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Nitrogen dynamics in a nutrient-poor soil under mixed-species plantations of eucalypts and acacias

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ABSTRACT

Introducing nitrogen-fixing species (NFS) in eucalypt plantations is a useful practice to compensate nitrogen loss at harvest, reduce fertilizer inputs, improve soil fertility and sustain forest productivity in low input systems. Nitrogen (N) and carbon (C) were evaluated in the active part of soil organic matter (SOM) i.e., the particulate organic matter (POM), which was obtained after soil (0–0.05 m) physical fractionation at the end of a first 7-year rotation (R1Y7) and at year 2 of a second rotation (R2Y2) in pure acacia (100A), pure eucalypt (100E) and mixed-species (50A50E) stands in an experimental plantation established on an Arenosol in the Congolese coastal plains. N concentration (in g kg^{-1} of soil) was higher in coarse POM (cPOM, 4000–250 μm) in 100A and 50A50E compared to 100E at 2YR2, while no difference was found in fine POM (fPOM, 250–50 μm) and in the organo-mineral fraction (OMF, < 50 μm). N content in cPOM was more than 3 times higher at R2Y2 than at R1Y7. A slight increase was also observed in fPOM, while no difference was observed in OMF between R1Y7 and R2Y2. Lower C:N ratios in the two POM fractions in 100A and, to a lesser extent, in 50A50E compared to 100E suggests an improved soil N status after acacia trees have been introduced in eucalypt plantations. The lack of difference in N content of coarse POM between 100A and 50A50E at R1Y7, despite higher amount of N in the forest floor and N returning to the soil (harvest residues and litterfall) in 100A than in 50A50E, suggests a faster cycling of nitrogen under acacia than under eucalypt.

1. Introduction

Nitrogen fixing species (NFS) are increasingly used in forest plantations, either alone or in mixture, because of their documented improvement in soil nitrogen (N) status through atmospheric N fixation and the positive impact on tree growth and forest productivity (Binkley, 1992; Kaye et al., 2000; Resh et al., 2002; Forrester et al., 2013; Koutika et al., 2014). In Puerto Rico, *Leu-* experimental plantation of nitrogen fixing tree species compared to non-NFS forest counterparts in subtropical area of southern China (Wang et al., 2010).

Introducing acacia in eucalypt plantations of the Congolese coastal plains increases wood biomass production (Bouillet et al., 2013; Epron et al., 2013). Being a reservoir of nutrients, SOM dynamics is closely linked to plant growth and crop production (Paustian et al., 1990; Sikora et al., 1996). Both the amount and the

the case in eucalypt plantations (d'Annunzio et al., 2008; Epron et al., 2015), especially at the sapling stage, when the decomposition of organic residues and uptake of nutrients from soil is most crucial to tree nutrition and growth (Laclau et al., 2005).

To date, POM has been evaluated only in monocultural eucalypt plantations established on a nutrient-poor soil of the Congolese coastal plains (d'Annunzio et al., 2008; Epron et al., 2015). This study demonstrated that removing both aboveground forest floor and harvest residues had a negative impact on C-POM status and on C accretion. Adding acacia might improve soil status as its litter has a faster decomposition rate than eucalypt due to faster mineralization of soluble compounds (Bernhard-Reversat, 1993). This is probably related to: a higher activity of macroarthropods, particularly cockroaches in acacia litter as opposed to ants in eucalypt litter (Bernhard-Reversat, 1993); changes in microbial communities and activities (Huang et al., 2014); and, a higher proportion of bacteria-feeding nematodes community in acacia than in eucalypt stands (Robin et al., personal communication). It is thus important to evaluate POM, since it has never been done in acacia plantations, either growing alone or in mixture with eucalypts.

We evaluated POM N and C content and POM contribution to total soil N and C at the end of the 7-year first rotation (R1Y7) and in year 2 of the second rotation (R2Y2). We related these to the amount of N in litterfall, in organic residues left at harvest and in forest floor litter in pure acacia (100A) and eucalypt (100E) stands and mixed-species stands of these two species (50A50E, 50% acacia and 50% eucalypt) during the first 2 years of the second rotation. Two hypotheses were tested: (i) N content of coarse POM (cPOM, 4000–250 μm) and its contribution to total soil N is higher in 100A and 50A50E than in 100E at R1Y7, due to a higher inputs of N through litter fall; and (ii), this trend will be even more pronounced at R2Y2, due to additional inputs through harvest residue and forest floor litter accumulated during the 2 years of the second rotation.

2. Materials and methods

2.1. Site description

The study 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 a geological bedrock composed of thick detritic layers of continental origin dated from plio-pleistocene. These soils are characterized by a low CEC (<0.5 cmolc kg^{-1}), a high sand content (>90% of the mineral soil), very low clay and silt content (6 and 2%, respectively) and low iron oxides content (<1.5% of the bulk soil, Mareschal et al., 2011). They are acidic with low C and N content (Koutika et al., 2014). The climate in the area is subequatorial with high mean annual air humidity and air temperature (85% and 25 °C, respectively) and low seasonal variation (about 2% and 5 °C, respectively). Annual precipitation averages 1200 mm with a dry season

2016).

2.2. Soil sampling

Soil sampling (0–0.05 m) was done at the end of the first seven-year rotation (R1Y7) in December 2011 and at two years of the second rotation (R2Y2) in March 2014 in 3 out of the 5 blocks. Three transects in 100A and 100E plots and six in 50A50E plots were setup in the inner part of each plot, starting at the base of a tree and ending in the center of the area delimited by four trees. Three cores (5 cm diameter) were sampled on each transect, each sampling point being separated by 0.7 m from each other along each transect. Nine samples were collected in each of the 3 blocks in 100A and 100E and 18 in each of the 3 blocks in 50A50E. Soil samples were air-dried and sieved at 4 mm.

2.3. POM fractionation

Twenty grams of air-dried and sieved soil, five glass beads and 50 ml of distilled water were put in 100 ml plastic bottle and shaken for 16 h at 20 °C in an end-over-end shaker at 40 rotations per minute to ensure physical fractionation of soil organic matter (Koutika et al., 2007; Epron et al., 2015). Soil was wet-sieved to separate the suspension in 3 fractions: 4000–250 μm, 250–50 μm and 0–50 μm. The organic components of the two largest fractions were separated from the mineral fraction by decantation. The following fractions were collected: coarse (cPOM, 4000–250 μm) and fine POM fractions (fPOM, 250–50 μm) fractions, coarse and fine mineral fractions (cMin and fMin), and the organo-mineral fraction (OMF, < 50 μm). All fractions were dried at 65 °C and weighed. Combined together, cMin and fMin accounted for 89% of soil mass and the recovery of fractionation (sum of all fraction mass divided by soil mass) was 1.0047. N and C concentrations in cPOM, fPOM and OMF were analyzed using an elemental analyzer (Carlo-Erba, Milan, Italy).

2.4. Above-ground litterfall, forest floor and harvest residues

Above-ground litterfall was collected every two weeks in litter traps (75 × 75 cm) from October 2010 to September 2011 (R1Y7) and from June 2013 to May 2014 (R2Y2), pooled separated by species and components, oven-dried at 65 °C and weighed. Four traps were installed in 100A and 100E in each block, and 6–8 traps in 50A50E. Annual litterfall N flux (kg m^{-2} year^{-1} of N) was obtained by summing biweekly mass of litterfall, multiplied by the N concentrations of the different litter components. The forest floor was collected in October 2011 (R1Y7) with a square metallic frame (50 × 50 cm), oven-dried and weighed on 4–6 locations within each plot. The forest floor nitrogen (kg m^{-2} of N) was computed from the dry mass and N concentration (see Epron et al., 2013; for details). Nitrogen content in the harvested residues (foliage and between stand types for each sampling date and between times for each stand type. One-way Anova followed by Tukey's HSD were used to estimate the effects of stand types on nitrogen in litterfall, forest floor and harvest residues.

3. Results

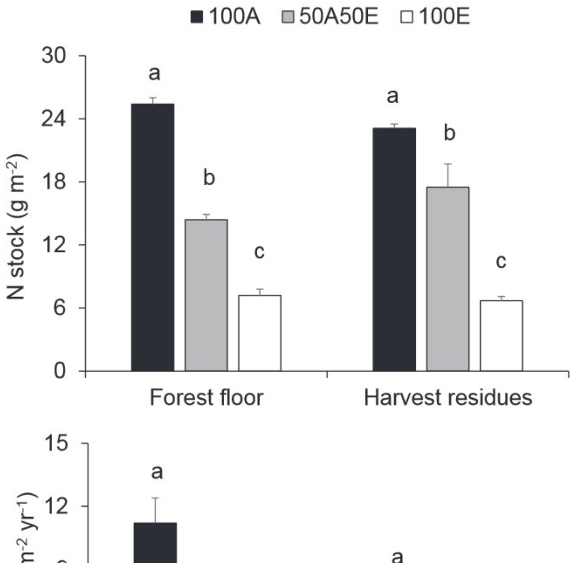
3.1. N in litterfall, forest floor and harvest residues

The amount of nitrogen in the forest floor at R1Y7 was higher in 100A (+253%) and in 50A50E (+100%) compared to 100E, while higher amounts of N were left on site at harvest in 100A (+245%) and in 50A50E (+161%) compared to 100E (Fig. 1). The amount of nitrogen returning to the soil through aboveground litterfall was also higher in 100A (+273%) and in 50A50E (+110%) compared to 100E at the end of the first rotation and during the second year of the second rotation (+276% for 100A and +95 for 50A50E, Fig. 1).

3.2. Characteristics of POM fractions and OMF at R1Y7 and R2Y2

The mass of the two POM fractions, expressed in g kg^{-1} of soil, increased after harvest, between R1Y7 and R2Y2, while that of OMF decreased (Table 1). However, there was no difference in mass for all fractions between stand types at R1Y7, while a significantly higher mass at R2Y2 was found in 50A50E compared to 100E for cPOM and in 50A50E compared to 100A for fPOM.

N concentration (in g kg^{-1} of fraction) in cPOM (4000–250 µm) was significantly higher in 100A (+30%) and in 50A50E (+10%) compared to 100E at R1Y7, while there was no significant difference in fPOM (250–50 µm) (Table 1). At R2Y2, N concentration in cPOM was still significantly higher in 100A (+23%) and in 50A50E (+11%) compared to 100E, and N concentration in fPOM was significantly higher in 100A than in 100E (+15%). No significant difference was found in N content of OMF (0–50 µm) between stand types at either R1Y7 or R2Y2. Between the end of the first rotation and at 2 years of the second rotation, the N concentration significantly increased for all fractions, up to 33% for fPOM in 100A (Table 1).



There was no difference in C concentration between stand types in cPOM and OMF at R1Y7, and in all fractions at R2Y2 (Table 1). However, C concentration of fPOM in100A was 10% lower than in 100E and 13% than in 50A50E at R1Y7. The C:N ratio was lower in 100A at both R1Y7 and R2Y2, especially in cPOM at R1Y7 (−36% compared to 100E) and in all fractions at R2Y2 (Table 1). The C:N ratio of cPOM decreased between R1Y7 and R2Y2 in 50A50E and in 100E, and those of fPOM in 100A.

3.3. Contribution of POM fractions and OMF to total soil N at R1Y7 and R2Y2

When N content was expressed in g kg^{-1} of soil, no difference was found between stands for either cPOM, fPOM or OMF at R1Y7 (Fig. 2). However, the N content significantly increased between R1Y7 and R2Y2 in all stand types in both POM fractions, but especially in cPOM (more than +300%), being significantly higher in 100A and 50A50E (+34%) compared to 100E at R2Y2 (Fig. 2). In contrast, no significant difference in N content was found at 2YR2 in fPOM and in OMF. The large increase in cPOM N content was partly due to the above-mentioned increase in N concentration, but mostly because of an increase in the mass of cPOM in all stand types (Table 1). While cPOM were contributing to 10–14% of total soil N at R1Y7, their contribution significantly increased between R1Y7 and R2Y2 for all stand types, reaching 36% in 100A and 34% in 50A50E at R2Y2. A much limited increase was observed in fPOM, and thus the contribution of OMF to soil N decreased from 70 to 74% at R1Y7 to 46–51% at R2Y2, mirroring the increase in the two POM fractions (Fig. 3).

4. Discussion

4.1. Soil nitrogen status seven years after the introduction of acacia trees alone or in mixture in a eucalypt plantation

While N concentration, expressed in g kg^{-1} of soil, is two to three times higher in POM than in OMF, the latter contained more than 70% of total N in a nutrient-poor sandy soil under tree plantations established in the Congolese coastal plains. This is consistent with previous studies showing that most of N is stored in the fine particles fractions in West African savannah (Lehmann et al.,

Table 1

Means (with their standard error) of the mass (g kg^{-1} of soil) of coarse (cPOM, 4000–250 μm) and fine (fPOM, 250–50 μm) particulate organic matter fractions and of the organo-mineral fraction (OMF, < 50 μm) from top soil (0–0.05 m) sampled in pure acacia (100A) and eucalypt (100E) stands (9 samples in 3 blocks) and in mixed-species stands (50A:50E, 18 samples in 3 blocks) either at the end of 7-year first rotation (R1Y7) and at year 2 of the second rotation (R2Y2). Nitrogen and carbon concentration (g kg^{-1} of fraction) of the different fractions, and their C:N ratio, are given. For each sampling time (R1Y7 or R2Y2), mean values followed by different letters indicate significant difference at P = 0.05 between stand types. For a given stand type, mean values followed by an asterisk indicates a significant difference between the two sampling times.

	R1Y7			R2Y2		
	100A	50A50E	100E	100A	50A50E	100E
Mass of fractions (g kg^{-1} of soil)						
cPOM	5.5 ± 0.6a	7.7 ± 1.1a	4.9 ± 0.5a	20.6 ± 1.7ab*	24.6 ± 1.4a*	18.0 ± 1.3b*
fPOM	9.9 ± 1.0	9.5 ± 0.9	8.3 ± 0.9	11.4 ± 0.6b	15.1 ± 0.7a*	14.3 ± 1.2ab*
OMF	96 ± 2	93 ± 2	92 ± 4	74 ± 2b*	82 ± 1a*	79 ± 2ab*
N concentration (g kg^{-1} of fraction)						
cPOM	19.7 ± 0.8a	15.3 ± 0.4b	13.8 ± 0.5b	21.7 ± 0.4a*	18.6 ± 0.2b*	16.7 ± 0.2c*
fPOM	14.0 ± 0.6a	13.9 ± 0.3a	14.4 ± 0.6a	18.6 ± 0.3a*	16.2 ± 0.2b*	15.8 ± 0.3b
OMF	6.0 ± 0.3a	5.8 ± 0.2a	5.4 ± 0.2a	7.3 ± 0.3a*	7.3 ± 0.2a*	6.8 ± 0.3a*
C concentration (g kg^{-1} of fraction)						
cPOM	375 ± 10a	354 ± 7a	361 ± 14a	404 ± 5a*	411 ± 3a*	416 ± 5a*
fPOM	326 ± 16a	368 ± 8b	358 ± 12ab	373 ± 7a*	396 ± 4a*	406 ± 8a*
OMF	82 ± 5a	89 ± 4a	82 ± 5a	105 ± 4a*	112 ± 3a*	111 ± 6a*
C:N ratio						
cPOM	19.4 ± 0.6a	23.9 ± 0.7b	26.5 ± 0.9c	18.6 ± 0.2a	22.2 ± 0.2b*	25.0 ± 0.3c
fPOM	24.1 ± 1.7a	27.1 ± 0.7b	25.4 ± 0.8ab	20.2 ± 0.3a*	24.7 ± 0.4b*	25.7 ± 0.4b
OMF	13.6 ± 0.3a	15.3 ± 0.3b	14.9 ± 0.4b	14.3 ± 0.2a	15.3 ± 0.2b	16.2 ± 0.3b*

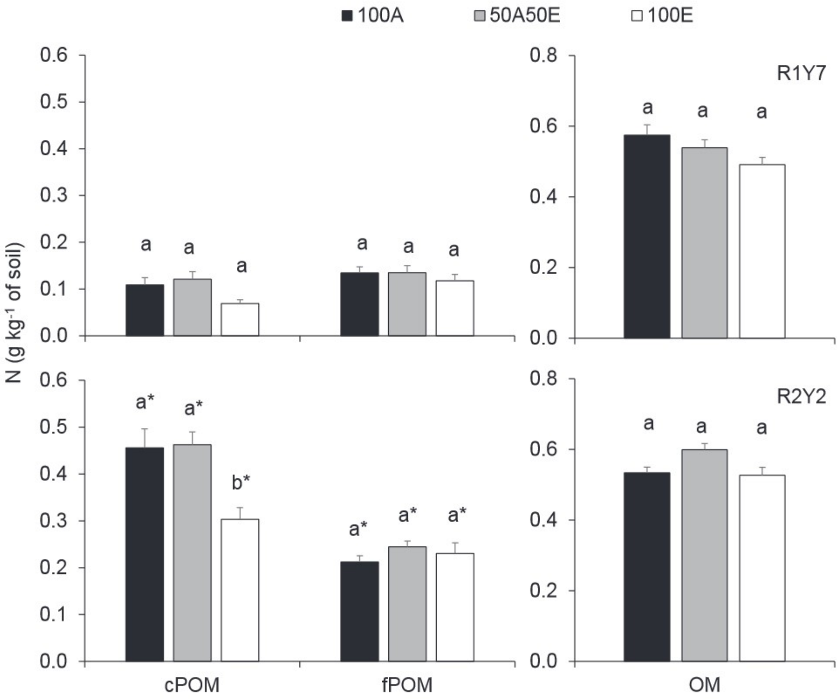


Fig. 2. Nitrogen content in coarse (4000–250 μm; cPOM) and fine (250–50 μm; fPOM) particulate organic matter fractions and in the organo-mineral fractions (0–50 μm; OMF) from top soil (0–0.05 m) sampled in pure acacia (100A) and eucalypt (100E) stands (9 samples in 3 blocks) and in mixed-species stands (50A:50E, 18 samples in 3 blocks) either at

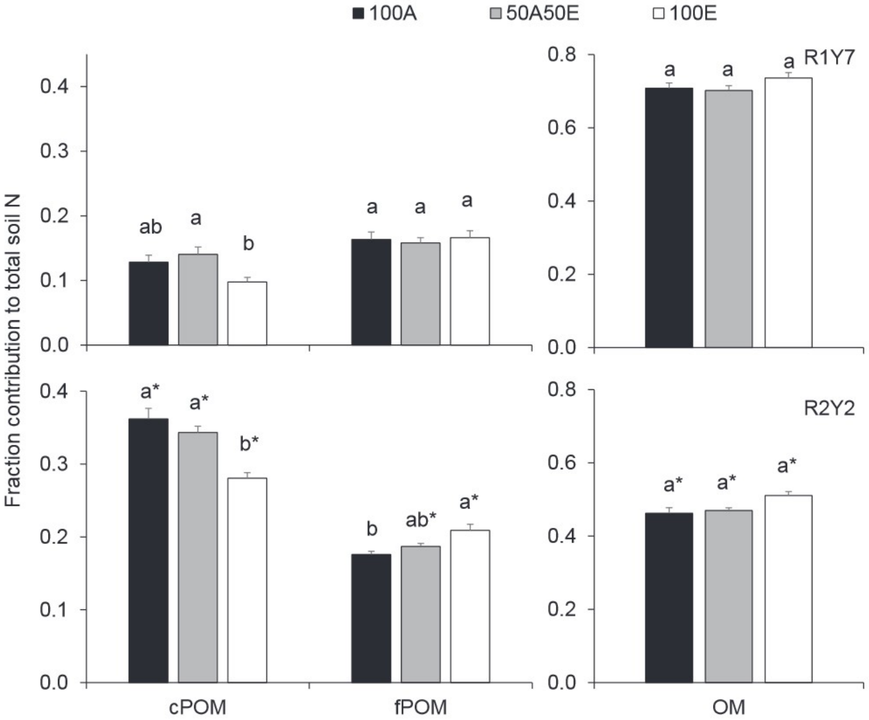


Fig. 3. Nitrogen contribution to total N in soil organic matter of coarse (4000–250 μm; cPOM) and fine (250–50 μm; fPOM) particulate organic matter fractions and of the organo-mineral fractions (0–50 μm; OMF) from top soil (0–0.05 m) sampled in pure acacia (100A) and eucalypt (100E) stands (9 samples in 3 blocks) and in mixed-species stands (50A:50E, 18 samples in 3 blocks) either at the end of a first rotation (R1Y7) and at year 2 of the second rotation (R2Y2). Vertical bars are the standard errors. For each sampling time (R1Y7 or R2Y2), different letters indicate significant differences at P = 0.05 between stand types. For a given stand type, an asterisk indicates a significant difference between the two sampling times.

100A and 100E was found i.e., 0.058 vs 0.050% in the 0–0.05 m (Koutika et al., 2014). This result confirms our second hypothesis and is in accordance with other findings revealing the specific effects of NFS on soil N status (Forrester et al., 2006; Crème et al., 2016).

However, despite the higher N input, the introduction of acacia trees alone or in mixture in a eucalypt plantation had not significantly increased the amount of soil N after seven years, i.e., R1Y7 in contrary of R2Y2. This result contrasted with previous data on the same site showing a slightly significant increase in total soil N stock down to 0.15 m depth in 50A50E stands (Koutika et al., 2014). Therefore, the lack of significant increase in cPOM contribution to soil N at R1Y7, likely highlights faster decomposition of acacia litter compared to eucalypt litter (Bernhard-Reversat, 1993). Faster rate of litter decomposition, thus higher nitrogen release from decaying litter through mineralisation, may result in lower accumulation of soil organic N under acacia trees than under eucalypt trees litter breakdown is known to provide N mainly to POM (Zeller and Dambrine, 2011). Our result indicates that the fine fractions are not yet nitrogen saturated (Castellano et al., 2012), even if N in these organic fractions (<50 mm) did not change with plantation age in nearby sites (d'Annunzio et al., 2008) and if carbon accretion was found to be limited by the low C saturation level of these sandy-structured soils (Epron et al., 2015). A higher contribution of cPOM, and to a lesser extend of fPOM, to total SOM and to soil N was observed. This was expected because of N derived from the first rotation remaining in the forest floor and of the input of N from harvest residues.

The lower amount of N in cPOM, and the lower contribution of this fraction to soil N, was expected in 100E compared to the other stands because of lower N stocks in the forest floor and in harvest residues due to the lack of symbiotic nitrogen fixation. However, we would have also expected a lower contribution of cPOM to soil N in 50A50E than in 100A for the same reason. Combined together, the

5. Conclusions

Introducing acacia in eucalypt plantations on the sandy and inherently nutrient-poor soils of the Congolese coastal plains were shown to have a beneficial impact on N status of cPOM, one of the most active SOM parts. Yet the contributions of cPOM to total soil N were not higher in 100A and 50A50E than in 100E at the end of the first rotation (R1Y7), despite higher inputs of N through litterfall. However, by year 2 of the second rotation (R2Y2), N content and contribution of cPOM and fPOM to total soil N were increased in all stands, and were higher in 100A and 50A50E than in 100E, reflecting the high N contents in organic residues left at harvest and in the forest floor accumulated during the first rotation and the 2 first years of the second rotation, thus confirming our second hypothesis. However, no difference was observed between 100A and 50A50E despite large difference in N stock in harvest residues and forest floor. Taken together, these two results indicate a faster turnover rate of N in stands where acacia is planted either alone or in mixture. Introducing NFS in tropical eucalypt plantations may be adopted as an ecological option to improve soil N status in areas with similar edaphic conditions worldwide. This study has shown once again that POM fractionation is a useful and simple tool to reveal SOM dynamics.

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