Research paper: Part of an invited issue on carbon allocation

Do changes in carbon allocation account for the growth response to potassium and sodium applications in tropical *Eucalyptus* plantations?

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Understanding the underlying mechanisms that account for the impact of potassium (K) fertilization and its replacement by sodium (Na) on tree growth is key to improving the management of forest plantations that are expanding over weathered tropical soils with low amounts of exchangeable bases. A complete randomized block design was planted with *Eucalyptus grandis* (W. Hill ex Maiden) to quantify growth, carbon uptake and carbon partitioning using a carbon budget approach. A combination of approaches including the establishment of allometric relationships over the whole rotation and measurements of soil CO_2 efflux and aboveground litterfall at the end of the rotation were used to estimate aboveground net production (ANPP), total belowground carbon flux and gross primary production (GPP). The stable carbon isotope ($\delta^{13}C$) of stem wood α-cellulose produced every year was used as a proxy for stomatal limitation of photosynthesis. Potassium fertilization increased GPP and decreased the fraction of carbon allocated belowground. Aboveground net production was strongly enhanced, and because leaf lifespan increased, leaf biomass was enhanced without any change in leaf production, and wood production (P_W) was dramatically increased. Sodium application decreased the fraction of carbon allocated belowground in a similar way, and enhanced GPP, ANPP and P_W, but to a lesser extent compared with K fertilization. Neither K nor Na affected $\delta^{13}C$ of stem wood α-cellulose, suggesting that water-use efficiency was the same among the treatments and that the inferred increase in leaf photosynthesis was not only related to a higher stomatal conductance. We concluded that the response to K fertilization and Na addition on P_W resulted from drastic changes in carbon allocation.

Keywords: carbon budget, *Eucalyptus grandis*, fertilization, soil CO_2 efflux, stable carbon isotope.

Introduction

Potassium (K) deficiency is known to be a key limiting factor for high crop production in developing tropical countries (Cakmak 2010). Potassium fertilization affects the yield of many crop species (Pettigrew 2008) and stimulates wood production in fast-growing tree plantations like *Populus* (Ache et al. 2010) and *Pinus radiata* (Smethurst et al. 2007) in temperate regions and

Eucalyptus in the tropics (Laclau et al. 2009). The *Eucalyptus* genus includes the fastest-growing species in tropical regions and they are among the most widely planted species in the tropics (FAO 2005). High productivity is attained in Brazil when *Eucalyptus* is grown on soil with sufficient amounts of nutrients and water (Gonçalves et al. 2008). The increase in global wood demand is leading to a rapid expansion of *Eucalyptus* plantations (300,000 ha year^{-1} in Brazil; ABRAF 2009) on weathered tropical soils that are often K deficient. Potassium plays many roles in plant physiology (Marschner 1995, Römheld and Kirkby 2010). Among them, K contributes to the osmotic mechanism that promotes stomata opening and closing, allowing a better diffusion of CO_2 inside the leaf mesophyll and thus favouring photosynthesis (Fischer 1968, Talbott and Zeiger 1996, Roelfsema and Hedrich 2005). Leaf photosynthesis declined when K deficiency developed in cotton (*Gossypium hirsutum*) plants (Bednarz et al. 1998). This was mainly explained by a decrease in stomatal conductance deduced from both leaf gas exchange measurements and foliar stable carbon isotope composition ($\delta^{13}C$). An increase in leaf area per tree (or in leaf area index at the ecosystem scale) also parallels an increase in gross primary production (GPP). This was evidenced in *Eucalyptus* plantations at early growth stages, and related to an increase in leaf lifespan (LLS) (Laclau et al. 2009). In addition, hydraulic conductance, which is essential for sustaining higher leaf stomatal conductance and gas exchanges, increased following short-term K fertilization of young *Laurus nobilis* plants (Oddo et al. 2011). Potassium is also an important solute that contributes to the maintenance of high turgor required for cell elongation in growing organs, especially the cambial zone for wood formation in trees (Wind et al. 2004, Fromm 2010). In addition, K^+ ions affected the rate of phloem loading in *Ricinus* plants, in particular by promoting the efflux of assimilates into the apoplast prior to phloem loading (Mengel and Haeder 1977, Doman and Geiger 1979). Taken together, all these mechanisms (carbon acquisition, assimilate loading in the phloem and carbohydrate requirements of growing sink organs) may affect carbon uptake, transport and allocation, and finally tree growth and wood production.

The relief of nutrient deficiency by fertilization may affect carbon allocation in several ways. Fertilization decreased the relative amount of carbon allocated belowground in *Pinus ponderosa* (Haynes and Gower 1995) and *Eucalyptus* plantations (Giardina et al. 2003). This might be explained by the high carbon cost of efficient nutrient uptake mechanisms in nutrient-poor soils. For instance, nitrogen addition decreased fine root production in *Liquidambar styraciflua* plantations, shifting carbon towards stem growth (Iversen and Norby 2008). Similarly, nitrogen deprivation enhanced the flux of recently assimilated carbon belowground in *Fagus sylvatica* seedlings (Dyckmans and Flessa 2001). Most of the studies to date have concentrated on nitrogen and phosphorus (see a recent review by Litton et al. 2007), while the effect of K or sodium (Na) on

carbon allocation in trees has been poorly investigated, especially in the field. We may however expect a similar shift in carbon allocation pattern when the added fertilizer relieves the main limiting factors for growth.

Fertilization also eliminates the necessity of having an efficient resource conservation strategy, which is often related to a long LLS for species adapted to nutrient-poor soils (Aerts and Chapin III 2000). A shorter LLS implies a higher leaf turnover rate and requires higher allocation to leaf growth, but it does permit higher leaf-area-based photosynthesis owing to the trade-off between LLS and leaf photosynthetic capacity (Wright et al. 2004). However, in a previous study, we showed that K fertilization enhanced LLS in young *Eucalyptus* trees and thus increased the maximum leaf standing biomass (Laclau et al. 2009).

Whereas K is the most abundant inorganic cation in plant tissue, Na is much less abundant. Except in halophytic plants, Na accumulation is often detrimental to tree growth due to both osmotic effects and salt toxicity (Kozlowski 1997). However, *Eucalyptus* may fulfil 15% of their water requirement from saline groundwater in some Australian plantations (Feikema et al. 2010). In addition, a positive effect of Na application on the growth of young *Eucalyptus* trees planted on a K-depleted soil has recently been reported, thus suggesting a putative physiological replacement of K by Na (Almeida et al. 2010). It is important to gain insights into the response of *Eucalyptus* to Na in areas with dry depositions of marine aerosols, and to investigate the possibility of including NaCl in the fertilization regimes of *Eucalyptus* plantations in a context of increasing costs for K fertilizers.

Our aim was to study the effects of K fertilization and Na addition on growth, carbon fluxes and carbon partitioning to above- and belowground in *Eucalyptus* trees at the stand level using a carbon budget approach (Keith et al. 1997). We hypothesized that K fertilization decreases the fraction of carbon allocated belowground and increases carbon uptake in trees both by increasing leaf biomass and by favouring stomatal opening that will, in turn, decrease water-use efficiency. In addition, we aim to confirm over the whole rotation that K fertilization enhanced LLS and that Na can partly replace K in K-deficient soils.

Materials and methods

Study site and experimental design

Seedlings of *Eucalyptus grandis* (W. Hill ex Maiden) were planted in April 2004 on a site afforested with *Eucalyptus* for >60 years at the Itatinga experimental station (23°02′S and 48°38′W, 850 m elevation). The soil is a deep Ferrasol lying on a Cretaceous sandstone (Laclau et al. 2009). At the Itatinga experimental station, mean annual air temperature and annual rainfall over the last 15 years (1996–2010) were 19 °C and

Table 1. Average annual air temperature (T_{mean}), maximum daily air temperature of the hottest month (T_{max}), minimum daily air temperature of the coldest month (T_{min}) and cumulative rainfall (P) for each 1-year period beginning from the first day of the month of planting (May 2004) and ending on 30 April 2011.

Age (months)	T_{mean} (°C)	T_{max} (°C)	T_{min} (°C)	P (mm)
0–12	19.1	37.6	1.6	1274
12–24	20.2	36.6	0.0	1503
24–36	19.6	35.5	0.0	1191
36–48	20.2	35.5	2.0	1352
48–60	23.9	34.9	1.0	1294
60–72	19.6	34.1	2.0	2105
72–84	19.3	33.1	2.4	1364

1360 mm, respectively, with a marked dry and cool season between June and September and substantial inter-annual variability (Table 1). The cumulative rainfall measured between 1 May 2009 and 30 April 2010 (when the stand was between 60 and 72 months old) was 2106 mm, while it had only been 1191 mm between 1 May 2006 and 30 April 2007 (stand age between 24 and 36 months).

A complete randomized block design was set up with seven treatments (81 trees per plot) and four blocks in order to assess the effects of K and Na additions on tree development (Almeida et al. 2010). Our study was carried out in three of the seven treatments and three of the four blocks. The fourth block was used for successive destructive sampling. The three treatments included a control treatment without K addition (C), a K fertilization treatment where 0.45 mol m^{-2} of KCl fertilizer was applied (K) and a Na addition treatment where 0.45 mol m^{-2} of NaCl was applied instead of KCl (Na).

All three treatments also received 0.86 mol m^{-2} of N (ammonium sulphate application split to match the periods of high tree demand), 0.11 mol m^{-2} of P and 0.2 g m^{-2} of dolomitic lime and micronutrients (applied once at planting). A quarter of the total amounts of K and Na was applied at planting and then at 6, 12 and 60 months of age. A complete analysis of all the fertilizers showed that total amounts of K and Na applied in all the plots with the basic fertilization were 4.2 and 3.1 kg ha^{-1}, respectively. The Na content in the KCl fertilizer was 1.0% and the K content in the NaCl fertilizer was 0.05% (< 0.15 kg K ha^{-1} contained in the NaCl fertilizer applied in treatment +Na).

Tree census and biomass measurements

Tree height (H, m) and circumference at breast height (C, cm) were measured every 6 months on 25 inner trees per plot (excluding two buffer rows). Aboveground biomass, i.e., stem wood (B_{SW}), stem bark (B_{SB}), living branches (B_{LB}), and dead branches and leaves (B_L), was measured at ages 11, 24, 35, 47, 60, 72 and 84 months by sampling eight trees selected to account for the distribution of cross-sectional area in each treatment. Coarse roots (diameter >10 mm) of each tree were

excavated at ages 60 and 72 months only. The fresh mass of all tree compartments was measured in the field and subsamples were brought back to the laboratory, oven-dried at 65 °C and weighed. Allometric relationships were established for each compartment:

$$B_X = a_X + b_X \times (D^2 \times H)^{c_X} \quad (1)$$

with B (kg) the biomass, D (m) the diameter computed from C, and a, b and c the allometric coefficients to be estimated for compartment X. The equations were fitted by treatment at each age using PROC NLP in the SAS 9.2 software (SAS Institute Inc., Cary, NC, USA), and maximum likelihood estimations were used as a criterion to decide whether a different set of allometric coefficients should be used for each of the three treatments or whether a single set should be used for all three treatments. The selected allometric relationships were then applied to the plot inventories at each age to estimate the biomass of each compartment at the plot level. Age-specific allometric relationships were established for branches and leaves by sampling eight additional trees per treatment at 84 months of age. B_{SW} and B_{SB} at 84 months were estimated using the allometric relationships established at 72 months.

Medium root biomass (2–10 mm diameter) was measured at 60 months of age by digging one 1.5 m^2 pit 1 m deep in each plot (three pits per treatment for the three blocks). Fine roots (diameter <2 mm) were retrieved at the same time from four soil cores per plot down to 1 m in depth (inner diameter of the root auger of 4.5 cm) in two blocks, and down to 3 m in depth in one block. We assumed that the vertical distribution of fine roots was similar in all blocks in order to extrapolate fine root biomass down to 3 m depth in the two blocks with 1 m deep pits.

Aboveground litterfall (F_{AL}, kg m^{-2} year^{-1}) was collected every month in five litter traps (52 × 52 cm) in each plot, separated into leaves (F_{LL}), bark and dead branches, oven-dried at 65 °C and weighed.

Soil CO$_2$ efflux and total belowground carbon flux

Soil CO$_2$ efflux (F_S, μmol m^{-2} s^{-1}) was measured every 2 weeks during two consecutive years between the ages of 60 and 84 months with a dynamic closed-path Li8100 system equipped with a 20 cm diameter Li8100-103 respiration chamber (LiCor Inc., Lincoln, NE, USA). Nine PVC collars were installed in each plot about 1 month before the beginning of the measurements. Soil volumetric water content in the 0–6 cm soil layer (SWC, m^3 m^{-3}) and within 5 cm of the collars was measured at the same time with a soil moisture probe (Theta Probe ML2X, Delta-T Device Ltd, Cambridge, UK). Cumulative annual soil CO$_2$ efflux (F_{CUM}, kg C m^{-2} year^{-1}) was estimated for each PVC collar using linear interpolations of F_S between each measurement date over the 2-year period of measurement.

Litter on the forest floor (L, kg m^{-2}) was collected at the ages of 18, 31, 43, 55, 61, 73 and 84 months in 4–6 quadrats (50 × 50 cm) in each plot and sorted into leaves, twigs, bark and miscellaneous, oven-dried at 65 °C and weighed. For forest floor samples, ash content was determined after combustion of pooled samples in a muffle furnace at 450 °C for 6 h, and used to correct the dry mass for soil contamination.

Total belowground carbon flux (TBCF, kg C m^{-2} year^{-1}) includes biomass increment of roots, turnover of fine roots, root exudation and respiration of roots and associated microorganisms. It can be estimated from F_{CUM} (Giardina and Ryan 2002):

$$\text{TBCF} = F_{CUM} + \Delta R \times C_R - F_{AL} \times C_{AL} + \Delta L \times C_L + \Delta C_S + E \tag{2}$$

where ΔR is the annual increment of root biomass, ΔL is the annual build-up of litter on the forest floor and ΔC_S is the annual increase in the carbon content of the mineral soil. C_R, C_{AL} and C_L are the carbon contents of roots, litterfall and litter on the forest floor and E is the flux of the C transported off the site by leaching of the dissolved organic and inorganic C, by erosion or by CH_4 emission. The annual increment of coarse root mass was calculated as the difference in biomass between two consecutive years. The annual increment of medium root mass was estimated assuming a similar relative growth rate for medium and coarse roots. We assumed a constant fine root (<2 mm) biomass and necromass at this age, no change in soil organic matter after >60 years of afforestation with *Eucalyptus*, and no exportation of carbon. Average TBCF between the ages of 60 and 84 months was calculated using 2-year average values of the above-mentioned parameters.

Aboveground net primary production and partitioning of GPP

Annual net production (kg m^{-2} year^{-1}) of stem wood was calculated as the difference in biomass between two consecutive years. Annual net productions of foliage, stem bark and branches were estimated as the yearly difference in standing biomass plus the amount of leaf, stem bark and dead branches recovered in the annual aboveground litterfall. Aboveground net primary production (ANPP, kg m^{-2} year^{-1}) was calculated as the sum of all annual net production of the aboveground compartments.

Gross primary production (GPP, g C m^{-2} year^{-1}) between the ages of 60 and 84 months was computed as the sum of ANPP, TBCF and aboveground respiration, which was assumed to be a constant fraction of ANPP:

$$\text{GPP} = \text{ANPP} + \text{TBCF} + \frac{(1 - \text{CUE})}{\text{CUE}} \times \text{ANPP} = \frac{\text{ANPP}}{\text{CUE}} + \text{TBCF} \tag{3}$$

where CUE is the carbon-use efficiency of aboveground production. We used 0.53, a value reported for *Eucalyptus saligna* in Hawaii (Giardina et al. 2003). Aboveground net production was converted into C flux using the carbon content of each aboveground compartment, which ranged from 0.43 g C g DM^{-1} in bark to 0.50 g C g DM^{-1} in leaves (data not shown).

Isotope composition of stem wood cellulose

A disk was collected at a height of 1.3 m on the trees felled in May 2010 (72 months old) to determine $\delta^{13}C$ as a proxy to provide time- and plant-integrated estimates of water-use efficiency (Farquhar et al. 1989). The circumference at breast height of the sampled trees had been recorded every year throughout the rotation. The limits of annual growth of the previous 5 years were drawn on the disk and annual rings were sampled with respect to this drawing. A safety margin was respected by discarding 25% of the annual increment around each ring boundary.

Alpha-cellulose was extracted from the wood using the acidified sodium chlorite extraction method (Bonal et al. 2011). The wood samples were oven-dried at 50 °C for a week and milled for 2–3 min at 30 Hz (MM301, Retsch GmbH, Haan, Germany). About 0.04 g of ground wood was put into individual pouches made of a Teflon filter with a pore size of 0.45 μm (Alltech, Templemars, France). Wood extractives were removed through Soxhlet extractions, first with a 2:1 (v/v) mixture of toluene 95% and ethanol 95%, and then with ethanol 95% alone. Inorganic salts were removed by boiling samples in de-ionized water for 6 h. In order to remove the lignin, the pouches were placed in distilled water with sodium chlorite (2.5 g per gram of wood) and glacial acetic acid (volume adjusted to reach a pH solution within the range of 3–4) at 70 °C for 24 h. Hemi-cellulose was removed by reaction with a 0.4 % (w/v) sodium hydroxide solution at room temperature for 1 h. After each step, the samples were rinsed with de-ionized water in an ultrasonic bath at 45 Hz for 10 min. Finally, the cellulose was oven-dried at 50 °C for 24 h.

For $\delta^{13}C$ analysis, about 1 mg of extracted α-cellulose was weighed into 4 × 6 mm tin capsules (Elemental Microanalysis, Okehampton, UK). The isotopic analyses were conducted using a continuous flow isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany) at the stable isotope facility at INRA (Nancy, France). $\delta^{13}C$ of wood α-cellulose was expressed relative to the Pee Dee Belemnite standard (VPDB). The analytical precision was ±0.12‰ (standard deviation for 20 repetitions of a standard working laboratory).

Statistical analyses

Statistical analyses were performed with R software version 2.13.0 (R Development Core Team, 2011). Mixed-effect models were used to estimate the effect of fertilization and age as
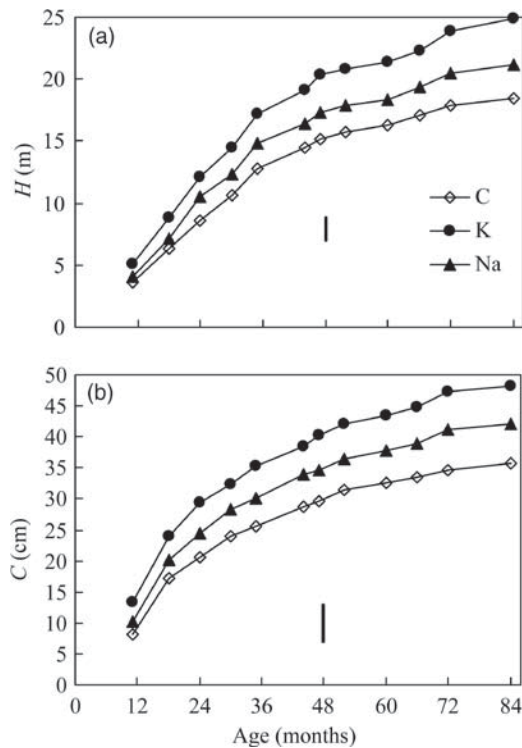
Figure 1. Tree height (H, a) and circumference (C, b) at different stand ages (in months) in the control (open diamonds), K-fertilized (closed circles) and Na-supplemented (closed triangles) plots. Measurements were taken on 25 trees in three plots per treatment. The vertical bars represent the standard deviation of the residuals. Statistics are given in Table 2.

fixed effects on tree dimension (H and C) and on $\delta^{13}C$ of stem wood cellulose (with trees within blocks as a random effect), on F_S (with collars within blocks as a random effect), on tree compartment biomass, on net production of the different tree compartments, and finally on F_{AL}, L and ANPP (with blocks as random effects). Mixed-effect models were also used to estimate the effect of fertilization as a fixed effect on F_{CUM}, TBCF and GPP (with blocks as a random effect). Contrasts were used to test relevant differences between treatment and/or age when the overall model was significant.

Results

Aboveground biomass and net primary production

Tree height and circumference were significantly enhanced at all ages by fertilization, with a stronger effect of K than of Na (Figure 1, Table 2). At 84 months, the differences in height between fertilized trees and control trees were 6.5 m for K fertilization and 2.7 m for Na addition. The differences for tree circumference were 12.3 and 6.2 cm, respectively.

Leaf biomass was significantly higher in the K treatment compared with the control at all ages while the increase in leaf biomass in the Na treatment was only significant at 35 and 47 months and vanished thereafter (Figure 2a, Table 2). Despite the higher leaf biomass in the K treatment, there was no significant difference in leaf litterfall among treatments (Figure 2b). Leaf production was only enhanced at 11 and 35

Table 2. Effects of the treatment [T: control (C), K fertilization (K) and Na addition (Na)], the age of the plantation (A) and their interaction for all variables depicted in Figures 1–3, 5 and 6: tree height (H), tree circumference (C), leaf biomass (B_L), stem wood (B_{SW}), stem bark (B_{SB}), living branches (B_{LB}), aboveground biomass (B_{AG}), aboveground net primary production (ANPP), wood production (including stem wood, stem bark and branches, P_W), leaf (F_{LL}) and total aboveground litterfall (F_{AG}), litter on the forest floor (L) and carbon isotope composition of stem wood α-cellulose ($\delta^{13}C$). Differences between treatments for each age tested by contrast analyses are given when the probability of rejecting equality when it is true is <0.05.

	Fixed effects (P values)			Contrast analyses ($P < 0.05$)		
	T	A	T ×A	K ≠ C	Na ≠ C	K ≠ Na
H	<0.001	<0.001	<0.001	All ages	24–84	18–84
C	<0.001	<0.001	<0.001	All ages	24–84	All ages
B_L	<0.001	<0.001	<0.001	All ages	35 and 47	24–84
F_{LL}	0.045	<0.001	<0.001	–	–	–
P_L	0.014	<0.001	<0.001	11, 35 and 72	35	–
LLS	<0.001	<0.001	<0.001	24–84	–	35–84
B_{SW}	<0.001	<0.001	<0.001	24–84	47–84	35–84
B_{SB}	<0.001	<0.001	<0.001	24–84	35–84	35–84
B_{LB}	<0.001	<0.001	<0.001	11–84	35–60, 84	24–84
B_{AG}	<0.001	<0.001	<0.001	24–84	47–84	35–84
ANPP	<0.001	<0.001	<0.001	24–84	35, 60–84	24–47, 72
P_W	<0.001	<0.001	<0.001	24–84	35, 60–84	24–47, 72
F_{AL}	<0.001	<0.001	0.015	60–84	72	–
L	–	<0.001	–	–	–	–
$\delta^{13}C$	0.050	<0.001	0.003	–	72[a]	–

[a]Note that the effect of treatment was just above the threshold of significance ($P = 0.0503$).
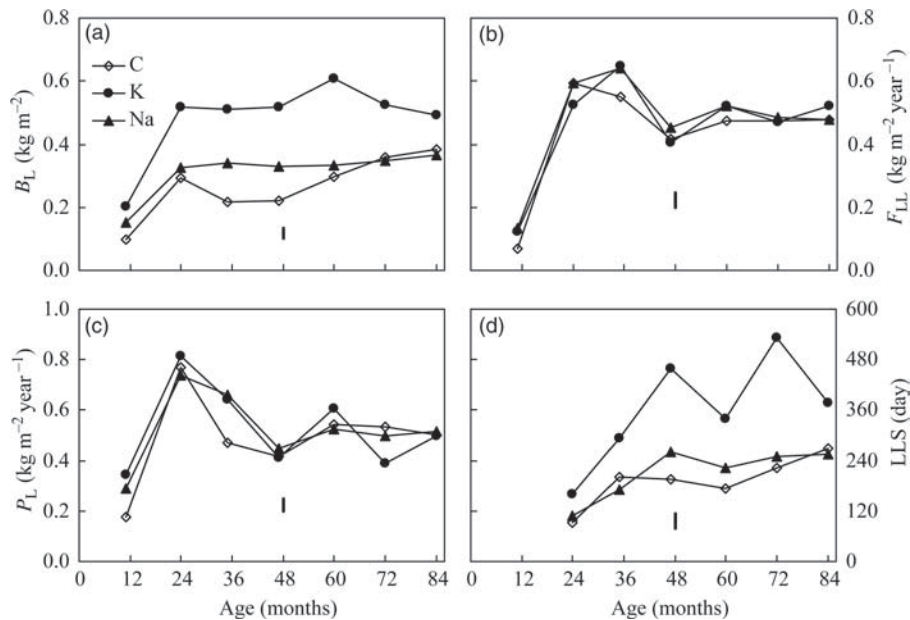
Figure 2. Leaf biomass (B_L, a, leaf litterfall (F_{LL}, b), leaf production (P_L, c) and leaf lifespan (LLS, d) at different stand ages (in months) in the control (open diamonds), K-fertilized (closed circles) and Na-supplemented (closed triangles) plots. Biomass and production were calculated using allometric relationships applied to 25 trees per plot and litter was collected in five litter traps in each plot. Each point is the mean of three plots per treatment (blocks). The vertical bars represent the standard deviation of the residuals. Statistics are given in Table 2.

months by K fertilization and at 35 months by Na addition (Figure 2c, Table 2). P_L was actually reduced by K fertilization at 72 months compared with the control. The increase in leaf biomass in response to K fertilization can therefore be explained by an increase in LLS from the age of 24 months that was not observed in response to Na addition (Figure 2d, Table 2).

Over all the treatments, stem wood, stem bark, branches and leaves respectively accounted for 82, 11, 4 and 4% of the total aboveground biomass at harvest. Stem wood biomass was significantly enhanced by K fertilization from the age of 24 months to harvest (+130% at 84 months compared with the C treatment) and by Na addition from the age of 47 months, though to a lesser extent (+55% at 84 months, Figure 3a, Table 2). Stem bark biomass responded similarly to K fertilization and Na addition (+101 and +44%, respectively, at 84 months compared with the C treatment, Figure 3b, Table 2). The effect of K fertilization on living branch biomass (Figure 3c, Table 2) was already significant at 11 months, while there was no clear response to Na addition. Aboveground biomass was significantly enhanced by K fertilization from the age of 24 months and by Na addition from 47 months (Figure 3d, Table 2). At harvest, aboveground biomass was enhanced by 121 and 51%, for K and Na, respectively.

Aboveground net primary production and wood production were significantly enhanced both by K fertilization from the age of 24 months and by Na addition from 35 months (except at 47 months), though the effect was stronger for K fertilization (Figure 3e and f, Table 2).

Total belowground carbon flow

Soil CO_2 efflux exhibited seasonal fluctuations with greater values during the wet/warm season than during the dry/cold season and paralleled the seasonal fluctuation in soil water content (Figure 4). Average cumulative soil CO_2 efflux for the three treatments was significantly higher in year 1 (1.41 kg C m^{-2} year^{-1}) than in year 2 (1.12 kg C m^{-2} year^{-1}). Over a 2-year period, F_{CUM} was slightly but not significantly higher ($P = 0.08$) in the fertilized treatments compared with the control (Table 3).

The total aboveground litterfall (F_{AL}) increased with stand age and was significantly higher in the fertilized treatments than in the control after the age of 48 months (Figure 5a, Table 2). The amount of aboveground litter (L) decreased slightly after planting until 43 months, and increased thereafter (Figure 5b). This pattern might result from thick depositions of dead branches from the age of 3 years onwards (data not shown). However, there was no significant difference among the three treatments (Table 2), nor was there any significant change between 61 and 84 months.

Coarse root biomass was significantly enhanced by K fertilization and Na addition at all ages (Table 4) while medium-sized root biomass and fine root biomass at the age of 60 months were not significantly different among treatments. Coarse roots therefore contributed more to total root biomass in the K treatment (73%) and the Na treatment (66%) than in the C treatment (57%). The increment in both coarse and medium root masses over the 2-year period (ΔR) was significantly different among the three treatments, with the greatest
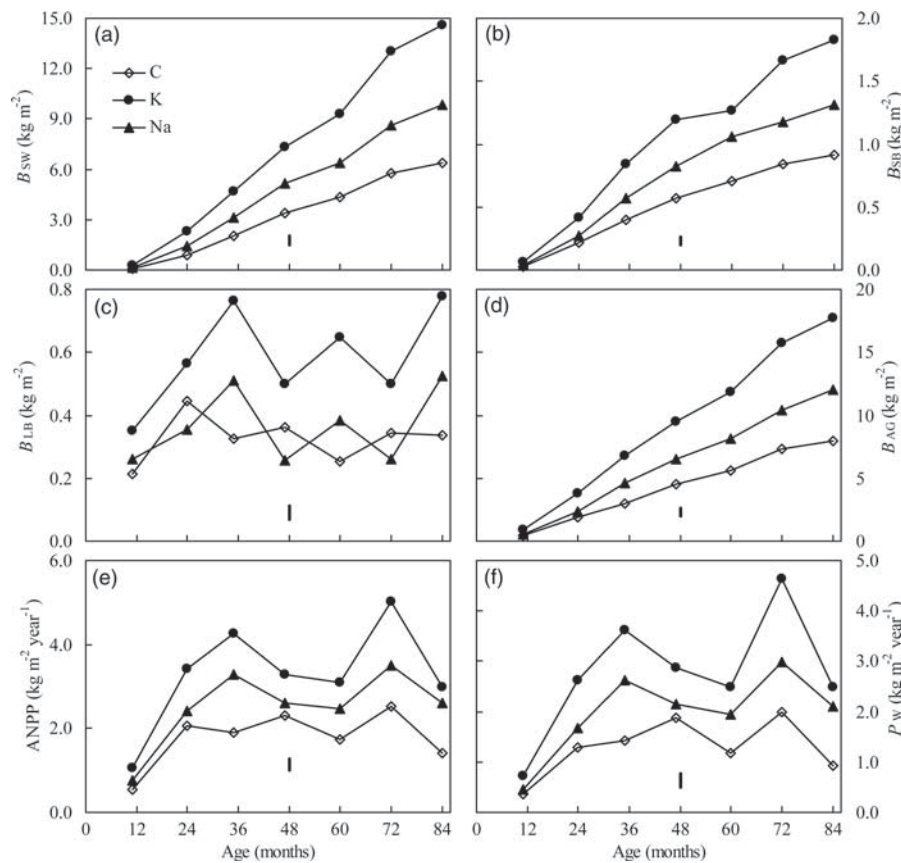
Figure 3. Stem wood biomass (B_{SW}, a), stem bark biomass (B_{SB}, b), living branch biomass (B_{LB}, c), aboveground biomass (B_{AG}, d), aboveground net primary production (ANPP, e) and wood production (P_W, f) at different stand ages (in months) in the control (open diamonds), K-fertilized (closed circles) and Na-supplemented (closed triangles) plots. Biomass and production were calculated using allometric relationships applied to 25 trees per plot. Each point is the mean of three plots per treatment (blocks). The vertical bars represent the standard deviation of the residuals. Statistics are given in Table 2.

increment in the K-fertilized plots and the lowest increment in the control plots (Table 3). The annual increase in root carbon stock over the 2-year period was therefore included in the TBCF calculation. Total belowground carbon flux enhancement by K fertilization (+20% compared with the control, Table 3) was not significant.

Gross primary production partitioning

Gross primary production was strongly enhanced by K fertilization (+74%, Table 3) while the effect of Na addition was much smaller (+36%). When expressed on a leaf mass basis, the difference in GPP:B_L was significant and more pronounced in the Na treatment (+41% compared with the C treatment) than in the K treatment (+27%).

In addition, there was a marked change in GPP partitioning in response to fertilization (Table 2) with less carbon allocated belowground for both the K and Na treatments (TBCF:GPP = 21 and 23%, respectively) compared with the control (31%), but also with less carbon allocated to leaf production, especially in the K treatment (P_L:GPP = 5%) compared with the Na (7%) and C (10%) treatments. As a consequence, partitioning to

wood production was strongly stimulated by K fertilization (P_W:GPP = 37% vs. 26% in the control) while it was intermediate in the Na treatment (34%).

Isotope composition of stem wood α-cellulose

The carbon isotope composition of stem wood α-cellulose varied with the age of the tree at the time of cellulose deposition from −25.9 to −28.2‰. Despite a nearly significant treatment effect ($P = 0.0503$), it did not exhibit any clear tendency (Figure 6).

Discussion

The Ferrasols on which the experiment was set up were acidic and had quite a low cation exchange capacity and very low amounts of exchangeable bases (Laclau et al. 2010). A positive effect of K fertilization (and potentially Na addition) was therefore expected, and indeed both higher GPP and net aboveground production confirmed this hypothesis. Clear changes in carbon allocation brought on by K fertilization and Na addition resulted in increased wood production.
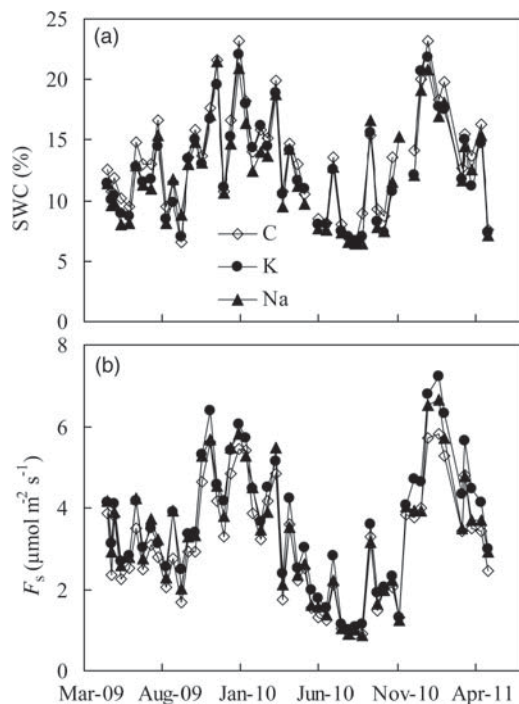
Figure 4. Soil water content (SWC, a) and soil CO_2 efflux (F_S, b) measured every 2 weeks for 2 years between the ages of 60 and 84 months in the control (open diamonds), K-fertilized (closed circles) and Na-supplemented (closed triangles) plots. Each point is the mean of 72 measurements per treatment (24 collars per plot and three plots per treatment). Fixed effects (treatments and dates) are highly significant ($P < 0.001$) for F_S and SWC as well as for the interaction ($P < 0.001$ for SWC and $P = 0.045$ for F_S).
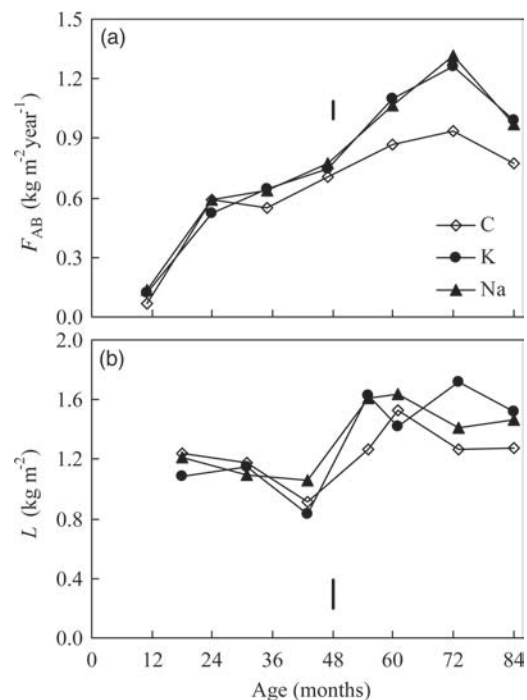

Figure 5. Aboveground litterfall (F_{AB}, a) and amount of litter on the forest floor (L, b) at different stand ages (in months) in the control (open diamonds), K-fertilized (closed circles) and Na-supplemented (closed triangles) plots. Litterfall was collected in five litter traps in each plot and litter on the forest floor was collected in 4–6 quadrats in each plot. Each point is the mean of three plots per treatment (blocks). The vertical bars represent the standard deviation of the residuals. Statistics are given in Table 2.

Table 3. Annual leaf production (P_L), aboveground wood production including stem wood, stem bark and branches (P_W), aboveground net primary production (ANPP), cumulative soil CO_2 efflux (F_{CUM}), coarse and medium root biomass increment (ΔR), total aboveground litterfall (F_{AL}), total belowground carbon flux (TBCF) and gross primary production (GPP) between the ages of 60 and 84 months in the control, K-fertilized and Na-supplemented plots. P_L, P_W and TBCF were also expressed relative to GPP and GPP relative to leaf biomass (B_L). Values are means with the standard deviation for the three blocks. Values followed by different letters are significantly different at $P = 0.05$.

	Control (kg C m^{-2} year^{-1})	K fertilization	Na addition	P values
P_L	0.26 ± 0.02 a	0.22 ± 0.02 a	0.25 ± 0.05 a	0.17
P_W	0.66 ± 0.10 a	1.62 ± 0.10 c	1.17 ± 0.07 b	<0.001
ANPP	0.92 ± 0.12 a	1.85 ± 0.12 c	1.42 ± 0.02 b	<0.001
F_{CUM}	1.16 ± 0.07 a	1.38 ± 0.11 a	1.26 ± 0.07 a	0.08
ΔR	0.06 ± 0.00 a	0.15 ± 0.02 c	0.10 ± 0.00 b	0.001
F_{AL}	0.43 ± 0.04 a	0.57 ± 0.05 b	0.57 ± 0.05 b	0.007
TBCF	0.79 ± 0.03 a	0.95 ± 0.15 a	0.79 ± 0.10 a	0.12
GPP	2.54 ± 0.22 a	4.44 ± 0.30 c	3.46 ± 0.14 b	0.001
P_L/GPP	0.10 ± 0.00 a	0.05 ± 0.00 c	0.07 ± 0.02 b	0.003
P_W/GPP	0.26 ± 0.02 a	0.37 ± 0.01 c	0.34 ± 0.01 b	<0.001
TBCF/GPP	0.31 ± 0.03 a	0.21 ± 0.02 b	0.23 ± 0.02 b	0.001
GPP/B_L	6.90 ± 0.63 a	8.73 ± 0.45 b	9.72 ± 0.39 c	0.003

Table 4. Coarse root biomass (diameter >10 mm) at the ages of 60, 72 and 84 months, and medium-sized biomass (2–10 mm diameter) and fine root (diameter <2 mm) biomass at 60 months for the control, K-fertilized and Na-supplemented treatments. Mean and standard deviations of three replicates (blocks) are given. Values followed by different letters are significantly different at $P = 0.05$.

	Age (months)	Control (kg m^{-2})	K fertilization	Na addition
Coarse root	60	0.58 ± 0.08 a	1.15 ± 0.07 c	0.80 ± 0.05 b
	72	0.71 ± 0.10 a	1.52 ± 0.11 c	1.04 ± 0.05 b
	84	0.78 ± 0.10 a	1.69 ± 0.13 c	1.16 ± 0.05 b
Medium root	60	0.22 ± 0.07 a	0.21 ± 0.04 a	0.19 ± 0.23 a
Fine root	60	0.22 ± 0.05 a	0.22 ± 0.04 a	0.23 ± 0.01 a

Potassium fertilization increases tree carbon uptake without affecting water-use efficiency

Annual GPP values between the ages of 60 and 84 months (Table 3) fell in the uppermost part of the range of values for forest ecosystems ([0.3–5.6 kg C m^{-2} year^{-1}], Litton et al. 2007). These values correspond well to those reported for 6-year-old *E. saligna* in Hawaii (Ryan et al. 2004) and are consistent with values estimated from eddy correlation measurements of
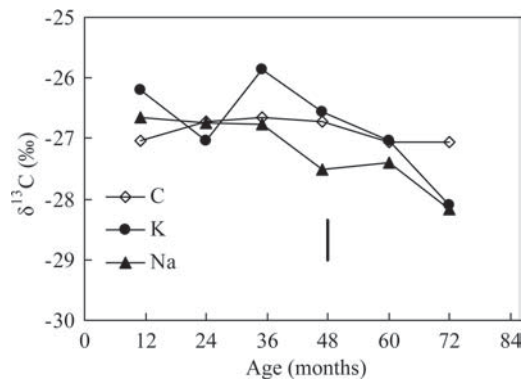
Figure 6. Carbon isotope composition of stem wood α-cellulose ($\delta^{13}C$) formed at different ages of the plantation recorded on the trees felled in May 2010 (72 months old) in the control (open diamonds), K-fertilized (closed circles) and Na-supplemented (closed triangles) plots. Each point is the mean of 25 measurements per treatment (five trees per plot and three plots per treatment) taken from stem disks collected at 1.3 m height and cut according to the circumference increment of each tree. The vertical bars represent the standard deviation of the residuals. Statistics are given in Table 2.

ecosystem carbon exchange in a nearby site (3.75 kg C m^{-2} year^{-1}, Cabral et al. 2011). Our estimation of GPP relies on the assumption that the CUE for aboveground growth is constant among treatments. A constant CUE among forest types has been questioned (DeLucia et al. 2007), but direct estimations of aboveground CUE are rather scarce and are often vitiated by uncertainties due to the cumulative error resulting from scaling chamber-based respiration measurements up to the stand scale. We used the average value of CUE (0.53) reported for *E. saligna* plots in Hawaii (Giardina et al. 2003), even though slightly higher values were reported for plots that received complete fertilization compared with unfertilized plots (0.51 vs. 0.55). Using these values rather than the average in our computation slightly reduced the difference in GPP between the control and the K-fertilized plots, and had only a marginal effect on GPP partitioning (<3%, data not shown). In addition, we might have slightly underestimated GPP because we used CUE values reported for trees (2–4 years old) younger than ours (4–6 years old) and because CUE was found to decrease with stand age (Ryan et al. 2004, DeLucia et al. 2007); however, this should not affect the overall trends among treatments.

Potassium fertilization enhanced the GPP of *E. grandis* more dramatically (+75%) than did a complete fertilization of *E. saligna* plots in Hawaii (34%, Giardina et al. 2003). Assuming that the increase in leaf biomass per ha mirrors a similar trend in leaf area index, the enhancement of GPP by fertilization can be explained by an increase in the amount of absorbed photosynthetic radiation. If the enhancement of GPP was solely due to a higher leaf area index, a constant GPP:B_L ratio (or a decrease due to leaf self-shading) would be expected but that was not the case. This suggests that K fertilization also enhances the canopy quantum-use efficiency (as defined by Landsberg and

Waring 1997) through an increase in CO$_2$ assimilation at the leaf level. Our argument implies a constant specific leaf area (SLA) among treatments that might not be true. However, because SLA decreases with tree height in *Eucalyptus* trees (Nouvellon et al. 2010), we may expect lower SLA in K-fertilized trees compared with the control; this would reinforce our argument that K fertilization enhances net leaf CO$_2$ assimilation.

Potassium is strongly involved in the osmotic control of stomatal movement (Fischer 1968, Roelfsema and Hedrich 2005), and the enhancement of leaf photosynthesis by K fertilization might be related to higher leaf stomatal conductance that would in turn decrease water-use efficiency. Several studies have reported contradictory relationships between soil fertility and $\delta^{13}C$ of wood or wood cellulose with a positive effect of site fertility on water-use efficiency (Weitner et al. 2007), a lack of significant relationships (Elhani et al. 2005) or interactions with soil moisture variations (Korol et al. 1999). The effect of K fertilization itself on water-use efficiency has seldom been assessed. Significantly lower $\delta^{13}C$ values in plant organic matter (which correspond to lower water-use efficiency) in K-fertilized cotton plants as compared with K-deficient ones have been reported (Bednarz et al. 1998). In contrast, we did not observe any significant difference in $\delta^{13}C$ of stem wood α-cellulose produced every year until the age of 72 months; this suggests that water-use efficiency might be the same among the treatments and that the increase in leaf photosynthesis was not only related to a higher stomatal conductance. A higher rate of phloem loading with improved K nutrition (Mengel and Haeder 1977) that increases the amount of C transferred to the active sinks might relieve some end-product, non-stomatal limitation of photosynthesis (Goldschmidt and Huber 1992). An increase in internal CO$_2$ concentration in the leaves of K-deficient almond trees (*Prunus dulcis*) and cotton plants revealed some biochemical limitations of photosynthesis (Bednarz et al. 1998, Basile et al. 2003) and an accumulation of glucose in the leaves of K-deficient cotton plants was reported (Pettigrew 1999).

Potassium fertilization decreases the fraction of carbon allocated belowground

The total belowground carbon flux in our study (0.7–0.95 kg C m^{-2} year^{-1}) was in the lower range of values reported for tropical *Eucalyptus* plantations (0.4–2.6 kg C m^{-2} year^{-1}; Giardina et al. 2003, Stape et al. 2008, Ryan et al. 2010) and accounted for only 21–31% of GPP, which was also in the lower range of values currently reported for forest ecosystems on the whole (Litton and Giardina 2008). We did not measure annual changes in fine root biomass and soil carbon content because we assumed that they would both be negligible over the 2 years studied at the end of the rotation. The net increase in fine root biomass (0–5 mm diameter) over a whole rotation in a *Eucalyptus* plantation in the Congo estimated with a set of age-related allometric equations was <0.02 kg DM m^{-2} year^{-1}

from the age of 60 months onwards (Thongo M'Bou et al. 2010), i.e., one order of magnitude lower than the coarse root biomass increment. In addition, a strong relationship between leaf and fine root biomass was observed in *E. grandis* plantations near our study site (Maurice et al. 2010), and the rather stable foliar biomass at the end of the rotation in our study suggests that fine root biomass remained roughly constant between the ages of 60 and 84 months. Change in soil carbon after afforestation with *Eucalyptus* depends on soil characteristics and previous land use (Paul et al. 2002, Zinn et al. 2002). The maximum rate of carbon accretion immediately after afforestation of carbon-depleted savannah soil with *Eucalyptus* was 0.09 kg C m^{-2} year^{-1} (Epron et al. 2009); furthermore, most carbon accretion is expected to take place during the first few decades after afforestation (Lima et al. 2006). Carbon accretion after 60 years of continuous cultivation and 60 months after replanting, if any, should therefore be several orders of magnitude lower than TBCF.

Whereas K fertilization did not significantly affect the total amount of carbon that flows belowground, the fraction of GPP allocated belowground was markedly reduced by K fertilization. This has often been reported for trees receiving complete fertilization (Giardina et al. 2003, Ryan et al. 2004), nitrogen fertilization (Chen et al. 2011) or phosphorus fertilization (Keith et al. 1997) but, to our knowledge, has never previously been documented for K. With K fertilization, a larger fraction of GPP was allocated aboveground. Assuming a constant CUE for aboveground growth, this contributed to the enhancement of ANPP (Giardina et al. 2003). The enhancement of ANPP in response to K fertilization was therefore explained by both an increase in GPP and a change in carbon partitioning towards aboveground growth, the increase in GPP being almost fully allocated aboveground. This finding is consistent with the theory that carbon allocation patterns optimize resource acquisition in order to maximize growth (Thornley 1972).

Potassium fertilization increases LLS and carbon allocated to leaf production

Our result confirmed the increase in LLS of *Eucalyptus* leaves in response to K fertilization at the early growth stage of the plantation (Laclau et al. 2009). Leaf lifespan increased with the age of the plantation in all treatments, and the effect of K fertilization was even more pronounced between the ages of 47 and 84 months (+186 days compared with the C treatment) than between the ages of 11 and 36 months (+91 days). This increase in LLS does not support the ecological concept of resource conservation strategy, which postulates that the lifespan of plant organs lengthens in resource-limited environments (Aerts and Chapin III 2000). The longevity of Douglas-fir and grand fir needles decreased in response to a combination of nitrogen and K fertilization (Balster and Marshall 2000). This response might be specific to nitrogen fertilization. A decrease

in LLS was reported in a tropical forest in Hawaii after nitrogen addition while LLS was not affected by phosphorus fertilization (Cordell et al. 2001). Our results on *Eucalyptus* are consistent with the acceleration of leaf senescence observed in K-deficient almond trees (Basile et al. 2003).

The dramatic increase in LLS associated with a relatively limited increase in leaf production accounts for the 50% decrease in the relative amount of carbon allocated to leaf production (P_L:GPP) in response to K fertilization compared with the control plots. The decrease in P_L:GPP together with an increase in ANPP explains the substantial increase in wood production (P_W) in response to K fertilization (+145% between the ages of 60 and 84 months compared with the C treatment). An increase in P_W is a common response to fertilization in *Eucalyptus* (Keith et al. 1997, Ryan et al. 2004), but the increase we observed is one order of magnitude above those commonly reported (Litton et al. 2007).

The enhancement of P_W by K fertilization was maintained over the whole rotation of the plantation from 11 months to 84 months, even though a decreasing trend in P_W was observed after 36 months in all treatments, except between the ages of 60 and 72 months. This decrease in P_W with stand ageing is in agreement with previous observations on *Eucalyptus* both in Hawaii (Ryan et al. 2004) and in Brazil (Stape et al. 2010) where a decline in P_W related to a decline in GPP was observed after canopy closure. Although we did not estimate GPP over the entire rotation, the decline in δ^{13}C of wood formed after age 36 months did not support the hypothesis of a hydraulic limitation that would have reduced canopy photosynthesis by stomatal closure as the trees became taller (Hubbard et al. 1999). However, both ANPP and P_W between the ages of 60 and 72 were significantly higher than those estimated for the two previous 1-year periods and for the following 1-year period. In addition, the most depleted values of δ^{13}C of α-cellulose were recorded in stem wood produced between 60 and 72 months. Since during this period the cumulative precipitation was 46% higher than the average value (2106 mm compared with 1440 mm), this strongly suggests that ANPP and P_W were limited by the amount of available soil water in this rain-fed plantation, which is in agreement with the strong response of Brazilian *Eucalyptus* plantations to irrigation (Ryan et al. 2010, Stape et al. 2010). The enhancement of ANPP and P_W by K fertilization was also more important between the ages of 60 and 72, possibly indicating an interaction between K and water availability on *Eucalyptus* growth.

Potassium can only partly replace K

There is now growing evidence that Na can replace K as an osmoticum or an accompanying cation for negatively charged organic solutes, and can stimulate the growth of a few crop species (Subbarao et al. 2003). Our study clearly demonstrated

that Na application on K-deficient soil can significantly enhance the GPP, ANPP and P_W of *Eucalyptus* stands, but to an extent that is nonetheless lower than that achieved with K fertilization. This confirms over the whole rotation the preliminary results obtained at a younger stage (Almeida et al. 2010). However, we should keep in mind that our calculation of GPP assumed a constant CUE for aboveground growth, which might not be true for the Na-supplemented plots since the energetic requirements of cellular mechanisms involved in alleviating Na toxicity, such as the active transport of Na^+ ions into the vacuoles, may increase maintenance respiration (Schwarz and Gale 1981, Epron et al. 1999). It should be noted, however, that the amount of Na added to the soil (99 kg ha^{-1}) in our experiment was quite limited.

Surprisingly, Na addition has a stronger positive effect on the GPP:B_L ratio; this might reflect a strong stimulation of leaf photosynthesis. In addition, there is no indication of a higher stomatal limitation of photosynthesis that would have increased $\delta^{13}C$ in Na-supplemented trees compared with K-fertilized or control trees, in contrast to what was reported for both salt-tolerant and salt-sensitive plants (e.g., Brugnoli and Lauteri 1991).

Aboveground net production and P_W differed between the two treatments between the ages of 60 and 72 months (wet year) while they did not differ the previous year nor the following year when precipitation was close to the 15-year average. This clearly indicates that K fertilization and Na addition interact with water availability in a different way, and that the ANPP and P_W of K-fertilized plots were likely limited by soil water availability under the average precipitation regime for this region.

The effect of Na addition on the partitioning of GPP did not completely mimic the effect of K fertilization. While Na addition and K fertilization did not affect the total amount of carbon allocated belowground, they both reduced the fraction of GPP allocated belowground in a similar way (TBCF:GPP), thus similarly enhancing the fraction of GPP allocated aboveground. The partitioning of GPP between leaf and wood production differed significantly between both treatments. One of the reasons is that LLS was not enhanced by Na addition in contrast to the dramatic increase in LLS in response to K fertilization. Accumulation of Na with time in leaves may constrain LLS in Na-supplemented trees due to the toxicity of high Na concentrations in non-halophytic plant tissue (Greenway and Munns 1980). Lower LLS means that leaf biomass is not enhanced by Na addition in contrast to K fertilization, and canopy carbon uptake (GPP) is therefore limited.

Conclusion

Potassium fertilization and Na addition increased carbon uptake by trees both at the canopy level (higher leaf biomass) and at the leaf level (higher GPP:LB ratio) without decreasing water-use efficiency. Both K fertilization and Na addition decrease the fraction of carbon allocated belowground, which is a common

response of trees to improved belowground resource availability (Litton et al. 2007). This occurred without affecting significantly the total amount of carbon allocated belowground, which contradicts the idea that the flux of carbon to all compartments increases with increasing GPP. Indeed, all the excess GPP was allocated aboveground, thus strongly enhancing ANPP. Because LLS increases in response to K fertilization, but not Na addition, leaf biomass increases without any change in leaf production, and wood production is dramatically increased by K fertilization. Because P_W was more enhanced than B_L, the water cost of wood production might be reduced.

The role of K in phloem loading may be key to alleviating non-stomatal limitations of photosynthesis, but it might also speed up the long-distance transport of photosynthates from leaves to the sink organs, thus changing the pattern of carbon allocation within the tree. Pulse labelling of whole trees by ^{13}C is a promising tool for obtaining more insight into the mechanisms of within-tree carbon transfer (Plain et al. 2009, Dannoura et al. 2011). We had previously hypothesized that the increase in leaf photosynthesis explains the enhancing effect of K on LLS (Laclau et al. 2009) but, while Na addition also increases leaf photosynthesis, it does not change LLS. Understanding the role of K and Na in drought stress mitigation will be necessary to refine silvicultural practices in forest plantations that have expanded to less fertile soils in drier areas, in particular in a context of a changing climate.

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