* plantations have been managed since 1940 doubled N mineralization rates in the topsoil despite non-significant changes in N stocks in the 0–15 cm soil layer. This suggests a much faster turnover of N in the forest floor and topsoil under *A. mangium* than under *E. grandis*. Rates of litter decomposition and N release were higher under NFT than under *Eucalyptus* stands in studies carried out in the Congo, Puerto Rico and Australia (Bernhard-Reversat 1996; Parrotta 1999; Forrester et al. 2005). In our experiment, significantly lower C:N ratios in the topsoil under *A. mangium* than under *E. grandis* trees indicated that a change in the chemistry of the soil organic matter occurred over the 6 first years after planting. In situ resin incubations have shown a significant enhancement in N availability in 17-year-old plantations of N-fixing woody species in compar-

ison with non-NFT stands in Hawaii (Kaye et al. 2000) and 7 years after planting in southern Brazil (Siddique et al. 2008). A similar pattern was reported for laboratory incubations of Oxisol soils sampled under 13-year-old NFT and non-NFT plantation forests in southern China (Li et al. 2001). Many studies have shown that Raison's method used in this study can be a valuable indicator of soil N mineralization, and is much more sensitive than soil analyses for detecting changes in soil N status between treatments (e.g. Nzila et al. 2002; O'Connell et al. 2003; Jussy et al. 2004). The large seasonal variations in N mineralization rates observed in our study are well documented by others (e.g. O'Connell et al. 2004; Yan et al. 2009). Further attention should be given to effects of new tree species (e.g. *A. mangium*) on microbial communities in soils cultivated over a long period with *Eucalyptus* plantations, and in particular to fungal communities involved in soil C and N cycling.

Nitrogen contents in litterfall that are similar to the net production of mineral N in the upper soil layer suggested that the stocks of N were stable under the two tree species in our study. The rates of N cycling in litterfall in the mono-specific *Eucalyptus* plots (49 kg ha^{-1} yr^{-1}) were within the range reported for other *Eucalyptus* plantations (Binkley et al. 1992; Parrotta 1999; Laclau et al. 2010). Litterfall N amounted to 97 kg ha^{-1} yr^{-1} in the *A. mangium* plots in our study and was twice as much as in pure *Acacia mearnsii* de Wildeman plantations in Australia (Forrester et al. 2005), but half that found in mono-specific plantations of *Falcataria moluccana* Miquel in Hawaii (Binkley et al. 1992), *Leucaena leucocephala* Lam. (de Wit) in Puerto Rico (Parrotta 1999) and *A. mangium* in Malaysia (Inagaki et al. 2010). More favourable climatic conditions for *A. mangium* in Malaysia (annual precipitation of 2,572 mm and mean temperature of 27.9°C) than at our study site might account for the differences in litterfall between the two studies. Nitrogen returning to the soil in litterfall is much easier to measure than in situ soil N mineralization. The validity of this indicator of soil N availability in fast-growing tropical plantations would be worth assessing across a large range of soils, under monocultures and mixed-species plantations.

Consequences for forest management

Our findings suggest that one rotation of mono-specific NFT after a certain number of mono-specific eucalypt rotations could help to maintain plantation soil fertility in a way that is compatible with forest company practices. The number of eucalypt rotations between rotations of NFT would depend on the lasting effect of these species on soil properties, which are likely to be affected by the biochemical characteristics of the residues and soil preparation methods (Corbeels et al. 2003).

Other large scale management options to take advantage of facilitation processes between species, could include replacement of a proportion of *Eucalyptus* trees by NFT or introducing an understory of NFT in commercial *Eucalyptus* plantations. However, the success of these mixed-species plantations is largely dependent on the selected species (Forrester et al. 2006). Comprehensive studies dealing with the influence of NFT in ecological processes in fast-growing plantations established on highly weathered soils are scarce (Binkley et al. 2003; Hunt et al. 2006; Richards et al. 2010). More attention should be paid to this issue of major interest to achieve an ecological intensification of tropical plantation forests leading to sustained yields without raising the need for N fertilizer.

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