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Soil organic matter quality along rotations in acacia and eucalypt plantations in the Congolese coastal plains

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Abstract

Background: Afforestation of savannas in the Congolese coastal plains with eucalypt has provided wood pulp for industry and fuel energy for the local population. Typically, following afforestation, *Acacia mangium* are introduced to improve soil fertility and sustain productivity. Through investigations of particulate organic matter (POM), potential soil organic matter (SOM) quality was assessed in acacia and eucalypt plantations along rotations.

Methods: Nutrients in POM (4000–50 µm) in the 0–5 cm soil layer were measured after five years into the second rotation (R2Y5) in relation to soil pH and P availability. Data were compared to those at the end of the first 7-year-rotation (R1Y7) and after two years into the second rotation (R2Y2) to evaluate overall SOM quality in the topsoil.

Results: At R2Y5, soil pH was higher in the pure eucalypt stands (100E) than in stands containing acacia, either in monoculture (100A) or evenly mixed with eucalypt (50A50E). Coarse POM (cPOM, 4000–250 µm) beneath 100A had the highest N concentration (1.71%), followed by those beneath 50A50E (1.42%) and 100E (1.30%). Higher N was always found in the stands containing acacia. Lower sulphur (S) concentrations and P availabilities were observed in cPOM (50A50E). The greatest amount of coarse (414.7 g) and fine (214.5 g) forest floor litter were found in 100A stands, whereas higher C concentrations were found in the 100E stands for coarse forest floor litter (36.5%) and in the 50A50E stands for fine forest floor litter (38.7%). The decrease in cPOM N and C concentrations were lower than 20% (R1Y7) and 26% (R2Y5) relative to the younger stage (R2Y2). This tendency was more pronounced in fine POM (250–50 µm) and organo-mineral fraction (< 50 µm).

Conclusions: The main changes occurred in cPOM beneath stands containing acacia while higher weight of forest floor litter was found in 100A. Soil pH decreased in stands containing acacia. Overall N and C dynamics was enhanced in older stands (R2Y5) than in the younger stands (R2Y2). This may reveal a creation of more labile SOM with lower N and C concentrations in POM fractions in the surface layer, i.e., an ecosystem with a lower potential to mitigate climate change along rotations.

Keywords: Particulate organic matter, Nitrogen, Carbon, Phosphorus, Sulphur, Nitrogen-fixing species, Fast growing tree, Climate change mitigation

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Background

Soil organic carbon (SOC) is the main component of soil organic matter (SOM) (Platteau et al. 2006; Lal 2008). Fluxes of C from soil to atmosphere are partially regulated by the physical, and chemical characteristics of this SOM (Stewart et al. 2009; Kirkby et al. 2013), and is linked to other nutrients such as N, P and S (Binkley 1992; Sang et al. 2013; Kopittke et al. 2017). Factors such as soil texture, climate and relief have an undeniable impact on SOC status, i.e., on SOM quality and quantity (Pieri 1989; Hassink et al. 1993; Koutika et al. 1999; Galantini et al. 2004). SOC sequestration, i.e., taking up atmospheric CO₂ and converting it into soil C may potentially and simultaneously improve soil fertility, increase food and wood production and mitigate climate change (Lal 2004, 2008; www.4per1000.org). Land-use changes have a great impact on the status and dynamics of SOM and its further impact on SOC sequestration and climate change mitigation (Lal 2014). Land-use changes such as afforestation of unmanaged lands, pastures, savannas or cerrados alter SOM i.e., SOC inputs and decomposition, and, consequently, impact on C, N and P dynamics (Gonçalves et al. 2008; Pérez-Cruzado et al. 2012; Forrester et al. 2013; Epron et al. 2015; Dou et al. 2016; Tchichelle et al. 2017; Koutika 2019).

In the 1950's, native tropical savannas on inherently nutrient-poor soils in the Congolese coastal plains in the Republic of the Congo have been afforested using fast growing eucalypt (Makany 1964) with the primary goals of providing both wood for the pulp industry and fuel energy for the rural population (Delwaulle et al. 1978, 1981). Currently these plantations greatly contribute to preserving natural forests, since around 94% of Congolese homes use forest products as fuel in both wood and charcoal (Shure et al. 2010), and mitigate climate change by storing C in both soil and biomass i.e., increased soil C stocks and stand wood biomass (Epron et al. 2013; Koutika et al. 2014). Research conducted in the native tropical savannas in the Congolese coastal plains has regional importance. These savannas extend to around 6 million hectares in the countries of Central Africa, e.g., the Democratic Republic of the Congo, Gabon and the Republic of the Congo (Schwartz and Namri 2002). Nutrient supply in these inherently poor soils depends predominantly on decomposition of organic residues, since little or no replenishment is made with fertilizers (Laclau et al. 2005). Depletion in nutrient availability occurs rapidly after successive rotations inducing a decline in the productivity of fast-growing plantations (Laclau et al. 2005; Corbeels et al. 2005). As nitrogen-fixing tree species (NFS), acacia has been introduced since the 1990s to restore and improve soil fertility and sustain the productivity of eucalypt plantations in

the Congolese coastal plains (Bernhard-Reversat 1993; Bouillet et al. 2013; Epron et al. 2013; Koutika 2019).

During the past years, the benefits of mixed-species plantations of acacia and eucalypt in the Congolese coastal plains have been evaluated for multiple benefits, including (i) increased forest productivity (Bouillet et al. 2013; Epron et al. 2013); (ii) improved soil fertility i.e., increased cumulative net production of mineral N, soil N concentration and stock in bulk soil and fractions (Koutika et al. 2014; Koutika et al. 2017; Tchichelle et al. 2017); and (iii) sequestered C through increased soil C stock and concentration in bulk soil and fractions, and biomass (Epron et al. 2013; Koutika et al. 2014; Koutika et al. 2017). Both soil N and C accretions have been reported in mixed-species plantations of NFS and non-fixing species worldwide (Resh et al. 2002; Binkley 2005; Forrester et al. 2013; Bauters et al. 2015). This is due to enhanced below- and aboveground biomass and forest growth as result of higher soil N availability, i.e., N-fixation permits C accretion and tends to reduce C loss, thus contributing to climate change mitigation (Binkley 1992; Fornara et al. 2013). In addition to the above benefits reported in the Congolese coastal plains, an improvement in soil P status in the coarse fraction of POM (measured by an increase in resin extractable P in cPOM; 4000–250 µm) has been noticed in afforested stands with acacia or/and eucalypt relative to native savannas (Koutika and Mareschal 2017; Koutika 2019). However, probably due to the high P demand in supplying symbiotic root nodules and N₂ fixation processes of acacia trees (Binkley 1992; Inagaki et al. 2011), a decrease in P availability and soil pH in afforested stands with acacia alone or in mixture with eucalypt relative to pure eucalypt stands has been observed (Koutika et al. 2014; Koutika et al. 2016).

Particulate organic matter (POM) is an early SOM indicator for evaluating changes in different ecosystems, e.g., forests, pastures and cropping systems (Cambardella and Elliot 1992; Sikora et al. 1996). According to soil type and characteristics, POM status may be determined by physicochemical (Vanlauwe et al. 1999; Koutika et al. 2001) or physical procedures (Epron et al. 2015; Koutika et al. 2017). It is a useful parameter since it relates to labile SOM and responds readily to soil management when no changes have been yet noticed in the bulk soil (Koutika et al. 2001, 2002; Wander 2004). Removing both the aboveground litter and harvest residues reduces soil C accretion in monocultural eucalypt plantations in the long-term, where three rotations each of seven years, i.e., a total of 21 years were evaluated in the Congolese coastal plains (Epron et al. 2015).

This study determines SOM dynamics and changes in soil pH in the Ferrallic arenosol along with rotations of acacia and eucalypt plantations established in the

Congolese coastal plains. Five questions were addressed: (i) would SOM mineralisation be enhanced through POM (4000–50 μm) mainly in the coarse fraction (4000–250 μm); (ii) would P availability decrease beneath mixed-species plantations at year 5 of the second rotation? (iii) how would POM dynamics and soil pH change over time, i.e., from R1Y7 to R2Y2 and R2Y5, knowing that high amounts of litterfall, forest floor and harvest residues were left after the first harvest and had accumulated for five years into the second rotation? (iv) would the trend previously observed in the second rotation at R2Y2 compared with R1Y7, i.e., higher N concentration in coarse POM (4000–250 μm) in afforested stands with acacia (100A and 50A50E) than in 100E, persist at R2Y5? and (v) how would changes in SOM status affect the potential of the ecosystem to mitigate climate change? To answer these questions we conducted the following analyses: (i) assessment of N, C and S concentration in bulk soil, POM fractions and forest floor, soil pH and available P status at year 5 of the second rotation (R2Y5); and (ii) evaluation of the overall SOM dynamics through POM (N, C and S concentration) status and changes in soil pH, from the end of the first 7-year-rotation (R1Y7) to year 2 of the second rotation (R2Y2) till the current study (R2Y5).

Materials and methods

Site description

The experimental site is located on a plateau close to Tchissoko village in the Republic of the Congo, 35 km from Pointe-Noire (4°44'41'' S & 12°01'51'' E, 100 m Alt.) on a deep Ferralic arenosol laying on geological bedrock composed of thick detritic layers of continental origin dated from the plio-pleistocene era. These soils are characterized by a low CEC (< 0.5 $\text{cmol}_c\text{kg}^{-1}$), a high sand content (> 90% of the mineral soil), very low clay and silt content (6% and 2%, respectively) and low iron oxide content (< 1.5% of the bulk soil, Mareschal et al. 2011). They are acidic with low C (< 1.2%) and N content (> 0.06%) (Koutika et al. 2014). The climate in the area is sub-equatorial with high mean annual air humidity and air temperature (85% and 25 °C, respectively) and low seasonal variation (about 2% and 5 °C, respectively). Mean annual precipitation is 1200 mm with a dry season extending from June to September. The original vegetation was a native tropical savanna dominated by the C_4 poaceae *Loudetia arundinacea* (Hochst.) Steud, which was afforested in 1984 with planted eucalypt hybrids.

An experimental trial was installed in May 2004, which included pure stands of *Acacia mangium* (100A) and of *Eucalyptus urophylla* \times *E. grandis* (100E) and mixed-species stands with 50% of acacia and 50% of eucalypt (50A50E) in five replicates (randomized block design) at

a density of 800 trees·ha⁻¹. Each stand covered 1250 m² and contained 100 trees (10 \times 10) including two buffer rows and an inner part of 36 trees (Epron et al. 2013). This first rotation was harvested in January 2012 at seven years old. A second rotation was planted with the same design in March 2012 (Koutika et al. 2016; Tchichelle et al. 2017).

Forest floor

The forest floor litter was sampled in May 2017 with a square metallic frame (50 cm \times 50 cm) on 4–6 locations within each stand. The samples were oven-dried at 65 °C during 72 h and weighed. The forest floor nitrogen ($\text{kg}\cdot\text{m}^{-2}$ of N) was calculated from the dry mass. N, C and S concentrations was evaluated using a Macro VARIO Cube Elemental Analyser (Germany) (see Epron et al. 2013; for details), while and P availability was determined by resin extraction (see more details below).

Soil and forest floor analyses

Soil sampling

As most soil organic matter is concentrated in the upper layer in the area (d'Annunzio et al. 2008), soil was collected with an auger at 0–0.05 m depth in March 2017 at year 5 of the second rotation (R2Y5). Soil was sampled beneath three stands, the pure acacia (100A), and eucalypt (100E), and mixed-species (50% acacia and 50% eucalypt, 50A50E) in nine replicates by stands in three out of the five blocks. Three transects in 100A and 100E stands and six in 50A50E stands were set up in the inner part of each stand, starting at the base of a tree and ending in the center of the area delimited by four trees. Three cores were sampled on each transect, each sampling point being separated by 0.7 m from each other along the transect. The total number of sampling points was 27 (9 \times 3 blocks) in 100A and 100E and 54 (18 \times 3 blocks) in 50A50E. Soil samples were air-dried and sieved at 4 mm. Soil pH in H₂O and 1 mol·L⁻¹ KCl (sample:liquid ratio, 1:5) were measured using a S47 SevenMulti TM (Mettler Toledo, Switzerland) after the suspensions were shaken for 30 min and equilibrated for one hour.

Particulate organic matter fractionation

Since the studied soils are sandy and contain low Fe oxide (Mareschal et al. 2011), the physical fractionation of POM was done according to Epron et al. (2015), and was followed by decantation to separate organic from mineral material as follows: 20 g of air-dried soil (sieved < 4 mm), 5 glass beads and 50 mL of distilled water were put in a 100-mL plastic bottle, and shaken for 16 h at 20 °C in an end-over-end shaker at 40 rpm (6 rotations·min⁻¹). The suspension was wet-sieved to separate the 4000–250, 250–50 and 0–50 μm fractions. In the two

larger fractions, the organic components were separated from the mineral fraction by decantation. The following fractions were obtained: coarse POM (cPOM, 4000–250 μm), fine POM (fPOM, 250–50 μm), organo-mineral fraction (OMF, < 50 μm), and the coarse and fine mineral fractions (cMIN 4000–250 μm and fMIN, 250–50 μm). Of these, only cPOM, fPOM and OMF composed of organic material are considered in this study. All fractions were dried at 45 °C for 72 h and weighed.

The mass percentage of N, C and S concentrations in bulk soil, cPOM, fPOM, and OMF fractions were determined simultaneously using a Macro VARIO Cube Elemental Analyser (Germany). Available P in bulk soil, soil fractions and forest floor litter were determined by resin extraction. Two anion-exchange resin strips (BDH#551642S) each 20 mm \times 60 mm were added to 0.5 g (soil) or 0.1–0.2 g (POM fraction) and suspended in 30 ml distilled water. Phosphate adsorbed by the anion-exchange resin was recovered in 20 mL of 0.5 mol·L⁻¹ HCl after shaking for 16 h (100 revs·min⁻¹) according to the method of Tiessen and Moir (2008). Malachite reactive P was determined at 630 nm with a JENWAY 6305 Spectrophotometer (UK).

Statistical analyses

Statistical analyses were performed with R software version 3.2.4 (R Core Team 2016). Similar analyses were performed to compare data of 7 years of the first rotation to 2 years of the second rotation (Koutika et al. 2017). The total number of samples in this study was 108, i.e., 27 (9 \times 3 blocks) replicates were used for 100A and 100E stands, and 54 (18 \times 3 blocks) for the mixed-species stands (50A50E). For the analysis at 5 years of the second rotation (R2Y5), a mixed-effect model (Bates et al. 2015) with blocks as random effects was used to estimate the effects of stand types (100A, 100E and 50A50E) as fixed effect. For the analysis at the three dates, i.e., the end of the first 7-year rotation (R1Y7), 2 (R2Y2) and 5 years (R2Y5) of the second rotation, a mixed-effect model (Bates et al. 2015) with blocks as random effects was used to estimate the effects of stand types (100A, 100E and 50A50E) and time (R1Y7, R2Y2 and R2Y5) as fixed effects.

Results

Water and KCl pH

At year 5 of the second rotation (R2Y5), both afforested stands with acacia, i.e., 100A and 50A50E had significantly lower soil pH (H₂O) than pure eucalypt (100E) stands (4.2) (Table 1). There were no significant differences in pH-KCl between stands. The difference between pH-H₂O and pH-KCl i.e., delta pH (ΔpH) was significantly higher in the 100E stand (Table 1). Along rotations, i.e., from the end of the first 7-year-rotation (R1Y7) to year 2 (R2Y2) and 5 (R2Y5) of the second rotation, soil pH-H₂O was higher in 100E, and lower in 100A at R1Y7 and R2Y2. There was no significant difference between pH-KCl in the three stands along the rotations, except for the significantly lower value in 50A50E relative to others at R2Y2 (Table 1). A higher change in soil pH was found in 100E and a lower change in soil pH was found in 100A at both R1Y7 and R2Y5, while no difference between stands was observed at R2Y2 (Table 1).

Concentrations and quality of POM at year 5 of the second rotation (R2Y5)

Weight of POM and organo-mineral fractions

There was no difference in the weight of coarse (cPOM, 4000–250 μm), fine (250–50 μm) POM or organo-mineral (< 50 μm) fractions based on 20 g fractionated soil between all studied stands (Table 2). This tendency remained when the weight was estimated in gram per kg of soil (Table 2).

N, C, and S concentrations and P availability in bulk, POM and organo-mineral fractions

cPOM (4000–250 μm) beneath 100A had significantly higher N concentration (1.71%) than in either 50A50E and 100E stands (Fig. 1a). As for the bulk soil, no difference was found between stand types for fPOM and OMF. There was no difference in C concentration between stand types for bulk soil and all fractions (Fig. 1b). Sulphur concentration was significantly lower in the cPOM beneath 50A50E (0.17%) relative to 100A (0.21%) stands, while there was no difference between bulk soil and fractions (Fig. 1c). The significantly lower P availability was still observed in the cPOM in 50A50E (4.6

Table 1 Soil pH change along rotations from the end of the first 7-year-rotation (R1Y7), to year 2 of the second rotation (R2Y2) and 5 of the second rotation (R2Y5)

	R1Y7			R2Y2			R2Y5		
	100A	50A50E	100E	100A	50A50E	100E	100A	50A50E	100E
pH-H ₂ O	4.2 (\pm 0.03) c	4.4 (\pm 0.02) b	4.5 (\pm 0.04) a	4.4 (\pm 0.02) a	4.3 (\pm 0.03) a	4.4 (\pm 0.03) a	3.9 (\pm 0.05) b	4.0 (\pm 0.03) b	4.2 (\pm 0.04) a
pH-KCl	3.5 (\pm 0.02) a	3.5 (\pm 0.02) a	3.5 (\pm 0.03) a	3.3 (\pm 0.02) a	3.2 (\pm 0.02) b	3.3 (\pm 0.03) a	3.5 (\pm 0.02) a	3.5 (\pm 0.02) a	3.5 (\pm 0.04) a
Delta pH (ΔpH)	0.8 (\pm 0.03) c	0.9 (\pm 0.01) b	1.0 (\pm 0.02) a	1.1 (\pm 0.02) a	1.1 (\pm 0.02) b	1.1 (\pm 0.03) a	0.4 (\pm 0.06) ab	0.5 (\pm 0.03) b	0.6 (\pm 0.05) a

Different letters indicate significant differences at $P = 0.05$ between stand types at each rotation stage and along rotations

Table 2 Weight of particulate organic matter (POM 4000–50 μm) fractions at year 5 of the second rotation (R2Y5)

POM fraction weight /Stands	100A	50A50E	100E
After fractionation of 20 g of soil:			
cPOM	0.14 (\pm 0.01) a	0.18 (\pm 0.01) a	0.19 (\pm 0.03) a
fPOM	0.35 (\pm 0.02) a	0.39 (\pm 0.02) a	0.40 (\pm 0.03) a
OMF	2.01 (\pm 0.06) a	1.61 (\pm 0.17) a	1.81 (\pm 0.07) a
In g per kg of soil:			
cPOM	6.87 (\pm 0.52) a	8.93 (\pm 0.47) a	9.47 (\pm 1.42) a
fPOM	17.71 (\pm 1.01) a	19.53 (\pm 0.28) a	19.77 (\pm 1.42) a
OMF	100.7 (\pm 3.2) a	88.7 (\pm 1.6) a	90.3 (\pm 3.7) a

100A and 100E = pure acacia and eucalypt, respectively, while 50A50E = mixed-species (50% acacia and 50% eucalypt) stands. cPOM = coarse POM (4000–250 μm), fPOM = fine POM (250–50 μm) and OMF = organo-mineral fractions (< 50 μm). Different letters indicate significant differences at $P = 0.05$ between stand types at R2Y5

mg $\text{P}\cdot\text{kg}^{-1}$) relative to 100E and 100A stands (> 6 mg $\text{P}\cdot\text{kg}^{-1}$, Fig. 1d), and no difference was found elsewhere. P availability was extremely low at R2Y5 compared to other periods (Fig. 1).

Weight and C concentration of forest floor litter

The higher dry weights of forest coarse and fine litter were both found in 100A (414.7 and 214.5 g, respectively) relative to the other two stand types (< 200 and 160 g, respectively, Fig. 2a). When the weight was expressed in gram per m^2 , the tendency remained; the significantly higher value for both coarse (1.58 $\text{g}\cdot\text{m}^{-2}$) and fine (0.81 $\text{g}\cdot\text{m}^{-2}$) forest floor litter were still found in 100A (Fig. 2b). There was no difference in the N and S concentrations and P availability in both coarse and

fine forest floor litter in all studied stand types (Data not shown). C concentration in the coarse forest floor litter was significantly higher in the 100E stands (36.5%), while the higher in fine forest floor was observed in 50A50E (38.7%) (Fig. 2c).

SOM dynamics along rotations: from the end of the first 7-year-rotation (R1Y7) to year 2 (R2Y2) and 5 (R2Y2) of the second rotation

N and C concentration in bulk soil and soil fractions

Higher N concentration was found in bulk soil at year 2 of the second rotation (R2Y5) relative to year 7 of the first rotation (R1Y7) and year 2 of the second rotation (R2Y2) (Fig. 3a). Stands containing acacia had higher N concentrations, particularly in cPOM of the

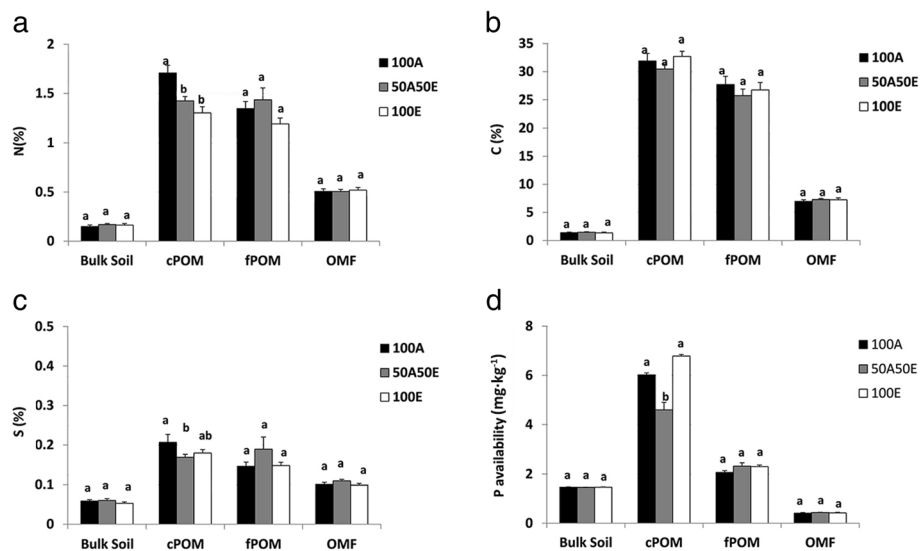
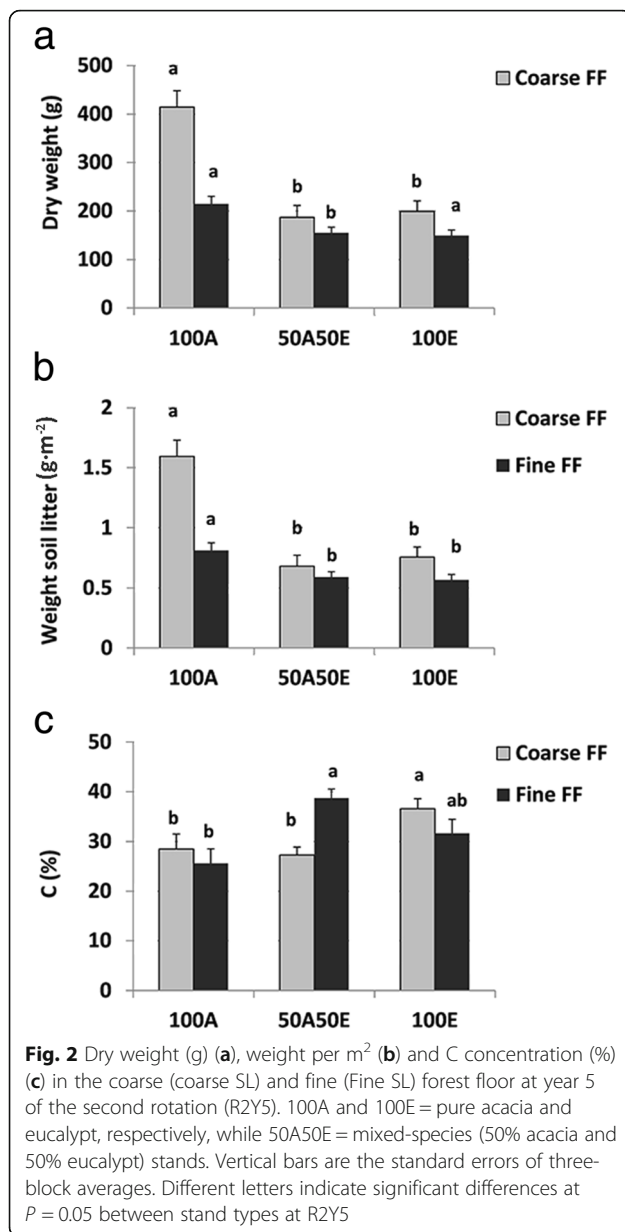


Fig. 1 Nitrogen (N) concentration (%) (a), C (b), sulphur (c) and phosphorus availability (d) in the bulk soil and particulate organic matter (POM 4000–50 μm) fractions at year 5 of the second rotation (R2Y5). 100A and 100E = pure acacia and eucalypt, respectively, while 50A50E = mixed-species (50% acacia and 50% eucalypt) stands. cPOM = coarse POM (4000–250 μm), fPOM = fine POM (250–50 μm) and OMF = organo-mineral fractions (< 50 μm). Vertical bars are the standard errors of three-block averages. Different letters indicate significant differences at $P = 0.05$ between stand types at R2Y5



pure acacia stands (100A) along rotations (Fig. 3b). For instance, higher N concentration was found in cPOM beneath 100A at R2Y2 relative to R1Y7 and R2Y5. This tendency was less pronounced in fPOM, except in 100A at R2Y2 (Fig. 3c). Higher N concentration in OMF was observed at R2Y2 of all stands, while R2Y5 had lower values (Fig. 3d). C concentration in bulk soil beneath 100A stand was significantly lower than 100E and 50A50E stands at R2Y2 (Fig. 4a). Higher C concentration was often observed in all stands at R2Y2 relative to R1Y7, with a more pronounced tendency to R2Y5 although at this stage, lower C concentrations were found in all soil fractions (Figs. 4b, c & d). Bulk soils had a low C to N ratios, ranging from 9.7 (100E) to 11.3 (100A) at

R2Y5 (Table 3), as a consequence of relative high N concentration (Fig. 3a). The mass fraction of cPOM in the three stand types at R2Y2 was significantly higher relative to R1Y7 and R2Y5, while the highest values for fPOM were at R2Y5 (Table 3). Overall both N and C concentrations decreased (Table 4). Both C and N concentrations were much lower at R1Y7 and R2Y5 than at R2Y2. The decrease in N and C (cPOM), at R1Y7 was less than 20%, compared with R2Y2 (Table 4). The higher values were observed at R2Y5 mainly in fPOM and ranged between 10%–35% for both N and C and 20%–35% for both N and C in OMF.

Discussion

SOM dynamics through cPOM

Even though there was no difference in mass of POM fractions between stands at R2Y5 in the current study (Table 2), N concentrations were higher in the cPOM (4000–250 μm) beneath 100A (Fig. 1a), and coarse and fine forest floor necromass were higher (Figs. 2a & b), but its C concentrations were both lower (Fig. 2c). Initially changes in POM (4000–50 μm) status, one of the most active parts of SOM, occurred in its coarse (4000–250 μm) fraction, through its quality, i.e., concentrations in N, C, P availability, etc. (Koutika et al. 2001; Koutika et al. 2017; Koutika and Mareschal 2017), or/and quantity i.e., mass (Epron et al. 2015). In a study on reforestation of tropical pastures in Puerto Rico estimating SOM dynamics through turnover times of C in the bulk soil and in density fractions during 80 years, Marin-Spiotta et al. (2009) identified the physically unprotected POM as the fraction most sensitive to land-use change. In another study in Central China, the effects of land-use change during afforestation of large uncultivated areas showed increased soil C and N storage, mainly in macroaggregates (> 2000 μm) (Dou et al. 2016).

The higher N concentration in cPOM and weight of coarse and fine forest floor litter in 100A observed in the current studies are in accordance with previous findings that the higher cumulative net production of mineral N over the two first years of the second rotation in 100A (343 $\text{kg}\cdot\text{ha}^{-1}$) relative to 100E (189 $\text{kg}\cdot\text{ha}^{-1}$) (Tchichelle et al. 2017). Even though P may be retranslocated along the tree growth period (Inagaki et al. 2011), the enhanced SOM dynamics in the pure acacia (100A) stands in the current study may be also linked to the high requirement of NFS for available P (Binkley 1992). This high requirement for P probably decreased the resin available P in the 0.15 m in 100A relative 100E stands and soil readily available inorganic P (resin and $\text{Pi}\cdot\text{HCO}_3$), i.e., 1.7 vs 2.17 $\text{mg}\cdot\text{kg}^{-1}$ for $\text{Pi}\cdot\text{HCO}_3$ previously reported at year 2 of the second rotation i.e., R2Y2 (Koutika et al. 2016), but also in cPOM of the mixed-species (50A50E) stands at R2Y2 (Fig. 1d). Even though

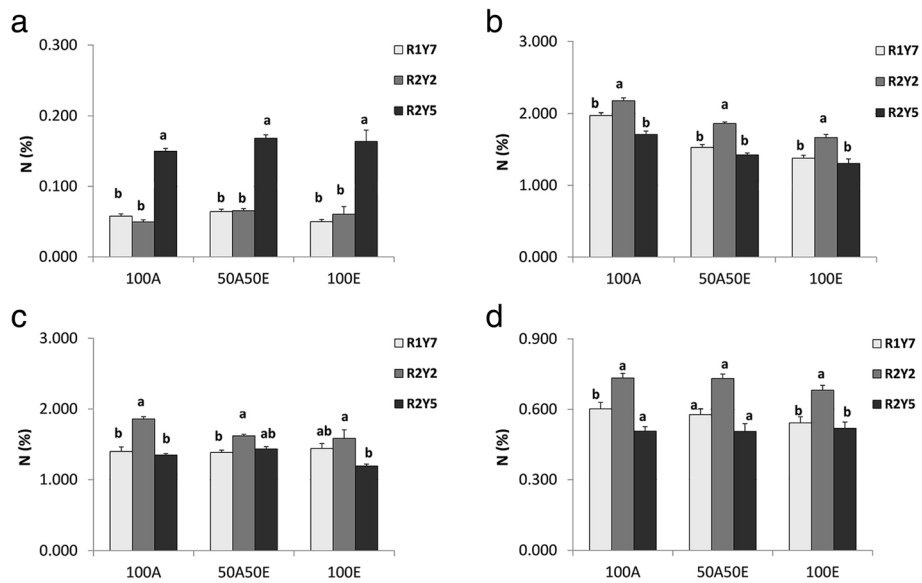


Fig. 3 N concentration (%) in bulk soil (a), coarse POM (cPOM 4000–250 μm, b), fine POM (fPOM 250–50 μm, c) and organo mineral fraction (OMF 0–50 μm, d) along rotations. 100A and 100E = pure acacia and eucalypt, respectively, while 50A50E = mixed-species (50% acacia and 50% eucalypt) stands. R1Y7 = Year 7 of the first rotation; R2Y2 = Year 2 of the second rotation and R2Y5 = Year 5 of the second rotation. Vertical bars are the standard errors of three-block averages. Different letters indicate significant differences at $P = 0.05$ between stand types at each rotation stage and along rotations

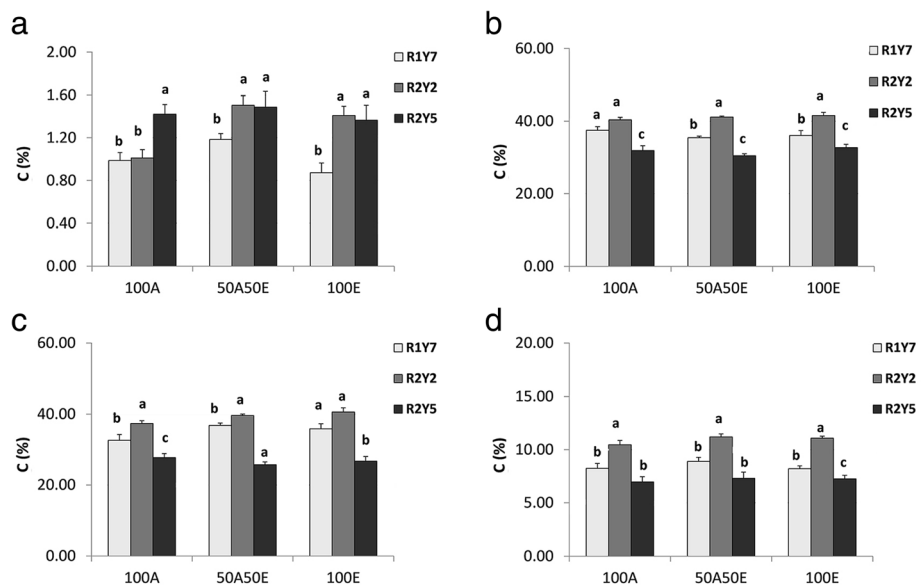


Fig. 4 C concentration (%) in bulk soil (a), coarse POM (cPOM 4000–250 μm, b), fine POM (fPOM 250–50 μm, c) and organo mineral fraction (OMF 0–50 μm, d) along rotations. 100A and 100E = pure acacia and eucalypt, respectively, while 50A50E = mixed-species (50% acacia and 50% eucalypt) stands. R1Y7 = Year 7 of the first rotation; R2Y2 = Year 2 of the second rotation and R2Y5 = Year 5 of the second rotation. Vertical bars are the standard errors of three-block averages. Different letters indicate significant differences at $P = 0.05$ between stand types at each rotation stage and along rotations

Table 3 CN ratio of bulk soil and soil fractions (cPOM; fPOM and OMF) and mass of fractions along rotations from the end of the first 7-year-rotation (R1Y7), to year 2 of the second rotation (R2Y2) and 5 of the second rotation (R2Y5)

Soil/fractions	R1Y7			R2Y2			R2Y5		
	100A	50A50E	100E	100A	50A50E	100E	100A	50A50E	100E
CN bulk soil	16.9 (± 0.054) a	17.8 (± 0.48) a	17.1 (± 0.51) a	20.2 (± 0.43) a	22.7 (± 0.19) b	25.0 (± 0.27) a	11.3 (± 0.90) b	10.4 (± 0.66) b	9.7 (± 0.95) a
CN cPOM	19.4 (± 0.056) a	23.9 (± 0.68) b	26.5 (± 0.91) c	18.6 (± 0.16) a	22.2 (± 0.19) b	25.0 (± 0.27) a	18.8 (± 0.36) b	21.5 (± 0.27) b	25.9 (± 0.88) a
CN fPOM	24.1 (± 1.68) a	27.1 (± 0.72) ab	25.4 (± 0.81) a	20.2 (± 0.32) a	24.7 (± 0.37) b	25.7 (± 0.41) a	20.8 (± 0.60) b	20.3 (± 0.77) b	22.8 (± 0.79) a
CN OMF	13.6 (± 0.034) a	15.3 (± 0.27) b	14.9 (± 0.43) b	14.3 (± 0.17) a	15.3 (± 0.18) b	16.2 (± 0.30) a	14.1 (± 0.33) b	14.9 (± 0.32) b	14.2 (± 0.31) a
cPOM (fraction per g)	0.11 (± 0.001) a	0.15 (± 0.02) b	0.10 (± 0.01) a	0.41 (± 0.03) a	0.49 (± 0.03) b	0.34 (± 0.03) a	0.14 (± 0.01) b	0.18 (± 0.01) b	0.19 (± 0.03) a
fPOM (fraction per g)	0.20 (± 0.002) b	0.19 (± 0.02) b	0.17 (± 0.02) a	0.23 (± 0.01) a	0.30 (± 0.01) b	0.28 (± 0.02) a	0.35 (± 0.02) b	0.39 (± 0.02) b	0.40 (± 0.03) a
OMF (fraction per g)	1.91 (± 0.005) b	1.86 (± 0.04) a	1.83 (± 0.07) a	1.47 (± 0.03) a	1.64 (± 0.03) b	1.56 (± 0.05) a	2.01 (± 0.06) b	1.77 (± 0.03) b	1.81 (± 0.07) a

100A and 100E = pure acacia and eucalypt, respectively, while 50A50E = mixed-species (50% acacia and 50% eucalypt) stands. cPOM = coarse POM (4000–250 µm), fPOM = fine POM (250–50 µm) and OMF = organo-mineral fractions (< 50 µm). Different letters indicate significant differences at $P = 0.05$ between stand types at each rotation stage and along rotations

there was no difference in C concentration of bulk soil and POM fractions between the three studied stands, the lower C concentration in coarse and fine litter beneath pure acacia may be explainable by larger C net ecosystem productivity in eucalypt stands relative to acacia stands. For example, in Guangdong Province of southern China, Chen et al. (2011) found net ecosystem productivity of $1960 \pm 178 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ for *E. urophylla* but $330 \pm 76 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ for *A. crassicarpa*.

Contrary to our findings, Pereira et al. (2018) found differences in mass of POM fractions at the juvenile stage of a first rotation (27 to 39th months of the trees). SOM dynamics depends on different factors including soil texture, climate and relief; for instance, soils at Tchissoko in the Congolese coastal plains are Ferralic arenosols (with a ratio of clay: silt: sand of 3:6:91), while those at Itatinga in Brazil are Ferralsols (with a ratio of clay: silt: sand of 13:3:84). All differences between the two sites, including climate and relief, have been well documented in Epron et al. (2013), and led to a positive effect of growing eucalypt with acacia on stand wood production at Tchissoko but not at Itatinga. In the same line, Voigtlaender et al. (2012) did not find any differences in N and C stocks beneath the pure acacia (100A), eucalypt (100E) and mixed-species (50A50E) stands in the 0–15 cm soil layer after 6 years of plantation at Itatinga. Koutika et al. (2014) reported an increase in both C and N stocks in the 0–25 cm beneath 50A50E relative to 100E after 7 years of plantation in the Congolese coastal plains.

Enhanced SOM dynamics inferred by cPOM status were also highlighted by the lower concentration in S and available P in the 50A50E relative to 100A and 100E

stands at R2Y5 (Fig. 1c & d). Besides, the common high requirement in available P for the symbiotic fixation of atmospheric N_2 by NFS (Binkley 1992), changes occurring in 50A50E stands are probably due to enhanced bacterial community composition (Pereira et al. 2017). This may be due probably to high biomass wood production previously observed by Epron et al. (2013) in mixed-species stands of the plantation established in the Congolese coastal plains. In addition to above benefits in the 50A50E stands, an increase in bulk soil N and C stocks down to 0.25 m in 50A50E have been also reported at the end of the first 7 years in the Congolese coastal plains, as mentioned in the previous section (Koutika et al. 2014).

High soil N contents can enhance SOC accretion due to increases in both below- and aboveground biomass and forest growth (Binkley 1992; Epron et al. 2013; Fornara et al. 2013). In addition to the effect of N on SOM quantity, N-fixing trees might also impact on SOM dynamics due to biological changes stimulated by the input of N-rich litter (Binkley 2005) or enhanced bacterial community composition or changed in microbial indicators (Pereira et al. 2017, 2018). In the Congolese coastal plains, the faster cycling of soil N beneath stands containing acacia relative to eucalypt has been already reported at the end of 7 year-first rotation (R1Y7) by the 30% and 10% higher N concentration in cPOM in 100A ($19.7 \pm 0.8 \text{ g} \cdot \text{kg}^{-1}$) and 50A50E ($15.3 \pm 0.4 \text{ g} \cdot \text{kg}^{-1}$), relative to eucalypt ($13.8 \pm 0.5 \text{ g} \cdot \text{kg}^{-1}$) (Koutika et al. 2017). This tendency remained at year 2 of the second rotation (R2Y5) with N concentration, which was still higher in cPOM in soil of stands containing acacia by 23% ($21.7 \pm 0.4 \text{ g} \cdot \text{kg}^{-1}$ for 100A) and 11% ($18.6 \pm 0.2 \text{ g} \cdot \text{kg}^{-1}$ for

Table 4 Decrease (in percentages) in N and C concentrations in soil fractions along rotations from the end of the first 7-year-rotation (R1Y7), to year 2 of the second rotation (R2Y2, values used as references) and 5 of the second rotation (R2Y5)

Fractions	cPOM (4000–250 μm)				fPOM(250–50 μm)				OMF (0–50 μm)						
	Reference (R2Y2)		R2Y5		Reference (R2Y2)		R2Y5		Reference (R2Y2)		R2Y5				
	N%	C%	N decrease (%)	C decrease (%)	N%	C%	N decrease (%)	C decrease (%)	N%	C%	N decrease (%)	C decrease (%)			
100A	2.2	40	9	7	21	21	21	13	27	26	26	18	21	31	33
50A50E	1.9	41	18	13.9	24	26	7	11	25	35	35	21	21	31	35
100E	1.7	42	17	13.2	22	21	12	25	34	34	20	20	26	24	35

100A and 100E = pure acacia and eucalypt, respectively, while 50A50E = mixed-species (50% acacia and 50% eucalypt) stands. cPOM = coarse POM (4000–250 μm), fPOM = fine POM (250–50 μm) and OMF = organo-mineral fractions (> 50 μm). Different letters indicate significant differences at P = 0.05 between stand types at each rotation stage and along rotations

50A50E) relative to eucalypt ($16.7 \pm 0.2 \text{ g}\cdot\text{kg}^{-1}$). This responds to our first question, i.e., SOM dynamics is enhanced through POM status and mainly its coarse fraction (4000–250 μm), while available P decreased in cPOM beneath mixed-species stands at year 5 of the second rotation (R2Y5).

The link between N dynamics and C sequestration depends on several factors such as soil texture, water availability and pH and the importance of N in C sequestration processes and has been highlighted in several studies (Binkley 2005; Lal 2014; Li et al. 2012). The decrease in C concentration of POM fractions and OMF noticed at R2Y5 relative to the younger stage of rotation (R2Y2) i.e., between 20%–26% for cPOM, 25%–35% for fPOM and 20%–35% for OMF may probably be due to an enhanced C mineralisation and decreased soil pH values (Table 1), involved by the high input of new organic residues along rotations, i.e., with the age of rotation. Studies conducted in the monoculture eucalypt plantations located in the Congolese coastal plains have shown that the input of labile substrate does alter the microbial community composition, potential metabolic activities, and the SOC pools utilisation (Derrien et al. 2014). Overall N and C dynamics in bulk soil and fractions is, therefore, showing an enhanced SOM i.e., N and C mineralization along rotations i.e., from R1Y7 to R2Y2 and R2Y5. For instance, when N dynamics is evaluated only through bulk soil, a higher N concentration was found at R2Y5 (Fig. 3a) along with very low CN ratio of bulk soil varied between 9.7 (100E) and 11.3 (100A) (Table 3), unusual in these soils. However, through POM dynamics, i.e., N and C concentrations in POM fractions, it appears that both N and C mineralization is enhanced with the age and length of rotation. This may reveal that introducing acacia in eucalypt plantations did involve N and C accretion in soil and POM fractions at the end of the first rotation (R1Y7) and at year 2 of the second rotation (R2Y2) as reported previously (Koutika et al. 2014; Koutika et al. 2017). However, the impact of edaphic factors and climate (sandy soil, high temperature and atmospheric humidity) in the Congolese coastal plains and the effects of new organic residues rich in N enhanced SOM dynamics i.e., mineralization of both N and C, especially C (Table 4), but also decreased soil pH values (Table 1). Even though higher soil N availability usually permits C accretion and tends to reduce C loss and potentially contributes to climate change mitigation (Binkley 1992; Fornara et al. 2013), high N status at R2Y5 (Fig. 3a) did involve higher C concentration beneath 100A relative to other periods (Fig. 4a). Previous studies in the area reported that SOM decomposition may be enhanced due to climate and edaphic factors, but also to newly add

organic residues (d'Annunzio et al. 2008; Epron et al. 2013; Derrien et al. 2014). This also shows that changes in POM dynamics and soil pH along rotations, i.e., from R1Y7 to R2Y2 and R2Y5, have been affected by the high amounts of litterfall, forest floor and harvest residue left after the first harvest and accumulated along the second rotation, and responded to the third question of our paper.

Even though N and C increased in the mixed-species stands at the end of the first 7-years of rotation (R1Y7) and in 100A stands at year 2 of the second rotation (R2Y2) relative to 100E, N and C accretion appeared to have reached a threshold at R2Y5. SOM dynamics in the mixed-species plantations may therefore also be limited by the potential low SOM saturation of these sandy soils (Hassink 1997; Marin-Spiotta et al. 2009; Epron et al. 2015). Enhanced N and C mineralization along rotations with increasing age suggests that the ecosystem is fragile and both N and C accretion have a limit, reducing the potential beneficial effect of the practice on both soil fertility improvement and climate change mitigation, and responds to the fourth and fifth question of this paper. Soil acidification has been observed along rotations through a decrease in soil pH H_2O values in stands containing acacia relative to pure eucalypt counterparts (Table 1), while P availability reached a very low level never previously recorded in these soils. These findings are in accordance with previous studies worldwide showing that soil acidification or a decrease in available P occurs when NFS fixing atmospheric N_2 are introduced (Binkley 1992; Binkley 2005; Inagaki et al. 2011; Koutika et al. 2014)

Conclusions

Even when changes occurring in SOM quality were not yet detectable in the bulk soil, they could be observed through changes in POM status, especially its coarse fraction (4000–250 μm) in stands containing acacia (100A and 50A50E). This has been shown by higher N concentration in the pure acacia (100A) or the lower S concentration and P availability in the mixed-species (50A50E), probably due to an enhanced both organic residues and mineralisation, decline in soil pH values and shift in microbial community composition and microbial indicators. SOM mineralisation was enhanced, while soil pH and P availability decreased with the age of stand, i.e., the length of rotation. This indicates an ecosystem containing more labile SOM, even though N and C accretion was noticed at the end of 7 years of the first rotation, and shows the importance of evaluating SOM quality, soil pH and P availability over a long period. The challenge of this study was to show that the introducing acacia in eucalypt plantations on the sandy and nutrient-poor soils involved soil N and C accretion and

an increased potential of ecosystem to mitigate climate change. However these benefits may be limited in the long-term. This may be due to climate, edaphic factors, and the effects of new organic residues rich in N on SOM dynamics and soil pH, diminishing the benefits of this practice on the ecosystem, and even threaten its equilibrium in a longer period, since after 12 years the ecosystem seems to contain more labile SOM and more acidic soils than after short time (7 years).

Abbreviations

100A: pure acacia or 100% acacia; 100E: pure eucalypt or 100% eucalypt; 50A50E: 50% acacia and 50% eucalypt or half acacia and half eucalypt; C: carbon; cPOM: coarse particulate organic matter; fPOM: fine particulate organic matter; N: nitrogen; NFS: nitrogen fixing species; OMF: organo-mineral fraction; P: phosphorus; POM: particulate organic matter; R1Y7: year 7 of the first rotation; R2Y2: year 2 of the second rotation; R2Y5: year 5 of the second rotation; SOM: soil organic matter

Acknowledgements

All who contributed towards the article who does not meet the criteria for authorship including anyone who provided professional writing services or materials have acknowledged. The authors thank Professor Daniel Epron (Université de Lorraine) for the statistical analyses, staffs of CRDPI, Pointe-Noire, Republic of the Congo (JC Mazoumbou, T. Matsoumbou, B. Tchicaya, A. Nzoulou, E. Banguissa, A. Diamesso, A. Dzomambou, A. Kinana), for their contribution during the establishment of the experimental trial, and sampling of forest floor and soil and Professor Lindsey Norgrove (Bern University of Applied Sciences, School of Agricultural, Forest and Food Sciences) for editing the final version. The trial has been established and funded by the project Ecological Intensification of Plantation Forest Ecosystems i.e., R1Y7 and R2Y5 (Intensification de la recherche, France /Projet-ANR-10-STRA-0004). Soil chemical analyses (R2Y5) have been funded by ENEA Research Center, Rome Italy. This study was supported by the TWAS –ENEA Research Training Fellowships Programme to Lydie-Stella Koutika. Authors greatly acknowledge the ENEA Biotechnologies and Agroindustry Division for its support and administrative tasks.

Authors' contributions

LSK planned the project, made P analyses and wrote the first draft and contributed to the final stage; SN made POM fractionation; LC made soil analyses (C, N and S); AB contributed to the planning of the soil analyses, provided funds for soil chemical analyses, supervised the TWAS-ENEA International Research Fellowship and provided comments and recommendations for the final version. All authors read and approved the final manuscript.

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Funding

LSK was supported by a TWAS-ENEA Research Training Fellowship Programme. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

Not applicable. Journal style has been followed and dataset identifiers including DOIs have been expressed as full URLs.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 3 April 2019 Accepted: 14 August 2019

Published online: 04 September 2019

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