

**Fine Root Production and Turnover Estimation by Ingrowth Core Method in Two Tropical Forests of Congo Basin**

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**Abstract.** In the context of global warming and its escalating consequences, forests have proven to be crucial for mitigating the impact of climate change. Therefore, we conducted a study on the impact of ingrowth core's method on carbon transfer into the soil in two tropical forests in the Republic of Congo, the Patte d'oie forest in Brazzaville and the forest of Lésio-Louna reserve in Batéké Plateau. Forest plots were installed in these areas, and fine root biomass and productivity were determined by sampling soil cores over the course of one year at two horizons: H<sub>1</sub>= 0–15 cm; H<sub>2</sub>= 15–30 cm. Moreover, three classes of root diameter were considered for quantifying standing fine root biomass: class I = [0-2 mm] (fine roots); class II = ] 2-4 mm] (medium roots); and class III = ] 4-10 mm] (large roots). For fine root productivity and root turnover studies, only class I was considered. We recorded the following fine root turnover values for horizons H<sub>1</sub> and H<sub>2</sub>: 1.66 y<sup>-1</sup> and 1.77 y<sup>-1</sup>, respectively, at Lésio-Louna, and 1.19 and 1.56, respectively, at Patte d'oie forest. Concerning the root turnover value, we obtain in H<sub>1</sub> a root turnover of 1.66 yr<sup>-1</sup> in the Lésio-Louna forest against 1.19 yr<sup>-1</sup> in the Patte d'oie forest; in H<sub>2</sub>, the turnover is 1.77 yr<sup>-1</sup> in the Lésio-Louna forest against 1.56 yr<sup>-1</sup> in the Patte d'oie forest. Furthermore, the forests are structurally different, which can influence fine root biomass and production but not the differences in root life between both forests. Finally, our results helped to conclude that these two forests did not transfer the same amount of carbon to the soil compartment.

**Keywords:** fine root, fine root turnover, root production, tropical region, Batéké Plateau

### Introduction

The latest IPCC report on the state of global climate highlighted the significant role that changes in land use have played in global greenhouse gas emissions—on average, the Earth has warmed 0.87°C between 1850–1900 and 2006–2015, and it may have exceeded the 1.2°C average due to the high temperatures recorded in July 2019 (IPCC, 2019). Moreover, the report demonstrated that a considerable amount of greenhouse gases can be avoided by reducing the degradation of forests, peat lands, and mangroves (since they currently account for 10–15 % of total emissions), which is particularly important because terrestrial ecosystems absorb approximately 29% of anthropogenically emitted greenhouse gases—mostly CO<sub>2</sub> (IPCC, 2019).

Tropical forests account for approximately one-third of the Earth's terrestrial gross primary productivity, and one-half of the Earth's carbon is stored in terrestrial vegetation (Lewis *et al.*, 2015). This is due to photosynthesis and the high carbon stock stored in trees but also because plants play a significant role in carbon sequestration into soils through the decomposition of leaf litter and fine root turnover (Gill & Jackson, 2000)—both of which are among the main pathways for carbon entry into forest soils (Nadelhoffer & Raich, 1992; Gill & Jackson, 2000; Lebègue *et al.*, 2004; Epron & Osawa, 2017). Globally, fine root turnover

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transfers approximately 33% of the annual net primary production into the soil in terrestrial ecosystems (Jackson *et al.*, 1997), and fine roots play an important role in the carbon cycle by contributing to a substantial fraction of the ecosystem's net primary production (Nadelhoffer & Raich, 1992).

Transferring carbon into the ground follows two paths: aerial litter and rhizodeposition, the latter of which involves root mortality, exudates, and the destruction of root extremities (Raich & Nadelhoffer, 1989; Lebeque *et al.*, 2004; Ifo & Nganga, 2011). In some tropical forest types, fine root production may be equivalent to or even greater than that of aerial litter, while root production has been suggested to contribute about half of the carbon being cycled annually in many forests (Vogt *et al.*, 1996) and 33 % of the global annual net primary production (Nadelhoffer & Raich, 1992; Jackson *et al.*, 1997). However, fine-root biomass and net primary productivity have been significantly altered by anthropogenic perturbations in natural forest ecosystems (Sundarapandian & Swamy, 1998): according to Gill and Jackson (2000), mean fine root (those less than 2 mm in diameter) turnover varies from 0.5 to 3 y<sup>-1</sup> in all forest ecosystems, from boreal to tropical. Nonetheless, there is still a lack of fine-root-turnover data from different forest types across the planet, probably due to difficulties associated with the choice of sampling methods and calculations—which are still controversial—especially in tropical forest ecosystems (Jourdan *et al.*, 2008; Yuan & Chen, 2010).

Notably, fine root productivity is one of the most complicated ecosystem parameters to measure (Ifo *et al.*, 2015) because of the lack of a simple and straightforward technique (Hamzah *et al.*, 1983). Moreover, the production of fine roots and their distribution in forests exhibit spatial and temporal trends, making them exceedingly difficult to characterize and/or measure (Wan *et al.*, 2004). There are two major approaches for studying biomass and fine root production: direct and indirect, both of which have advantages and disadvantages (Joslin & Henderson, 1982, 1987; King *et al.*, 1996; Hendricks *et al.*, 2006).

Some of the direct methods include a) the sequential coring method (Nadelhoffer & Raich 1992; Makkonen & Helmisaari 1999; Ostonen *et al.*, 2005); b) root recolonization, or ingrowth core (Caldwell & Virginin, 1991; Ostonen *et al.*, 2005); and c) the minirhizotron method (Bates, 1937). The sequential coring method determines the production of fine roots and their mortality from the changes in biomass observed during the periodic collection of soil cores over a year (Grier *et al.*, 1981). This method implies that the increase in living roots represents better productivity, whereas an increase in the number of dead roots is associated with higher mortality rates (Santantano & Hermann, 1985). It also assumes that fine root production and mortality do not occur simultaneously; therefore, the method may underestimate fine root production and turnover if any random variation in the distribution influences fine root estimates in terms of real gain or loss among sampling periods (Publicover & Vogt, 1993).

On the other hand, root recolonization or ingrowth cores are among the most widely used methods to determine biomass and fine root production (Ifo, 2010). For this method, a soil core was sampled at point x<sub>1</sub>, all living roots were extracted, and then the rootless soil was returned to the same point to follow recolonization by new roots over a period of time. This method is recommended in ecosystems where fine root growth is fast, such as tropical rainforests and fast-growing forest plantations (Vogt *et al.*, 1998). However, the ingrowth core method has two major disadvantages: first, the removal and subsequent reintroduction of rootless soil at the original location can alter soil characteristics (Makkonen & Helmisaari, 1998). Moreover, when the soil cores are set, the root auger cuts the roots, which can activate root production (Oliveira *et al.*, 2000, cited by Ifo, 2010).

Lastly, the minirhizotron method is a nondestructive method used to observe root growth and mortality over time using photos (Bates, 1937; Vamerali *et al.*, 2012; Mohamed

*et al.*, 2017). This approach is advantageous because roots are not cut—thereby favoring root production—and it helps avoid the problem of insufficient spatial sampling since multiple observation tubes can be placed in the ground. This technique can also be used to obtain quantitative information on root length, density, and dynamics (McMichael & Taylor, 1987), as well as qualitative information on root color (Upchurch & Richie, 1983; Smucker *et al.*, 1987).

In contrast, indirect methods for studying biomass and fine root production include the following: a) the nitrogen stock method (Nadelhoffer *et al.*, 1985), and b) the radiocarbon isotopic method (Joslin *et al.*, 2006).

First, the radiocarbon isotopic method was used to estimate the mean age of fine root carbon by comparing radiocarbon ( $^{14}\text{C}$ ) content in fine root structural materials with the measured record of change of  $^{14}\text{C}$  in atmospheric  $\text{CO}_2$ . In temperate forests, it can take 3–18 years for atmosphere-fixed carbon to be transferred into roots, which is longer than the estimates for root lifetime previously reported in the literature (Gaudinski *et al.*, 2001); however, estimates of fine root production and turnover rates computed by indirect methods are much higher (87–124%) than those computed using direct methods (Yuan & Chen, 2010).

Finally, considering the aforementioned methods and to better understand fine-root dynamics of tropical forests in the Congo Basin, we studied fine roots using two different direct approaches at two sites in the country: the Patte d’oie forest in Brazzaville and the Lésio-Louna reserve in Batéké Plateau. We had three specific objectives: (i) to compare fine root biomass and standing litter mat values in both forests; (ii) to study the seasonal trends of fine root biomass; and (iii) to evaluate the effects of forest structure on fine root productivity and fine root turnover in these two forests.

## Materials and Methods

### Study Areas

This study was conducted at the Patte d’oie forest, Brazzaville, and at the Lésio-Louna reserve. The Patte d’oie covers 90 ha and is located at the center of the city of Brazzaville. Mean annual rainfall here is 1200 mm, with a dry season between June and September, and a mean annual temperature of 25.5 °C. The soils are ferralitic, strongly desaturated and impoverished, with low exchangeable and very permeable bases because of the sandy substrate, which allows the migration of ground solutions (Makany, 1976). This is a degraded forest, dominated by *Millettia laurentii* De Wild, because of logging and hunting that occurred during the Congo Civil War in the 1990s. Currently, human activity is limited to research and recreation, such as bird watching and walking. Lésio-Louna, on the other hand, has an extension of 173,000 ha and is found in the central Batéké Plateau region of Congo, 160 km north of Brazzaville. Mean annual rainfall in the reserve is 2100 mm, with a dry season between June and September, and the mean annual temperature is 26 °C. The soils of Batéké Plateau are deep and sandy, which results in low water availability—even if it is a tropical wet climate (Makany, 1976). Altitude ranges here from 300 to 750 m, and the gallery forests found in Lésio-Louna, which are relatively undisturbed, are dominated by *Pentaclethra eetveldeana* De Wild, *Chaetocarpus africanus* Pax, and *Barteria fistulosa* Mast.

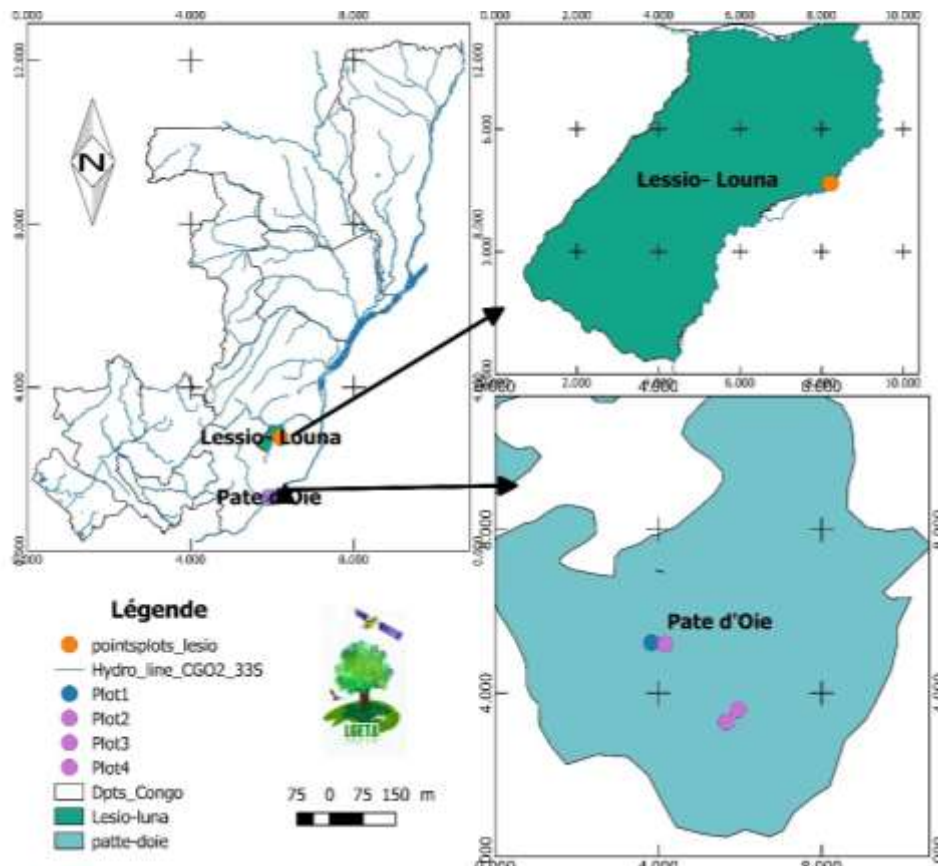


Figure 1: Study area

## Methodology

### *Root biomass at T<sub>0</sub> and fine root production*

We installed three 50 m × 50 m plots at Lésio-Louna, and four 20 m × 20 m plots at Patte d'oe. Smaller plots were set up in Patte d'oe because of its small size and the number of footpaths crossing the park. For ingrowth core method, we collected several cores at each plot (No. ingrowth cores were 20 in ZP forest and 21 in WR forest), and samples from two horizons (H<sub>1</sub>: 0-15 cm and H<sub>2</sub>: 15-30 cm) were collected using a root auger (8 cm in diameter). After the removal of the soil core, *in situ* sorting was performed to separate alive and dead fine roots, which were then brought back to the laboratory (Laboratoire de télédétection et d'écologie forestière, Marien N'gouabi University), where these roots were oven-dried at 70 °C for five days to constant mass, and then weighed using a balance with 0.01 a precision. Using an electronic sliding foot, dried roots were then classified as class I: 0-2 mm (fine roots), class II: 2-4 mm (medium roots), and class III: 4-10 mm (large roots). Nevertheless, for fine root production and turnover, we only considered class I.

Furthermore, for the ingrowth core method, fine root production was calculated for periods T<sub>0</sub> and T<sub>2</sub> using the following equation:

$$\emptyset = \frac{\Delta B(T_2 - T_1)}{T_2 - T_1} \times 365 \quad (1)$$

Where  $\emptyset$  is annual fine root production and  $\Delta B$  is the variation of dry biomass over a period of time (T<sub>2</sub> - T<sub>1</sub>). Fine root production was expressed as MgDM.ha<sup>-1</sup>.y<sup>-1</sup>.

Fine root turnover was also calculated using the following equation:

$$FRT = \frac{\Phi(\text{class } 1:0-2 \text{ mm})}{BO(\text{class } 1:0-2 \text{ mm})} \quad (2)$$

Where FRT is fine root turnover,  $\Phi$  is annual fine root production, and BO is fine root biomass obtained after sampling  $T_0$ . FRT was expressed as  $\text{yr}^{-1}$ .

Finally, the lifespan of the roots was obtained as follows:

$$\text{Lifespan} = \frac{-1}{FRT} \quad (3)$$

At the beginning of this project, similar number of cores were installed in the both sites, but some were destroyed by wildlife mainly in ZP Brazzaville.

#### **Forest inventory**

When the plots were established, stand structure was calculated in each plot by measuring tree diameter at 1.3 m along the stem from the ground up (or above buttresses, if present) for each tree equal to or more than 10 cm in diameter. Moreover, trees were identified to species level when possible, following the Plant List ([www.theplantlist.org](http://www.theplantlist.org)), and samples of unidentified trees were collected for identification and deposited at the herbarium of the University of Brazzaville along with their names in Baka. However, some unidentified morphospecies could not be identified to species level because of the poor quality of the collected samples, and their vernacular names were used for tree diversity calculations.

#### **Estimating above-ground biomass**

Aboveground biomass (AGB) was calculated using the equation by Fayolle *et al.* (2018), which includes tree diameter and mean wood density. This equation was chosen because it was created using the most extensive destructive sampling method available for the Congo Basin.

$$AGB = \exp[0.046 + 1.156 \cdot \ln(\text{WSG}) + 1.123 \cdot \ln(D) + 0.436 \cdot (\ln(D))^2 - 0.045 \cdot (\ln(D))^3] \quad (4)$$

Using the best taxonomic match, the mean wood density of each stem was obtained from a global database (Chave *et al.*, 2009; Zanne *et al.*, 2009) following Lewis *et al.* (2013). In addition, the extrapolation of aboveground biomass values per hectare was performed using an expansion factor indicating the area represented by each plot (Walker *et al.*, 2011).

#### **Amount of litter on the forest floor**

At Patte d'oie, four points were sampled, and three points were sampled at Lésio-Louna. The collected points were randomly selected and sampled for litter during the rainy and dry seasons. Furthermore, a 50 cm × 50 cm metal frame was used to collect dead leaves, stems, bark, fruits, flowers, seeds, and other wood debris. The various components of the litter were then separated, oven-dried at 70 °C for five days, until constant weight was achieved, and weighed with a precision balance of 0.01 g.

#### **Data analysis**

The sum or average is presented with its standard errors (SE). Moreover, confidence intervals were established and used for all measurements by choosing a 95% confidence level, and the average was expressed as the confidence interval. Student's *t*-test was used to evaluate significant differences between means, and other statistical tests were also performed to determine any differences in aboveground biomass, fine root biomass, amount of litter on the floor, and between forest sites.

## Results

### Distribution of the Percentage of Fine Roots in the Two Forests

The percentage of fine roots varied among classes, but also among forest types. For classes I and II, fine root rates were lower at Patte d'oe for both horizons: in class I, H<sub>1</sub> and H<sub>2</sub> presented values of 29.69% and 26.32%, respectively, whereas for class III, the rates were higher at Lésio-Louna compared to Patte d'oe, with 51.53% for H<sub>1</sub> and 61.40% for H<sub>2</sub>.

### Initial Root Biomass

Average root biomass was calculated considering all three root classes: fine, medium, and large roots, and results were obtained from the first fine root collection at T<sub>0</sub>. For class I, this study revealed that Lésio-Louna had the highest average root biomass per unit area compared to Patte d'oe for both H<sub>1</sub> and H<sub>2</sub> horizons: fine root mean biomass values at Patte d'oe varied between 1.68±0.35 Mg.ha<sup>-1</sup> and 2.26±0.48 Mg.ha<sup>-1</sup> for H<sub>1</sub>, and they ranged from 0.42±0.14 Mg.ha<sup>-1</sup> and 0.78±0.29 Mg.ha<sup>-1</sup> for H<sub>2</sub>. For class II, root biomass varied between 0.92±0.59 Mg.ha<sup>-1</sup> and 1.63±0.74 Mg.ha<sup>-1</sup> for H<sub>1</sub>, and between 0.09±0.09 Mg.ha<sup>-1</sup> and 0.37±0.54 Mg.ha<sup>-1</sup> in H<sub>2</sub>. In contrast, the difference in mean biomass values among plots was very large in class III: for H<sub>1</sub>, these values varied from 0.93±0.91 Mg.ha<sup>-1</sup> to 4.98±6.55 Mg.ha<sup>-1</sup>, and from 0.47±0.83 Mg.ha<sup>-1</sup> to 3.21±2.50 Mg.ha<sup>-1</sup> for H<sub>2</sub>. Furthermore, total root biomass was 4.81 Mg ha<sup>-1</sup> in Lésio-Louna and 3.63 Mg ha<sup>-1</sup> in Patte d'oe, with significant differences across sites. At both sites, there was more root biomass in H<sub>1</sub> than in H<sub>2</sub>; nonetheless, in Patte d'oe, unlike in Lésio-Louna, larger roots contributed significantly more to root biomass than of smaller roots did. However, considering the first diameter class (0-2 mm), the sum of the average of fine root biomass in horizons H<sub>1</sub> and H<sub>2</sub> was 1.88 Mg.ha<sup>-1</sup> in Lésio-Louna, whereas it was 1.05 Mg.ha<sup>-1</sup> in Patte d'oe. Therefore, the total amount of fine root obtained for H<sub>1</sub> in Lésio-Louna was 1.44±0.032 Mg.ha<sup>-1</sup>, and 0.81±2.03 Mg.ha<sup>-1</sup> in Patte d'oe forest. In horizon 2, on the other hand, the amount of fine root obtained was 0.50±0.011 in Lésio-Louna forest and 0.24±0.006 in Patte d'oe.

Values are presented as mean ± standard error. Different letters (a, b, ab) within one horizon and column indicate significant differences (p<0.01). Different letters across horizontal rows indicate to significant differences across sites at p<0.01, but also across types of fine root diameter.

### Production and Turnover of Fine Roots

#### *Case of the Patte d'oe forest*

The results show the different production values in the Patte d'oe forest plots. For horizon 1, we notice a higher production in plot 2 with 2.36±0.55 Mg.ha<sup>-1</sup>.y<sup>-1</sup> then plot 4, with 2.36±1.23 Mg.ha<sup>-1</sup>.y<sup>-1</sup>. The lowest values are obtained in plots 1 and 3, with productions of 1.67±0.51 and 1.52±0.22 Mg.ha<sup>-1</sup>.y<sup>-1</sup> respectively. The observation is identical for the second horizon. Plots 2 and 4 are the most productive with 1.19±0.24 and 0.94±0.38 Mg.ha<sup>-1</sup>.y<sup>-1</sup> respectively. The lowest production is found in plot 1 with 0.63±0.14 Mg.ha<sup>-1</sup>.y<sup>-1</sup>. These low values can be explained by the low density of trees per hectare in this forest, as the most productive plots are found in the densest forests and vice versa. As root production is dependent on biomass, it is obvious that a high tree density would lead to a high biomass of fine roots.

#### *Case of the Lesio Louna forest*

In contrast to the initial biomass, the results show a very high value for fine root production in plot 3. We obtain 5.41±4.14 Mg.ha<sup>-1</sup>.yr<sup>-1</sup> in this plot and 4.50±1.70 Mg.ha<sup>-1</sup>.yr<sup>-1</sup> in plot 1, for only 2.28±0.38 Mg.ha<sup>-1</sup>.yr<sup>-1</sup> in plot 2 for the first horizon. The same observation

is made for horizon 2 with  $2.22 \pm 0.63$ ,  $1.51 \pm 0.42$  and  $1.29 \pm 0.30$   $\text{Mg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  in plots 3, 2 and 1 respectively.

### Average Production between the Two Forests

The production remains higher in the Lésio-Louna forest than in the zoological park. The average production of the Lésio-Louna forest is 2.05 times higher than that of the zoological park in the first horizon; then 1.98 times higher in the second horizon. Thus, for the first horizon, we have  $4.06 \pm 1.53$   $\text{Mg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  in the Lésio-Louna forest compared to  $1.98 \pm 0.46$   $\text{Mg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  in the zoo; for the second horizon, we have  $1.68 \pm 0.30$   $\text{Mg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  in the Lésio-Louna forest, but only  $0.85 \pm 0.18$   $\text{Mg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  in the Patte d'oie.

### Fine Root Turnover

The turnover of fine roots is shown in figure 30. For both forests, we observe a higher turnover in the second horizon, regardless of the method used.

We obtain in horizon 1 with the recolonisation method a root turnover of  $1.66 \text{ yr}^{-1}$  in the Lésio-Louna forest against  $1.19 \text{ yr}^{-1}$  in the Patte d'oie forest; in horizon 2, the turnover is  $1.77 \text{ yr}^{-1}$  in the Lésio-Louna forest against  $1.56 \text{ yr}^{-1}$  in the Patte d'oie forest.

### Relationship between Root Biomass and Other Forest Parameters

Lésio-Louna forest was found to have significantly higher stem density, but lower basal area and above ground biomass values than Patte d'oie (Table 1). Lésio-Louna also had a greater litter fall than Patte d'oie, and fine root biomass had a significant positive correlation with AGB at both sites and with stem density in Lésio-Louna (Table 1).

**Table 1: Studied Forest parameters and their relationship with fine root biomass**

Structural Parameters	Lesio Louna forest	Patte d'oie forest
FRB (0-30 cm, 0-2 mm, MgDM/ha)	$4.32 \pm 0.59$	$2.64 \pm 0.37$
Average biomass	Horizon 1	Horizon 1
	Horizon 2	Horizon 2
AGB ( $\text{Mg} \cdot \text{ha}^{-1}$ D.M)	$122.86 \pm 2.40$	$173.33 \pm 29.64$
BA ( $\text{m}^2 \cdot \text{ha}^{-1}$ )	$26.74 \pm 0.63$	$35.38 \pm 1.60$
Tree density (tree number/ha) >10 cm DBH	$631 \pm 90$	$263 \pm 14$
Leaf litter fall ( $\text{tha}^{-1} \cdot \text{year}^{-1}$ )	$1063 \text{ gDMm}^{-2} \cdot \text{year}^{-1}$	$731 \text{ gDMm}^{-2} \cdot \text{year}^{-1}$
% of leaves in litter fall	95 %	64%

## Discussion

### Fine Root Biomass

The results showed that fine root biomass was greater at Lésio-Louna than Patte d'oie, which is likely linked to higher stem density and higher litter fall in the former. In fact, several biotic and abiotic factors, such as tree density per hectare, basal area, soil fertility, forest stand age, number of small and big trees, number of sampling points, the type of forest, the type of soil and its structure, climate, tree density, basal area, aerial tree biomass, ground litter stock, rate of litter decomposition, availability of bioelements in soils, and especially litter quality, are important factors for fine root biomass production (Visalakshi, 1994; Gill & Jackson, 2000; Eapen *et al.*, 2005; Becel, 2010; Ifo *et al.*, 2015). Although our study cannot focus on the main factors that determine fine root production at these two sites, we agree that the two forests have different characteristics.

Moreover, this study is the second one conducted at the Lésio-Louna site, after the one conducted by Ifo *et al.* (2015), that included a class diameter size of 0-2 mm. In addition, for a total horizon of 0-30 cm, the sum of the average of fine root biomass at this site for horizons H<sub>1</sub> and H<sub>2</sub> was 1.88 Mg.ha<sup>-1</sup>, whereas its value at Patte d'oie forest was 1.05 Mg.ha<sup>-1</sup>. Our fine root biomass estimates, as well as our estimates for fine root average ( $2 < \phi \leq 4$  mm at 0-15 cm and 15-30 cm soil depth) were accurate for both Patte d'oie and Lésio-Louna, and they also fall within the fine root average range reported in other studies carried out in Africa. Notably, it is difficult to compare results for root biomass among studies due to the differences in the depths of collection of fine and medium roots: for example, Addo-Danso *et al.* (2018) reported 1.42 Mg ha<sup>-1</sup> for an undisturbed forest and 3.04 Mg ha<sup>-1</sup> for logged forests in Ghana (0–30 cm soil depth); Sierra-Cornejo *et al.* (2020) reported 3.7 Mg ha<sup>-1</sup> for undisturbed montane forests in Tanzania (0-40 cm soil depth); and Ibrahima *et al.* (2010) reported between 1.74 Mg ha<sup>-1</sup> and 7.43 Mg ha<sup>-1</sup> for undisturbed forests in Cameroon (0-25 cm soil depth). However, our estimates were also within the range of the results reported in other tropical forest studies (Ngarnougber, 2016; Thongo Mbou, 2008; Ponton *et al.*, 2008; Defrenet, 2012).

Ifo *et al.* (2015) also raised the problem of sample representativeness for the averages obtained; with this in mind, the number of sampling points in this study was even higher than that used by Ifo *et al.* (2015) in a previous study, in which eighteen soil cores were sampled at a rate of six cores per sampling plot using the ingrowth core method. In addition, the stock of root mats at Lésio-Louna was lower than that obtained by Ifo (2010), as it was 6.10 Mg.D.M ha<sup>-1</sup>. This could be explained by two factors: the presence of the mixed litter compartment and the thickness of the litter, which ranged from 8 to 13 cm in Ifo's (2010) study and was 4 to 8 cm in ours. These differences suggest, on the one hand, that litter production in the Lésio-Louna forest is declining, or that the rate of litter decomposition is currently faster than in it was during the first experiment. Second, the high density of litter roots can also be explained by the fact that the poverty of tropical forest soils triggers adaptive mechanisms, including the rapid recycling of bioelements during litter decomposition (Went & Stark, 1968).

Further, the near absence root mat at Patte d'oie was due to the low density of trees in the study plots, the low basal area in this ecosystem, and anthropogenic pressure. During this time, much of this forest had been burned and trees were felled for charcoal right after this period of armed conflict, all of which reduce the number of trees. Indeed, the more trees there are, the greater the fallout from litter, hence the high availability of bioelements and, therefore, fine roots on the surface (Lebègue *et al.*, 2004).

### **Fine Root Production, Turnover, and Lifespan**

In our study, the results of root biomass production, turnover, and lifespan were all greater for the ingrowth core method than those obtained after sequential coring, as has been reported in the literature (Vogt *et al.*, 1998; Jourdan *et al.*, 2008). Moreover, the ingrowth core method used in this study is recommended for forest ecosystems with fast-growing trees (Vogt *et al.*, 1998); however, no devices for the monitoring of tree growth have been installed at our two study sites to conclude whether they are fast-growing, thereby making it difficult to draw any conclusions on this.

Additionally, our fine root biomass production estimates, which ranged between 0.22 and 4.06 Mg ha<sup>-1</sup>y<sup>-1</sup>, depending on the site and method, are lower than the estimates previously reported for montane forests in Rwanda (9-19 Mg ha<sup>-1</sup>y<sup>-1</sup> using in-growth cores; Hansoon *et al.*, 2014), but they are within the range of values described for forests in India (0.8-3.1 Mg ha<sup>-1</sup>y<sup>-1</sup>, soil core method; Barbhuiya *et al.*, 2012) and Ecuador (0.7-1.3 Mg ha<sup>-1</sup>y<sup>-1</sup>, minirhizotron technique; Finer *et al.*, 2011). Regarding turnover, our estimates ranged



from 0.19 to 1.77 years. Sierra-Cornejo *et al.* (2020) reported 1–4.6 years for undisturbed montane forests in Tanzania. Thongo-M'bou (2008), who studied coastal *Eucalyptus* plantations in Pointe Noire (south west of the Republic of Congo), found values of 2.43 to 3.44  $y^{-1}$  with the ingrowth core method. The low values of fine root turnover obtained by the sequential coring method indicated that this method underestimated the root turnover of our study forests and many others; therefore, this method might not be suitable for forests that undergo small variations in root biomass, such as our study forests.

On the other hand, the significant value of root turnover at the H<sub>2</sub> level can be explained by the fact that, in terms of depth, the low presence of bioelements could be a stressor that might induce a rapid replacement of fine roots to balance their maintenance and maintenance costs (Pritchard *et al.*, 2001): fine roots absorb nutrients from the soil, and their growth and maintenance require energy (Epron & Osawa, 2017). In addition, root turnover varies widely among species and across ecosystems (Eissenstat & Yanai, 1997), which could explain the difference in fine root turnover between both forests. In Patte d'oie forest, *M. laurentii* De Wild, *Terminalia superba*, and *Ficus exasperata* were the main forest species found in the different plots; all trees have a large diameter, meaning that they are old considering the correlation between diameter and tree age in tropical regions.

Although some authors have claimed that the sequential coring method underestimates the production of fine roots, we believe that more studies are needed in our ecosystems to compare both sequential coring and ingrowth core approaches. This is more intriguing because, in our case, both methods were applied simultaneously in both forests, which was advantageous as both methods were simultaneously influenced by the same environmental factors regardless of their nature. However, considering the numerous limitations of the sequential coring method, we can affirm that the results obtained by this method underestimate fine root production due to the spatial variation of fine root biomass, which is influenced by the spatial distribution of trees within and outside of the experimental plots. This method is suitable for measuring standing biomass, but has several limitations when used for assessing root turnover and requires assumptions about root growth and mortality that can be difficult to ascertain (Majdi *et al.*, 2005).

Lastly, the results we obtained also raise the problem of estimating the true amount of carbon transferred into the soil through fine root turnover, which should be taken into account as this is one of the most important roles that tropical forests play, which can help mitigate the effects of climate change.

## Conclusions

This study allowed us to obtain fine root turnover values from two forests: the Patte d'oie forest, located at the center of the city of Brazzaville, and the Lésio-Louna forest reserve in Batéké Plateau. Considering the effects of global warming and climate change, we need to better understand the role of forest ecosystems and the role soil could play in the mitigation of CO<sub>2</sub> excess emissions via fine root turnover.

The production of fine roots at Lésio-Louna was 2.05 times higher than that at Patte d'oie for horizon H<sub>1</sub> and 1.98 times higher for horizon H<sub>2</sub>. We propose to estimate the turnover rate for more types of tropical forests existing in the Republic of Congo before drawing conclusions about the potential of African tropical forests for climate mitigation.

Additionally, this study provides a strong argument for improving the sustainable management of tropical forests in the Republic of Congo and in all the tropic, as any intensive logging impacts fine root turnover, thereby reducing the amount of carbon transferred into the soil. However, this requires further research and quantification of root turnover in more mature tropical forests in the Republic of Congo, as well as comparative

studies between secondary and mature forests historically undisturbed by anthropogenic activities.

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### Conflict of Interest

The authors declare that there are no conflicts of interests.

### Author Contributions

Study idea and design: SAI; field and lab work: SAI and MNP, FM, PJMM; data analysis: SAI and MNP; paper concept and writing: SAI, MNP, FM.

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