

# Soil P availability under eucalypt and acacia on Ferralic Arenosols, republic of the Congo



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## ABSTRACT

Introducing nitrogen fixing species (NFS) in forest plantations reduces soil N-limitation, but also involves changes in phosphorus (P) availability in the Ferralitic Arenosols of the Congolese coastal plains or Batéké Plateaux in Central Africa. We evaluated soil-available P and total P in above-ground litters, leaves, bark and wood in pure (100A, 100E) and mixed-species (50A50E) stands of acacia (a NFS) and eucalypt plantations in the Congolese coastal plains at year 2 of the second rotation (Y2R2) compared to the end of the first 7-year rotation (EndR1). Soil available P was measured as resin P, bicarbonate-extractable inorganic (P<sub>i</sub>-HCO<sub>3</sub>) and organic (P<sub>o</sub>-HCO<sub>3</sub>). Soil resin-P values (15–19 mg P kg<sup>-1</sup>) in 100E were 80% higher relative to 100A (8–17 mg P kg<sup>-1</sup>) at Y2R2 against no difference for both 100E and 100A (8–12 mg P kg<sup>-1</sup>) at EndR1. Total P concentration was higher in acacia wood (0.61 g P kg<sup>-1</sup> of dry mass (DM)) than in eucalypt wood (0.57 g P kg<sup>-1</sup> of DM) in 50A50E at Y2R2, while higher stock of P and higher ratio of N:P ratios were found in the foliage of acacia than of eucalypt trees. Our data suggests that the risk of shifting from N-limitation to P-limitation system is minor. However, in the long term, P-limitation may eventually occur in pure acacia plantations, due to mining of soil available P by acacia's higher P uptake relative to eucalypt and additional requirement for symbiotic fixation of atmospheric N<sub>2</sub>.

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## 1. Introduction

The productivity of fast-growing tree plantations declines in second and subsequent rotations owing to a reduction in soil fertility when nutrients exported at harvest are not supplied as fertilizers (Corbeels et al., 2005; Laclau et al., 2005; Nambiar and Kallio, 2008; Tiarks and Ranger, 2008). In the Congolese coastal plains, eucalypt plantations deplete soil fertility in the highly-weathered inherently nutrient poor sandy Ferralitic soils, due to the large nutrient exported in harvested biomass, and the subsequent leaching of nutrients after harvest at the end of each rotation (Laclau et al., 2005). These soils are typical of large areas of savannah in central Africa, especially in the Batéké Plateaux spanning over 6 million hectares in Gabon, the republic of the Congo and the DR Congo (Schwartz and Namri, 2002). The retention of harvest residues (Corbeels et al., 2005; Kumaraswamy et al., 2014), the plantation of trees fixing nitrogen from the atmosphere, so called N<sub>2</sub> fixing species (NFS) such as fast-growing exotic acacia species or their introduction in eucalypt plantations may improve soil fertility by reducing N

depletion and sustaining forest productivity (Khanna, 1998; Binkley et al., 2000; Forrester et al., 2006; Epron et al., 2013; Koutika et al., 2014). These practices have been implemented in the Congolese coastal plains in the republic of the Congo (Nzila et al., 2002; Bouillet et al., 2013) as well in the Batéké Plateaux in the DR Congo (Kasongo et al., 2009).

In addition to soil N supply, phosphorus (P) is an essential nutrient for forest productivity in most tropical soils due to its major role in biological nutrient cycling (Hinsinger, 2001; Yang et al., 2013). Without regarding P export and potential leaching at harvest, soil P availability to plants is greatly reduced by strong adsorption of P due to the large amounts of Al and Fe oxide surfaces present in weathered soils (Sanchez and Uehara, 1980; Hinsinger, 2001). Soil P availability is especially critical in mixed-species plantations containing NFS owing to the high P requirement for symbiotic fixation of atmospheric N<sub>2</sub> (Binkley, 1992; Hinsinger, 2001; Inagaki et al., 2011). Thus, increasing N availability in mixed-species plantations including NSF trees may shift the system from N-limitation to P-limitation, with a potential negative feedback on N<sub>2</sub> fixation after several rotations, even though the phosphatase activity of NFS may enhance P availability (Blaser et al., 2014).

Due to the low input of fertilizers in commercial forest plantations established on the Congolese coastal plains, soil fertility, tree nutrition and early growth mainly rely on the decomposition of organic residues (Laclau et al., 2005; Versini et al., 2013). During the juvenile stage of a

Abbreviations: NFS, nitrogen fixing species; P, phosphorus; EndR1, end of the first 7-year rotation; Y2R2, year 2 of the second rotation; Pi-HCO<sub>3</sub>, bicarbonate inorganic phosphorus; Po-HCO<sub>3</sub>, bicarbonate organic phosphorus; N, nitrogen.

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eucalypt plantation up to canopy closure, the uptake of nutrients from the soil reserves supplied most of tree growth requirements (Laclau et al., 2003). At a site located on the coastal plains of the Congo, previous results of the first rotation (2004–2011) of a mixed plantation of acacia and eucalypt have shown that eucalypt growth benefits from the  $N_2$  fixed by acacia (Bouillet et al., 2013; Epron et al., 2013). However, a decrease in soil resin P in the topsoil of the mixed-species stands relative to the pure eucalypt stands was observed at the end of the first 7-year rotation (EndR1) (Koutika et al., 2014).

These results obtained at the end of the first rotation raise concerns about the relationship between soil fertility e.g., P availability and the sustainability of mixed-species plantations of acacia and eucalypt. Two questions have emerged: (1) would the decrease in resin P values observed in the mixed-species stands (half eucalypt and half acacia) at the end of the first 7-year rotation amplify at year 2 of the second rotation? (2) Will a low available soil P decrease P uptake by NFS trees during the first 2 years of the second rotation? In this study, soil P availability at two key stages of eucalypt–acacia plantation rotations (EndR1 and Y2R2) was compared. Soil P availability was characterized by quantifying the resin-extractable P,  $P_i\text{-HCO}_3$  and  $P_o\text{-HCO}_3$  fractions from the Hedley soil P sequential extraction procedure that are considered readily available to plants (Tiessen et al., 1984; Tiessen and Moir, 2008). The amount of P in the tree biomass and in litterfall was also estimated at year 2 of the second rotation, a juvenile stage when the nutrients uptake from the soil reserves supplied most of tree growth requirements, especially in low-input systems.

## 2. Materials and methods

### 2.1. Site description

The study site is located about 35 km outside Pointe-Noire city on the coastal plains close to Tchissoko village in the Republic of Congo ( $4^\circ 44' 41''\text{S}$  &  $12^\circ 01' 51''\text{E}$ , 100 Alt.). The climate of the area is subequatorial with high mean annual air humidity and air temperature (85% and  $25^\circ\text{C}$ , respectively) and low seasonal variation (about 2% and  $5^\circ\text{C}$ , respectively). Annual precipitation averages 1200 mm with a dry season extending from June to September. The soils in this area are deep Ferralic Arenosols overlying geological bedrock composed of thick detritic layers of continental origin (sandstone) dating from the Plio–Pleistocene. They are characterized by a low cation exchange capacity ( $\text{CEC} < 0.5\text{ cmol kg}^{-1}$ ), a high sand content (>90% of the mineral soil) and a very low clay and silt content 6 and 2%, respectively (Mareschal et al., 2011). The soil at the site has a low total N content (<0.07%) and C content (0.4–1.18%). The mean total phosphorus (P),

aluminium (Al), iron (Fe) and manganese (Mn) analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, soil analyses laboratory, INRA, Arras, France) are respectively  $0.06 \pm 0.01\%$ ,  $1.02 \pm 0.03\%$ ,  $0.99 \pm 0.03\%$  and  $4.8 \pm 0.2\%$  in the 0–5 cm.

The original vegetation was native tropical savannah dominated by the poaceae *Loudetia arundinacea* (Hochst.) Steud. The area was first afforested in 1984 with pure eucalyptus hybrids. In May 2004, mixed *Eucalyptus urophylla* x *grandis* hybrid (18–52) and *Acacia mangium* stands were established with a starter fertilization of  $43\text{ kg ha}^{-1}$  of N as ammonium nitrate. Pure acacia (100A), mixed-species with 50% acacia and 50% eucalypt trees (50A50E, the two species being alternately planted in the row and between adjacent rows) and pure eucalypt (100E) stands were compared within a randomised block design with five replicates and a stocking density of  $800\text{ trees ha}^{-1}$ . Each stand ( $1250\text{ m}^2$ ) consisted of an inner plot comprising 36 trees ( $6 \times 6$ , Fig. 1) and two buffer rows. The first rotation ended after seven years which is a full length rotation for eucalypt plantations established in this area (Laclau et al., 2000). Trees were harvested in January 2012. The debarked commercial-sized boles were removed at harvest while all remaining residues i.e., branches, bark and leaves were left and equally distributed on the soil surface in each stand. The stock of available P ( $\text{g P m}^{-2}$ ) in the soil (0–15 cm) at the end of the first rotation together with the amounts of P in the slash left after harvest calculated from the amount stored in the biomass at the end of the first rotation (Koutika et al., 2014) are given in Table 1. The second rotation was planted in March 2012 using the same design, with a closely related *Eucalyptus urophylla* x *grandis* hybrid (18–147) and *Acacia mangium* but without any N fertilizer added. Potassium (K) was supplied three months after planting ( $150\text{ kg ha}^{-1}$  as KCl) owing to the risk of K depletion on highly weathered tropical soils (Epron et al., 2012).

### 2.2. Soil sampling

Soil was sampled in December 2011 (end of the first rotation after seven years, EndR1) and in March 2014 (after two years of the second rotation, Y2R2) in three out of the five blocks. On each occasion, nine soil samples (18 in 50A50E) were collected in each stand from three soil depths (0–5 cm, 5–10 cm and 10–15 cm) using  $5 \times 5\text{ cm}$  sampling cylinders. In each stand, three transects (six for the 50A50E) were set up starting at the base of a tree and ending in the centre of the area delimited by four trees (Fig. 1). The three sampling points were separated by 0.7 m from each other on each transect. The total number of sampling points was 27 ( $9 \times 3$  blocks) for 100A and 100E and 54 ( $9 \times 2$  species  $\times 3$  blocks) in 50A50E. The soil samples were air-dried, sieved at 4 mm and root fragments were removed.

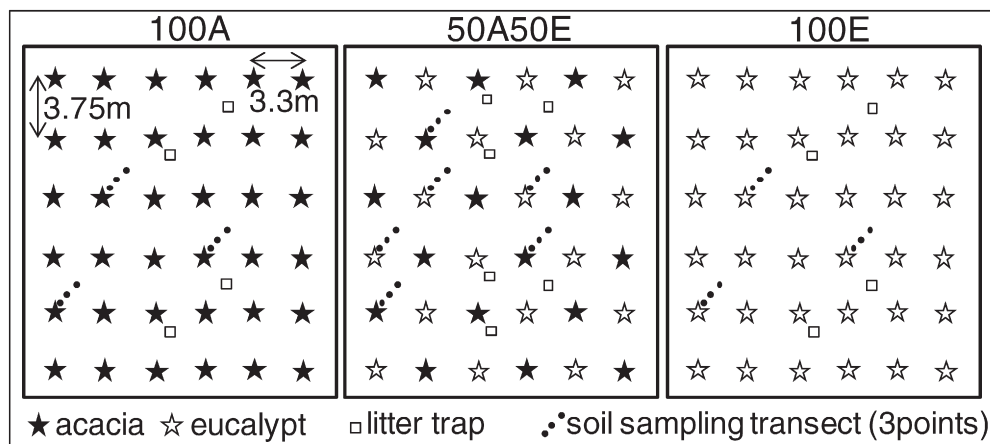


Fig. 1. Schematic representation of the planting and sampling designs showing the inner plot comprising 36 trees ( $6 \times 6$ ) of pure acacia (100A), mixed-species with 50% acacia and 50% eucalypt trees (50A50E) and pure eucalypt (100E) replicated in five blocks.

**Table 1**

Stock of P ( $\text{g P m}^{-2}$ ) in soil (0–15 cm) at the end of the first rotation (EndR1, in Koutika et al., 2014) and at the age of two years of the second rotation (Y2R2), and amount of P in slash left after harvest ( $\text{g P m}^{-2}$ ) in pure acacia (100A), mixed-species (50A50E) and pure eucalypt (100E) stands. Soil stocks are mean values (with standard errors) of 27 ( $9 \times 3$  blocks) replicates for the pure stands and 54 replicates ( $18 \times 3$  blocks) for the mixed-species stand. Amounts of slash left after harvest are mean values (with standard errors) of two blocks. The different letters indicate that means are significantly different between stands, and stars indicate significant differences between the two ages for the stock of P in soil ( $p < 0.05$ ).

	100A	50A50E	100E
Soil (EndR1)	$2.0 \pm 0.1\text{b}$	$1.7 \pm 0.1\text{a}$	$2.0 \pm 0.1\text{b}$
Soil (Y2R2)	$2.3 \pm 0.2\text{b}$	$2.8 \pm 0.2\text{a}^*$	$3.4 \pm 0.2\text{c}^*$
Slash left after harvest	$2.9 \pm 0.0\text{b}$	$2.8 \pm 0.3\text{b}$	$1.6 \pm 0.2\text{a}$

### 2.3. Soil analysis

Soil resin P was determined using two anion exchange resin strips (BDH#551642S, 20 mm  $\times$  60 mm) added to 0.5 g of dried and sieved soil suspended in 30 ml distilled water. Phosphate adsorbed by the anion exchange resin was recovered in 30 ml of 0.5 M HCl after shaking for 16 h ( $100 \text{ revs min}^{-1}$ ) according to the method of Hedley et al. (1982) as described by Tiessen and Moir (2008) i.e. 16 h of contact between the soil suspension and the resin strips. Malachite green reactive P was determined at 630 nm with an UVmc® Safas Monaco spectrophotometer (Monaco, Principality of Monaco). Soil resin P in each soil layer were converted to  $\text{g P m}^{-2}$ , knowing the thickness and the bulk density of each soil layer, and summed to 0.15 m to estimate the stock of available P.

The bicarbonate P Hedley fractions were only determined in the 0–5 cm layer of the 3 stands since large differences in soil resin P were only observed in this layer at the end of the first 7-year rotation (Koutika et al., 2014). This is the layer where soil organic matter dynamics and biological processes are the fastest (Epron et al., 2009; Versini et al., 2013; Derrien et al., 2014). A composite sample per block was made using the nine replicate samples taken from each of the 3 blocks. The composite soil was fractionated following the modified method of Hedley et al. (1982) as described by Tiessen and Moir (2008) with further modifications. After anion-exchange resin P extraction on 0.5 g of dried and sieved soil as described above, 30 ml of 0.5 M  $\text{NaHCO}_3$  solution was added to the soil previously washed with deionized water. The soil suspension was shaken overnight, centrifuged at 25,000 g for 10 min at 0 °C and filtrated through a 0.45  $\mu$  membrane filter. Inorganic P ( $\text{Pi-HCO}_3$ ) in the filtrate was immediately determined calorimetrically using malachite green. Total P ( $\text{Pt-HCO}_3$ ) in the filtrate was then obtained after oxidizing the dissolved organic matter with ammonium persulfate (Tiessen and Moir, 2008), using also malachite green. Organic P ( $\text{Po-HCO}_3$ ) was estimated by subtracting  $\text{Pi-HCO}_3$  from  $\text{Pt-HCO}_3$ . In this study which aimed to evaluate potentially labile P to plants, the methodological steps that are further used to quantify P with low bio-availability extracted from more stable pools were not carried out because they were unlikely to be affected by the duration of rotation and tree growing period (Tiessen and Moir, 2008).

### 2.4. P in wood, bark, foliage and litter fall and N:P ratios in foliage

Ten trees of each species in both pure and mixed-species stands were selected over the 3 blocks and harvested at year 2 of the second rotation. The crown was divided into three equal-length sections. The fresh mass of all leaves of each section was measured and an aliquot of 20 randomly selected leaves was collected and weighed immediately. Leaf aliquots were dried at 65 °C, weighed and ground. The three aliquots of each tree were combined in a single composite sample according to the proportion of leaf fresh mass in each section of the crown. A disk section of the trunk of each tree was cut with a chain saw at 1.3 m from the base of the tree. Bark and wood were separated, oven-

dried, weighed and ground. Above-ground litterfall was collected every two weeks in litter traps (75 cm  $\times$  75 cm of area) for a year from June 2013 to June 2014. The number of traps was four in 100A and 100E plots of each block, and six in the 50A50E stand of each block. Litter collected in all traps within each block and treatment was pooled, oven-dried at 65 °C weighed and ground. Ground plant material (0.6 g) was mixed with 10 ml of concentrated  $\text{HNO}_3$  and heated for 10 h to mineralize P until approximately 3 ml of a clear mixture remained and its P content was measured according to the green malachite method as described above. P in aboveground litterfall and P stored in foliage, stem-bark and stem-wood were calculated by multiplying the concentrations with annual above-ground litterfall and with foliage, stem-wood and stem-bark biomasses estimated from allometric relationships applied to tree inventories, as described in Epron et al. (2013). N concentration in leaf samples was determined by combustion with an elemental analyser (NCS 2500, Thermoquest, Italy).

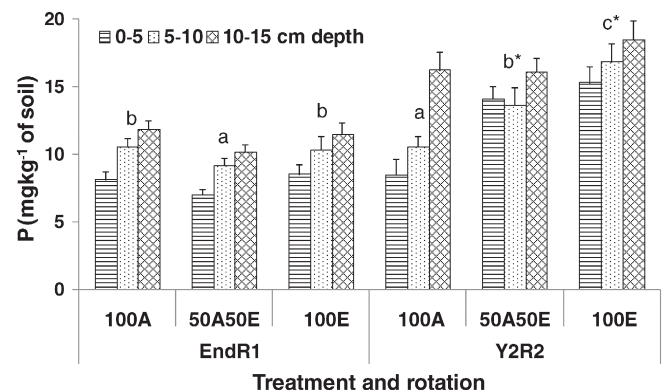
### 2.5. Statistical analyses

Statistical analyses were performed with R software version 2.13.0 (R Development Core Team, 2011). Mixed-effect models with blocks and sampling place within blocks as random effects were used to estimate the effects of stand treatment (100A, 100E and 50A50E), soil depth and time in rotation (EndR1 and Y2R2) as fixed effects on soil pH and soil resin P. Contrasts were used to test relevant differences between stands and between soil depths where there was an overall significant model effect (a probability level below 0.05). One way analyses of variance followed by Tukey's HSD tests were used to assess differences in P concentrations in bicarbonate-P Hedley fractions, in biomass and in P stock between the three stands. One way ANOVA was also used to assess difference in P concentration in above-ground biomass and litterfall, and N:P ratio in foliage, for the two species growing either in pure or in mixed-species stands.

## 3. Results

### 3.1. Soil resin P

The values of soil resin P down to 15 cm in 50A50E and 100E were significantly higher at Y2R2 relative to EndR1, while those of 100A did not significantly change with stand age (Fig. 2). At Y2R2, soil resin P down to 15 cm in 100E was significantly higher than those in 50A50E, while the latter was also significantly higher than those in 100A



**Fig. 2.** Soil available P to plants (resin P) down to 15 cm in 100A (pure acacia), 100E (pure eucalypt) and 50A50E (mixed-species) stands at the end of the first rotation (at age seven years, EndR1) and at age two years of the second rotation (Y2R2). Mean values of 27 ( $9 \times 3$  blocks) replicates for the pure stands and 54 replicates ( $18 \times 3$  blocks) for the mixed-species stand. Vertical bars represent standard errors. The different letters indicate that means are significantly different between stands at one age, and the stars indicate significant differences between the two ages ( $p < 0.05$ ). The increase in P with depth was significant with no significant interaction with stands and ages (not shown for clarity).

(Fig. 2). There was also more resin P in the 5–10 and 10–15 cm layers than in the 0–5 cm layer in all stands and at both EndR1 and Y2R2. This leads to stocks of soil resin P down to 15 cm of  $2.3 \text{ g P m}^{-2}$  in 100A,  $2.8 \text{ g P m}^{-2}$  in 50A50E and  $3.4 \text{ g P m}^{-2}$  in 100E (Table 1).

### 3.2. Bicarbonate-P Hedley fractions

The P concentration in the  $\text{Pi-HCO}_3$  fraction, measured on composite samples ( $n = 3$ ), were significantly higher at EndR1 varying between  $3.2$  (50A50E) and  $4.7 \text{ mg P kg}^{-1}$  (100A) than at Y2R2 where values ranged from  $1.7$  (100A) to  $2.7$  (100E)  $\text{mg P kg}^{-1}$  (Table 2). In Y2R2,  $\text{Pi-HCO}_3$  concentrations were significantly higher in 100E and 50A:50E stands than in 100A stands. The  $\text{Po-HCO}_3$  values ranged from 28 to  $42 \text{ mg P kg}^{-1}$  of soil at EndR1 and from 32 to  $45 \text{ mg P kg}^{-1}$  of soil at Y2R2 (Table 2). However, there was no significant difference in  $\text{Po-HCO}_3$  values between stands and between the two dates of soil sampling. Concentrations of bicarbonate-extractable organic P ( $\text{Po-HCO}_3$ ) were a magnitude greater than bicarbonate-extractable inorganic P ( $\text{Pi-HCO}_3$ ) in these stands.

### 3.3. P concentrations in wood, bark, leaf, litter fall, biomass and P stock in biomass at Y2R2

The highest P concentration was found in leaves and it ranged from  $2.22 \text{ g P kg}^{-1}$  of DM in eucalypt (50A50E) to  $1.61 \text{ g P kg}^{-1}$  of DM in acacia (100A). However, there were no significant differences in P concentration of leaves, bark and litterfall among stand treatment and species (Fig. 3). While there was no significant difference in wood and bark biomass at Y2R2, leaf biomass was significantly higher in 100A and in 50A50E than in 100E (Fig. 4). Therefore, despite the lack of a significant difference in leaf P concentration, the amount of P in the foliage of acacia was more than twice as high in 100A and in 50A50E than in the foliage of eucalypt in 100E and in 50A50E (Fig. 4). This was also reflected in greater litterfall biomass and P stock in 100A and 50A:50E than in 100E stands. There was no significant difference in P stock in wood and bark among stands at year 2 of the second rotation.

### 3.4. Ratio of nitrogen to phosphorus concentrations (N:P) in the foliage of acacia and eucalypt at EndR1 and Y2R2

The N:P ratio was significantly higher in the foliage of acacias than in the foliage of eucalypts, both at the end of the 7 year-first rotation (EndR1) and at year 2 of the second rotation (Y2R2). The N:P ratio in the foliage of acacia and eucalypt trees increased from the EndR1 and Y2R2, especially for trees growing in pure stands i.e., 100A and 100E (Table 3).

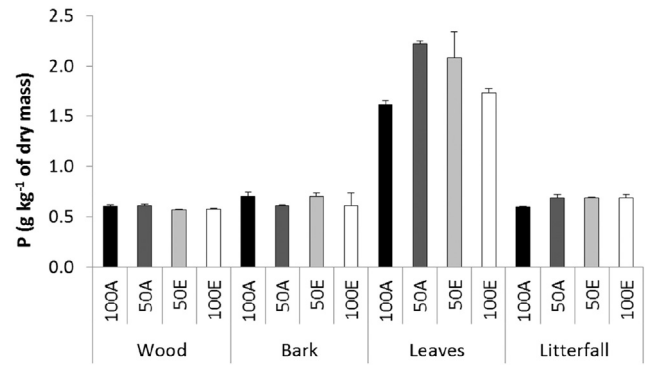
## 4. Discussion

Our previous studies of the first rotation (2004–2011) of a mixed-species plantation of acacia and eucalypt established on Ferralitic

**Table 2**

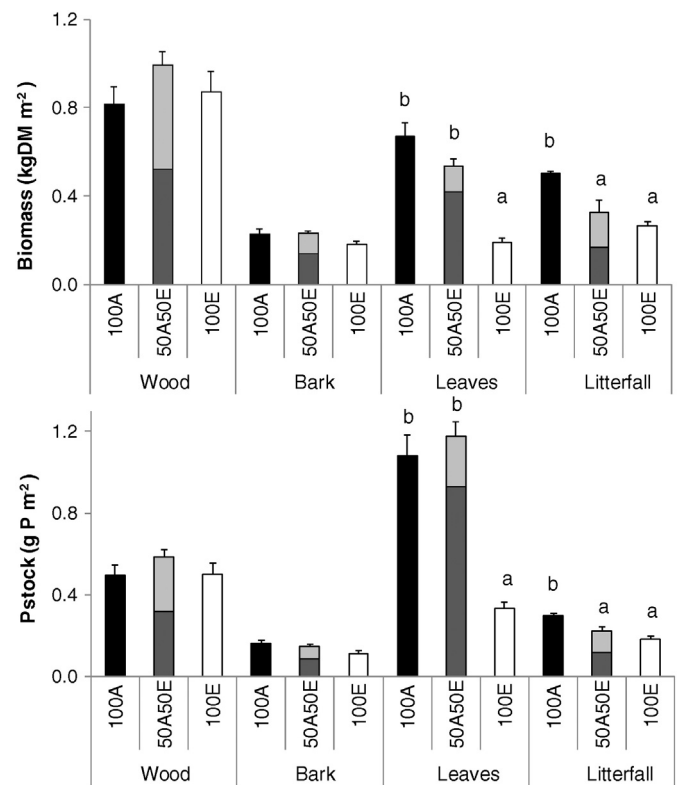
Hedley  $\text{P}_i\text{-HCO}_3$  and  $\text{P}_o\text{-HCO}_3$  concentrations ( $\text{mg kg}^{-1}$  of soil) in surface soil (0–5 cm) in pure acacia (100A), mixed-species (50A50E) and pure eucalypt (100E) stands at the end of the first rotation (EndR1) and at the age of two years of the second rotation (Y2R2). Each value is an average (with standard error) of three composite samples collected on three blocks (one per block). Stars indicate significant differences between the two ages ( $p < 0.05$ ). There was no significant difference between the three stands.

	100A	50A50E	100E
$\text{P}_i\text{-HCO}_3$			
EndR1	$4.7 \pm 1.0$	$3.2 \pm 0.2$	$3.8 \pm 0.8$
Y2R2	$1.7 \pm 0.7^*$	$2.9 \pm 0.4$	$2.7 \pm 0.5$
$\text{P}_o\text{-HCO}_3$			
EndR1	$42 \pm 16$	$28 \pm 1$	$32 \pm 8$
Y2R2	$45 \pm 12$	$42 \pm 10$	$32 \pm 18$



**Fig. 3.** P concentration in wood, bark, leaves and aboveground litter fall of acacia trees in pure (100A) or mixed-species stands (50A50E) and of eucalypt trees in pure (100E) and mixed-species (50E) stands at the age of two years of the second rotation. Mean values (with standard errors) of 4 replicates. Differences were not significant.

Arenosols of the Congolese coastal plains reveal that eucalypt growth benefits from the  $\text{N}_2$  fixed by acacia (Bouillet et al., 2013; Epron et al., 2013), even though soil resin available P decrease in the topsoil of the mixed-species stands relative to the pure eucalypt stands at the end of the first 7-year rotation (Koutika et al., 2014). Studies conducted on tropical unfertilized soils of naturally low fertility have shown that soil available P concentrations measured have an average content of only  $6.6 \text{ mg P kg}^{-1}$  (Cardoso et al., 2003; Gama-Rodrigues et al., 2014). The soils of the Congolese coastal plains have a higher average P content varying between 7 and  $15 \text{ mg P kg}^{-1}$  (Laclau et al., 2010; Koutika et al., 2014). The introduction of NFS such as acacia in forest plantations of Congolese coastal plains is known to lead to changes in P availability, as found in the other part of the world (Binkley, 1992; Kaye et al., 2000;



**Fig. 4.** Wood, bark and leaf biomass and aboveground litter fall of acacia tree in pure (100A) or mixed-species stands (50A50E) and of eucalypt tree in pure (100E) and mixed-species (50A50E) stands at the age of two years of the second rotation (upper panel) and P stocks in the same compartments (lower panel). Mean values (with standard errors) for five blocks. The different letters indicate that means are significantly different between stands for one compartment ( $p < 0.05$ ).



**Table 3**

Mean values (with standard errors) of the ratio of nitrogen to phosphorus concentrations (N:P) in the foliage of acacia trees in pure (100A) or mixed-species stands (50A50E) and of eucalypt trees in pure (100E) and mixed-species (50A50E) stands at the end of the first rotation (EndR1, in Koutika et al., 2014, n = 8) and at the age of two years of the second rotation (Y2R2, n = 4). The different letters indicate that means are significantly different between the two species growing either in pure or in mixed-species stands, and stars indicate significant differences between the two ages ( $p < 0.05$ ).

	100A	50A50E		100E
		Acacia	Eucalypt	
EndR1 <sup>a</sup>	14.6 ± 0.9b	12.9 ± 1.0b	9.9 ± 0.5a	9.4 ± 0.5a
Y2R2	18.5 ± 0.9b*	15.2 ± 2.2ab	11.3 ± 0.5a	13.1 ± 0.6a*

Yamashita et al., 2008). The effects of changes in soil P fertility are important for understanding overall plantation productivity over several rotations. Binkley et al. (2000) argued that P supply is not independent of P demand, since despite significantly lower soil P availability in *Albizia falcataria* stands (a NFS) relative to *Eucalyptus saligna* stands, *Albizia falcataria* did take up and cycle as much P as the eucalypt stand.

In response to our first question of a further decrease in available soil P in the mixed-species stands two year after the beginning of the second rotation, when most of the tree nutrient demand has to be absorbed from the soil (Laclau et al., 2003) where it is supplied by the decomposition of the harvest residues, we observed in contrast an increase in resin P in the pure eucalypt (100E) and the mixed-species (50A50E) stands compared to pure acacia (100A) stands at year 2 of the second rotation (Y2R2) relative to the end of the first 7-year rotation (EndR1). The lower value of soil resin P in 100A compared to 100E at Y2R2 probably indicates a higher acacia tree P uptake and a lower eucalypt P demand at Y2R2. This result is confirmed by the lower Pi-HCO<sub>3</sub> value in 100A at Y2R2 relative to EndR1. In contrast to eucalypt, acacia depleted more the available P (soil resin P and Pi-HCO<sub>3</sub>) at Y2R2 than at EndR1 (Fig. 2 and Table 2). This trend occurred even 100A stands had high P amount left after the harvest of the first rotation (2.9 g P m<sup>-2</sup>) than 100E stands (1.6 g P m<sup>-2</sup>, Table 1). This suggests that acacia stands may uptake soil inorganic P faster than it is released by the biological mineralisation of slash. Our findings are in line with Crews (1996), who argued that inorganic P fractions are critical sources of P in traditional agro-ecosystems, and probably the more targeted P fractions in low-input forest plantation systems such as ours, especially in the pure acacia stands.

In response to our second question, we observed, in contrast to our expectations, an overall increase in soil resin-P across 50A50E and 100E stands that may be related to the P released from harvest residues (Table 1). In fact, only the debarked commercial-sized boles were removed at harvest while branch, bark and leaves were left and equally distributed on the soil surface of each respective stand. Moreover besides the high P concentration in the slash of acacia left after harvest of the first rotation (Table 1), acacia stands seem to have a higher P uptake than do pure eucalypt stands (100E) as shown by: (i) their lower soil available P (both resin and Pi-HCO<sub>3</sub>; Fig. 2 and Table 2) relative to the pure eucalypt stands; (ii) a higher P stock in the foliage (1.08 mg P m<sup>-2</sup>) than in those of pure eucalypt (0.34 mg P m<sup>-2</sup>, Fig. 4); and probable higher rate of decomposition of acacia leaves compared to eucalypt leaves, as already shown in the same area (Bernhard-Reversat, 1993). This author has shown that, acacia litter has twice higher rate constant of decomposition compared to eucalypt litter (0.69 versus 0.35 yr<sup>-1</sup>) in spite of its higher lignin content, since its high N content led to a high rate of consumption by the meso and macrofauna.

Leaf N:P ratios (Table 3) are used to indicate N-limitation or P-limitation in ecosystems (Koerselman and Arthur, 1996; Güsewell, 2004; Han et al., 2005; Zhu and Cao, 2010; Fan et al., 2015). It is commonly assumed that a N:P ratio below 14 indicates N limitation while a N:P ratio above 16 suggests P limitation (Koerselman and Arthur, 1996). Thus, the high N:P ratio (18.5) in 100A stand at Y2R2 may suggest that P is becoming limiting for growth in relation with the observed

decrease in soil available P (Han et al., 2005). However, this N:P value was still below those encountered for many tropical forest species in P-deficient forest ecosystems (values above 20, Zhu and Cao, 2010), and much lower than the value reported for *Acacia mangium* in Malaysia (Inagaki et al., 2011). It is only occurring at the juvenile stage of the plantation when P may be less available than N, even though most of nutrient requirement is fulfilled by slash decomposition. High rate of N<sub>2</sub> fixation at young age may immobilize P in the root system. Interestingly, eucalypt trees seemed more N limited at EndR1 than at two years after the beginning of the second rotation. Lower N:P ratios in older eucalypt trees than in younger ones, despite lower soil available P, contrasted with results obtained in subtropical China on the same hybrids, where N:P increased from 17 at age 2 to almost 30 at age 6 (Fan et al., 2015). This further suggests that P is not limiting growth of eucalypt trees in our site.

It appears that in the studied low-input system of mixed-species plantations of acacia and eucalypt established on a nutrient-poor soil with less than 0.07% of N content (Koutika et al., 2014), soil P availability seems to depend not only on species identity or stand type, but also on stand age and rotation. At the end of the first rotation (2004–2011), it was shown that eucalypt growth has benefits from the N<sub>2</sub> fixed by acacia in these mixed-species stands (Bouillet et al., 2013; Epron et al., 2013) reducing N-limitation status of these poor soils (this study). In conclusion, despite a higher input of P in decomposing slash left after harvest, soil readily available inorganic P (resin and Pi-HCO<sub>3</sub>) was lower in stands containing acacia relative to pure eucalypt stands at Y2R2. This was due to higher P uptake by acacia trees, as suggested by the high P stock in acacia foliage related to high leaf biomass. However higher ratio of N:P concentrations in foliage of acacia stand at juvenile stage suggested that P is more limiting than N early in the rotation. While the threat of shifting from N-limitation to P-limitation after several rotations in the mixed-species stands as previously argued at the end of the first rotation is minor, our data however suggests that P-limitation may eventually occur in pure acacia stands in longer term. That raises questions about afforestation plans based on fast-growing exotic acacia monoculture and supports restricting its use in mixed-species plantations or in agroforestry systems.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi: <http://dx.doi.org/10.1016/j.geodrs.2016.03.001>. These data include the Google maps of the most important areas described in this article.

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