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Asynchronous xylogenesis among and within tree species in the central Congo Basin

Pauline Hicter[1,2*], Hans Beeckman[1], Basile Luse Belanganayi[2,3], Tom De Mil[3], Jan Van den Bulcke[2], Peter Kitin[4], Marijn Bauters[5,6], Kévin Lievens[1], Donatien Musepena[7], José Mbifo Ndiapo[7], Nestor K. Luambua[2,8], Félix Laurent[1,2], Bhély Angoboy Ilondea[9,10] and Wannes Hubau[1,2]

Abstract

Background Xylogenesis is synchronous among trees in regions with a distinct growing season, leading to a forest-wide time lag between growth and carbon uptake. In contrast, little is known about interspecific or even intraspecific variability of xylogenesis in tropical forests. Yet an understanding of xylogenesis patterns is key to successfully combine bottom-up (e.g., from permanent forest inventory plots) and top-down (e.g., from eddy covariance flux towers) carbon flux estimates.

Methods Here, we monitor xylogenesis development of 18 trees belonging to 6 abundant species during 8 weeks at the onset of the rainy season from March to April 2022 in a semideciduous rainforest in the Yangambi reserve (central Democratic Republic of the Congo). For each tree, the weekly cambial state (dormant or active) was determined by epifluorescence microscopy.

Results We find interspecific variability in the cambial phenology, with two species showing predominant cambial dormancy and two species showing predominant cambial activity during the monitoring period. We also find intraspecific variability in two species where individuals either display cambial dormancy or cambial activity. All trees kept > 60% of their leaves throughout the dry season and the monitoring period, suggesting a weak relationship between the phenology of the cambial and foliar. Our results suggest that individual trees in Yangambi asynchronously activate their cambial growth throughout the year, regardless of leaf phenology or seasonal rainfall.

Conclusion These results are consistent with global analysis of gross primary productivity estimates from eddy covariance flux towers, showing that tropical biomes lack a synchronous dormant period. However, a longer-term monitoring experiment, including more species, is necessary to confirm this for the Congo Basin. As Yangambi is equipped with facilities for microscopic wood analysis, a network of inventory plots and a flux tower, further research in this site will reveal how xylogenesis patterns drive annual variability in carbon fluxes and how ground-based and top-down measurements can be combined for robust upscaling analysis of Congo basin carbon budgets.

Keywords Cambial phenology, Tropical forest, Yangambi, Carbon storage, Congo Basin

*Correspondence:
Pauline Hicter
pauline.hicter@africamuseum.be
Full list of author information is available at the end of the article

Introduction

Tropical forests annually take up ~ 10% of anthropogenic CO_2 emissions [1]. However, accelerating changes in environmental conditions alter their carbon sequestration capacity. One important example is increasing tree mortality in the Amazon, which in turn increases heterotrophic respiration [2, 3]. Yet environmental variables also impact net primary productivity of tropical forest (NPP). Woody NPP is predominantly driven by tree growth [3–6] which is monitored on millions of trees across the tropics, through repeated tree censuses and the use of dendrometers [5, 7, 8]. In order to understand the seasonal tree-growth periodicity, its underlying processes, and its sensitivity to environmental conditions, we need to closely monitor wood formation (xylogenesis) [9], which is a periodic process and consists of four phases. Xylogenesis begins with the cell division of vascular cambium cells. The second phase is cell enlargement during which the newly formed cells (initials) enlarge and specialise into specific xylem tissue cells (most importantly fibres, parenchyma, or vessels in hardwood). These two first phases (division and enlargement) result in a tree diameter growth [10, 11]. During the third phase, the cell walls thicken and lignify [10, 12]. These two processes (thickening and lignification) are highly dependent on carbon absorbed during photosynthesis and allocated to places of wood formation [10]. Xylogenesis ends with programmed cell death, which results in mature cells. Similar to leaf production, xylogenesis covers several months of the year, during which cells collectively undergo changes driven by external (climate and soil) [13, 14] and internal factors (hormonal and genetic) [15, 16], resulting in a diversity of phenological patterns, both of the primary and secondary meristems. These indicators are essential to understand patterns and processes within a forest [9].

The spread of phases of xylogenesis over time results in a lag time between the tree-diameter increase and actual carbon uptake [10, 17, 18]. In regions with a distinct growing season, xylogenesis is relatively synchronous among trees, showing a consistent link with leaf phenology. As such, the lag time between radial growth and biomass production is also synchronous between trees leading to a forest-wide delay between growth and carbon uptake. This lag time must be taken into account in experimental sites comparing ground-based measurements of tree growth with measurements of carbon fluxes using eddy-covariance techniques on flux towers [17–19]. While continuous tree-diameter measurements with band dendrometers or repeated diameter measurements capture volume increase during cell division and enlarging phases, eddy covariance sensors mostly capture actual carbon uptake during the thickening and lignification phases. This explains the temporal mismatches between growth measured on the ground versus carbon uptake measured on the flux tower. In temperate and boreal forests in the northern hemisphere, the lag time varies from a few days (from one up two) to one month [10, 20].

However, very little is known about the existence or significance of the growth-carbon lag time around the equator as well as its relationship to leaf phenology patterns. These aspects of forest ecology are especially understudied in the Congo Basin, where both detailed tree monitoring and detailed eddy-covariance flux measurements are lacking. Existing growth studies adopt different spatial and temporal resolutions, ranging from permanent monitoring [1, 21] to dendrological approaches [21–23] or using the pinning method [22–25]. Most of the available studies focus on a limited number of species and on high-seasonal forests such as Mayombe [21–23, 26] or Miombo [24]. These studies generally show a cessation of cambial activity during the dry season and a resumption of wood formation during the rainy season [22, 23, 27], as in the case of *Terminalia superba* [27], *Prioria balsamifera* [21] or *Brachystegia spiciformis* [24]. However, a wide diversity of cambial patterns has been observed between species, sites [25] and sometimes even within species (e.g. *Terminalia superba* [26]). Although some studies have included several species, they have rarely considered climates with low seasonality such as in the central Congo Basin.

A step was made recently, with the operationalization of the first eddy-covariance flux tower (CongoFlux) in the Central African rainforest [28]. CongoFlux is located in the Yangambi Man-And-Biosphere Reserve in the heart of the Congo Basin (Fig. 1). Yangambi experiences a semi-humid climate and a bimodal rainfall seasonality with an intense dry season with low precipitation (< 100mm month^{-1}) (December-February) and a mild dry season (June-July) where precipitation varies between 100 and 150mm month^{-1} [29, 30]. CongoFlux is surrounded by an increasing number of geographically dispersed [7], intensive [5] and large-scale [8] permanent inventory plots, where tree diameters are measured continuously [1, 31–34]. Future research will attempt to upscale forest carbon fluxes by combining data from CongoFlux and permanent inventory plots. Robust upscaling is urgently demanded by Environment Ministries of central African nations [35] who need the information to improve their quantifications of Nationally Determined Contributions and Forest Reference Levels [36, 37]. Yet the success of combining data from CongoFlux and permanent inventory plots will depend on a good understanding of xylogenesis and especially the lag time between growth and carbon

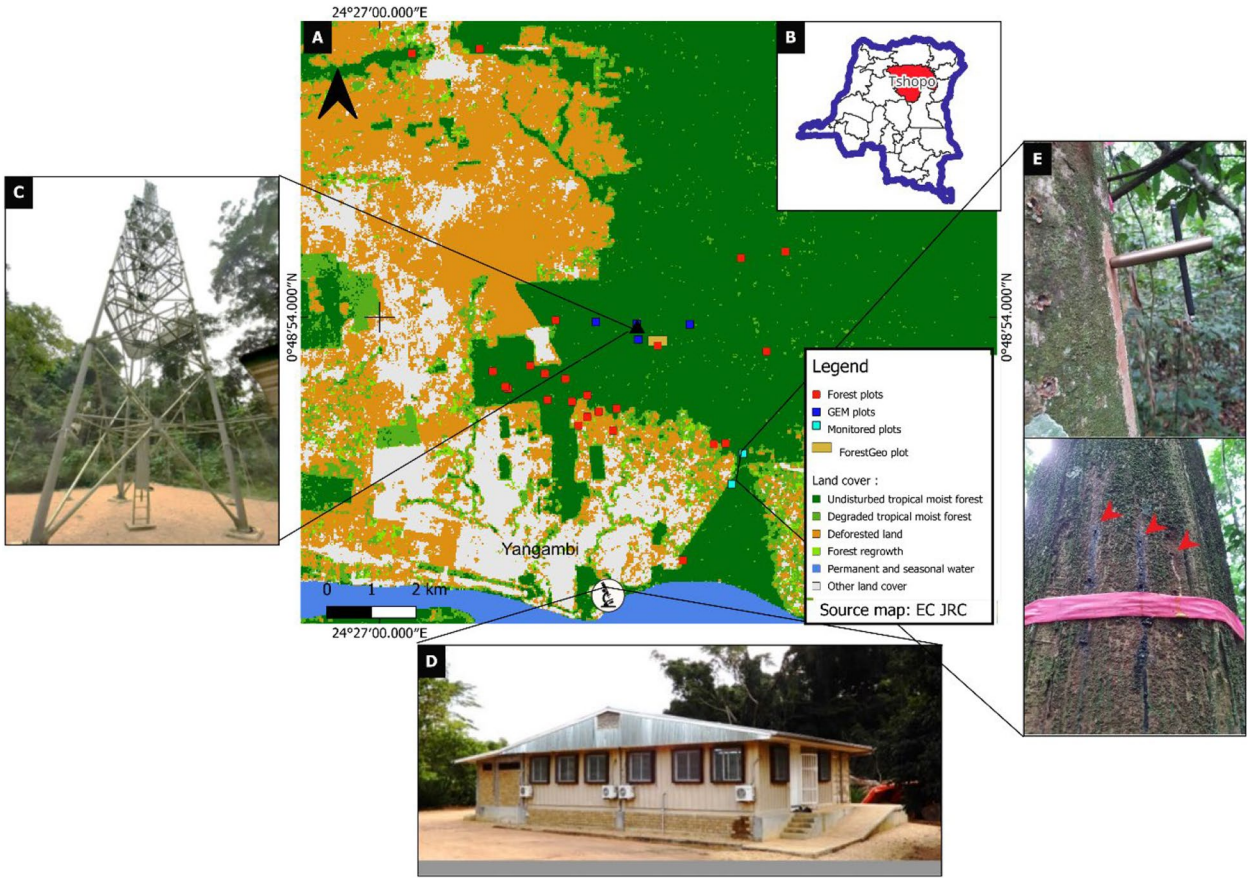

Fig. 1 Study site. **A** Location of the Yangambi Man and Biosphere Reserve [41] in the (**B**) Tshopo province (in red) within the Democratic Republic of Congo (blue limits). **A** Location of the CongoFlux Eddy Covariance tower (black triangle) surrounded by a 10-ha permanent inventory plot from the ForestGEO network (yellow rectangle) [8] four intensively monitored plots from the Global Ecosystem Monitoring (GEM) network (blue squares) [5], and 23 dispersed permanent inventory plots from the Forestplots.net network (red squares) [7]. The two plots containing the 18 selected trees are indicated as light blue squares. **C** The Congo Flux tower. **D** The Yangambi Woodlab. **E** Collection of micro-cores consists of a micro-coring process with a Trephor (top) according a spiral scheme (bottom: red arrowheads) over four collections [39]

uptake. Therefore, the main ambition of this paper is to explore whether the most commonly used methods for analysis of xylogenesis are applicable in a central African context.

Specifically, we first test the hypothesis that trees display synchronous cambial activity among and within species, following precipitation seasonality. As wood formation includes water-requiring turgor processes, inactive cambium (i.e. cambial dormancy) is expected during the dry season, while cambial activity is expected during the rainy season [13, 21]. Secondly, we test the hypothesis that there is a relation between rainfall seasonality, leaf phenology and cambial phenology. Yangambi is characterised by the presence and local abundance of (semi-) deciduous tree species [34, 38] due to rain seasonality. These species can shed all or part of their leaves during dry seasons to avoid drought stress (e.g. cavitation). In turn, significant leaf loss is expected to halt cambial activity through a lack of resources.

To verify these hypotheses, we applied frequently repeated sampling of microcores [10, 39] on the same set of 18 individuals belonging to 6 of the most abundant species in the Yangambi forest. On each tree, we collected 3 microcores every two weeks throughout March and April 2022, when precipitation in Yangambi intensifies from 100 to 142mm month^{-1} (in March) and exceeds the median monthly precipitation of 142mm month^{-1} (in April) (Supplementary Fig. 1) [30]. To determine the weekly cambial state (dormant or active) and the xylogenesis phase (cell division, cell enlargement, cell wall thickening and lignification), we prepared microscopic thin sections from each microcore using a rotary microtome. We then used epifluorescence microscopy to distinguish

cell types and measure the radial width of each xylogenesis phase [40]. For each tree, we determined foliar phenology (percentage of old and new leaves, and leafless crown) twice during the monitoring period.

Materials and methods

Study site and meteorological data

Data have been collected within the Yangambi Biosphere Reserve (0°46'N −24°29'E) located in the Tshopo province in the northeast of the Democratic Republic of the Congo (hereafter DRC). Forests in Yangambi are described as semi-deciduous and consist of a mix of deciduous, brevi-deciduous and evergreen species [38]. Other forest types occur in patches, such as pioneer or successional forests and old-growth forests dominated by the shade-bearing species *Gilbertiodendron dewevrei*. The latter is an edaphic forest type occurring along rivers in Yangambi [34]. Yangambi experiences an Af equatorial climate [42], with an annual mean temperature of 25.1 °C, and annually receives between 1450 and 2400mm rainfall for the 1960–2020 period [30]. It exhibits two dry seasons alternating with two rainy seasons (Supplementary Fig. 1) [30]. A long dry season extends from December to February, followed by a short rainy season from March to May (Fig. 2 and Supplementary Fig. 1). Then a short dry season covering the months of June and July is followed by a long rainy season from August to November (Supplementary Fig. 1). Dry season months are defined as those with median precipitation below the overall 1960–2020 median monthly precipitation (142mm month^{-1}). January and February are the driest months, with median precipitation of 61 and 79mm month^{-1} respectively (Supplementary Fig. 1) [30], reaching 31 and 79mm month^{-1} respectively in 2022 (Fig. 2). The cumulative monthly precipitations and average monthly temperatures were calculated from June 2021 until May 2022 (Fig. 2) using digital climate data collected by the Yangambi meteorological station to confirm rainfall seasonality patterns. This state-of-the-art station is part of the Trans-African Hydro-Meteorological Observatory (TAHMO) [43], and has provided data in quasi-real-time since 2018 at Climate Data—TAHMO.

Species and individual tree selection

We used available forest inventory data from 29 permanent inventory plots in Yangambi (blue and red squares

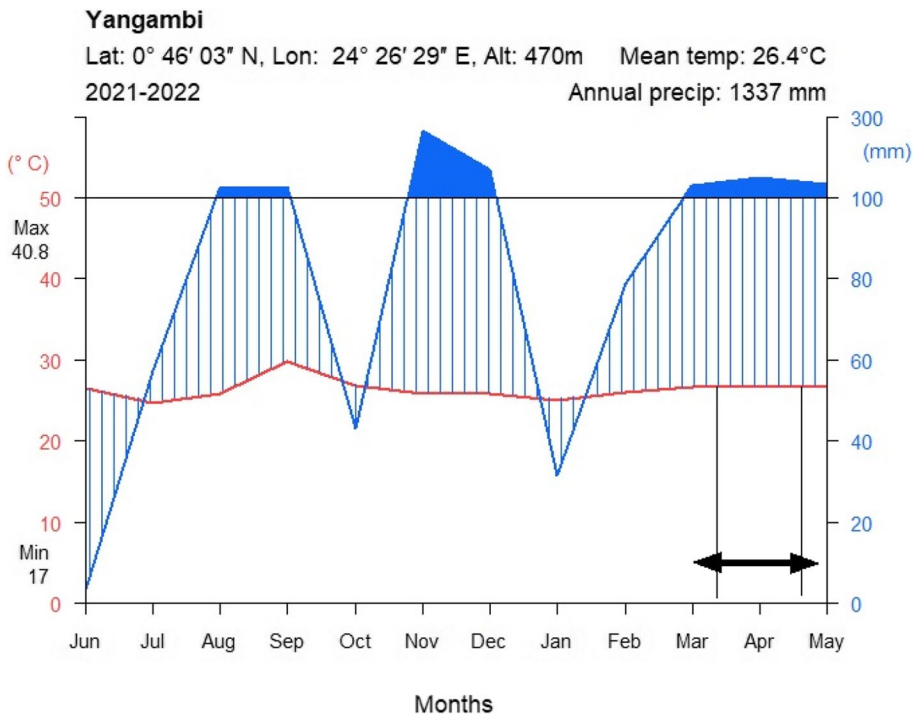

Fig. 2 Walter and Lieth climate diagram of Yangambi, DRC, from June 2021 to May 2022. The covered study period for cambial phenology monitoring and the two monitoring of leaf phenology are indicated by the black double-headed arrow and the vertical black lines, respectively. On the left ordinal axis, the monthly mean air temperature (T) is shown with the mean daily minimum (Min) and maximum (Max) air temperatures of the total period. The monthly total precipitation (Prcpt) is shown on the right ordinal. The (mild) wet periods (> 100mm month^{-1}) and intense dry periods (< 100mm month^{-1}) are represented by solid blue areas and vertical blue lines, respectively. Note that the scale of the right axis changes above 100mm. Digital data are part of the Trans-African Hydro-Meteorological Observatory (TAHMO) [43]

in Fig. 1) to calculate the proportion of basal area per species (in %) [34]. These plots are dispersed around the CongoFlux site and belong to the international Global Ecosystem Monitoring (GEM) network and the Forestplots.net network [5, 7]. Using the basal area proportions, we selected 3 species from the top 10 most abundant species in Yangambi: *Scorodophloeus zenkeri* (14.4% of basal area), *Panda oleosa* (5.8% of basal area) and *Leplaea thompsonii* (2.8%). As all these are typical canopy species, we also selected an abundantly occurring evergreen understory specialist species, *Synsepalum subcordatum* (0.8% of basal area) (Table 1). Finally, we selected two of the most abundant (brevi-) deciduous species: *Petersianthus macrocarpus* (4.8% of basal area) and *Trilepisium madagascariense* (2.3%).

We used repeated measurements of tree diameter at breast height (DBH) from the permanent inventory plots to calculate individual tree growth rates over an interval of three years (2017 and 2020). For each tree, we calculated biomass in 2017 and 2020 using allometric biomass-diameter relations according to standard methods [1, 44]. We then calculated species-specific biomass gains over the interval (in kg yr-1) and divided by total biomass gains to express in percentage. Of all species in these plots, *Scorodophloeus zenkeri* and *Petersianthus macrocarpus* contribute most to biomass productivity (12.0% and 9.0% respectively) while *Trilepisium madagascariense* and *Panda oleosa* are in the top ten (5.0% and 3.0% respectively) (Table 1). *Synsepalum subcordatum* and *Leplaea thompsonii* both just contribute 1% to biomass productivity.

We then considered two permanent inventory plots to choose trees for microcoring (light blue squares in Fig. 1). For each of the six species, we sampled three individuals within the two plots, so that we have a total sample size of 18 monitored trees (Supplementary Table 1). Within each species, we selected individuals of similar status (i.e. similar DBH, height, health, light competition) to exclude differences in growth performance that are driven by resource availability, health or other non-climatic factors. This choice was first based on inventory data, namely diameter at breast height (DBH) and the Crown Illumination Index (CII) [46]. We specifically selected trees with a DBH close to the average DBH of the plots (i.e. 32 cm) and individuals with a similar Crown Illumination Index (Supplementary Table 1). The final selection was based on a field prospection where we chose individuals that were in good health and did not have stem deformities at breast height.

Monitoring leaf phenology and collecting microcores

Leaf phenology of each tree was visually assessed on 18/03/2022 (just after the dry season) and 15/04/2022 (during the rainy season) (Fig. 2). Leaf phenology was quantified by estimating the proportion of the crown that contained old leaves, new leaves or no leaves at all (expressed in %). Classification was determined by the colour, the shape, and the abundance of leaf cover. New leaves display vivid colour with intact shape while old leaves often present perforated or cut leaves, appear dried out, and show colour changes. A visual estimation of the respective proportions of these three categories

Table 1 Ecological characteristics of selected species. The mean pattern of leaf phenology cycles and the relative occurrence of phenophase, i.e. turnover (T) and senescence (S), for each species are indicated by the annual mean percentage of individuals with phenophase and percentage of observed phenophase on the total observation years, respectively, based on data from Kearsley [45] The relative contributions of the monitored species compared with all species in terms of basal area (%BA) and biomass productivity (%Productivity) are based on data from the three-year permanent inventory plots (between 2017 and 2020)

Species name	Guild	Leaf type	Mean annual % of ind. with T/S events	% years with phase	Habitus	%BA	%Productivity
Synsepalum subcordatum	shade bearing	evergreen	3% S 3% T	9.8% S 14.6%	small tree 5–20 m)	0.8%	1.0%
Leplaea thompsonii	shade bearing	evergreen	NA	NA	big tree (> 20 m)	2.8%	1.0%
Panda oleosa	shade tolerant	evergreen	0% S 0% T	1.6% S 1.6% T	big tree (> 20 m)	5.8%	3.0%
Scorodophloeus zenkeri	shade tolerant	evergreen	3% S 0% T	11.1% S 3.7% T	big tree (> 20 m)	14.4%	12.0%
Petersianthus macrocarpus	non-pioneer light demander	deciduous	0% S 5–10% T	2.2% S 3.8% T	big tree (> 20 m)	4.8%	9.0%
Trilepisium madagascariense	non-pioneer light demander	(brevi-)deciduous	NA	0% S 0% T	big tree (> 20 m)	2.3%	5.0%
Total						**30.9%**	**31%**

was conducted by observing the overall crown of each tree (Supplementary Table 1).

Microcores were collected using a Trephor [47] according to a standardized spiral scheme starting at two meters from the ground and from the North direction for all the individuals and species. For each selected tree, microcores were sampled four times at two-week intervals [39, 48], between 5/03/2022 and 15/04/2022, just after the onset of the rainy season (Fig. 2). At each sampling date, we sampled three microcores per tree, each spaced 10cm apart. As such, we collected 216 microcores (18 individuals × 4 sampling dates × 3 cores), which were stored in Eppendorf tubes in a 50% ethanol solution.

Microtomy

Microcores were used to prepare microscopic thin sections of the transversal plane using standard protocols including the following steps: dehydration, embedding, microtomy, and staining [49, 50]. Microcore preparation was performed in the Yangambi wood biology laboratory (Fig. 1). First, we used a Spin Tissue Processor STP 120 (Leica, Nussloch, Germany) to immerse each microcore in dehydration baths of 2 h each (50%, 75%, 96%, and 100% ethanol). Then, the microcores were cleared twice with Histo-clear ® over 1h30 each and finally embedded 2 times in paraffin at 60 °C for 1h30 each. From each paraffin block, one to three transversal thin sections were cut with a Leica RM 2235 rotary microtome (Leica, Nussloch, Germany). Thin sections were then fixed with albumin, and cooled after being heated for 5 min at 60 °C. Thin sections were then successively cleared with 3 Histo-clear® baths of 6 min, dehydrated with ethanol 100% for 3 min, stained in a solution of Alcian Blue and Safranin (respectively, 65% and 35%) for 3 min, cleared with distilled water, and then dehydrated by successive 3 min baths of ethanol (50%, 75%, 96%, 100%). Finally, each thin section was fixed between a microscopic slide and a coverslip with Euparal (0.99).

Microscopy and identification of xylogenesis phases

Subsequently, microscopic slides were then observed using UV-filtered epifluorescence microscopy. We used a BX60 transmission light microscope (Olympus, Hamburg, Germany) equipped with a pE-300^{lite} LED epifluorescent illumination system (CoolLED, Andover, UK) and an ultraviolet filter (bandpass filter BP 330–385, dichromatic mirror 400nm, and barrier filter 420-480nm) [40]. To improve contrast, we also applied unfiltered transmitted light.

Epifluorescence microscopy highlights the contrast between lignified and non-lignified tissues [40] and the addition of transmitted light allows us to distinguish between the four xylogenesis phases. Xylogenesis starts with the activation of cells in the cambial zone (CZ), which is located between the mature xylem (MX) and mature phloem (MP). It includes the cambium *sensu* stricto, which is composed of 'initial' cells. The cambial zone also includes undifferentiated cells of the phloem (P) and xylem generated by the division and development of cambial cells, and their derivatives [51]. Similar to the initials, these cells have narrow radial shapes and thin non-lignified walls.

The xylogenesis process is detailed according to the four consecutive differentiation phases proposed by Wilson [39, 52–54]. The first phase is the cell division phase (DP) during which a cambial 'initial' cell divides into a new initial or "daughter cell" and a xylem mother cell. Sometimes, this nascent division process is visible thanks to the appearance of division plates, characterised by very thin cell walls. Each daughter cell is programmed to differentiate into a fibre, a parenchyma cell, or a conduit. The enlargement or expansion phase (EP) is recognised when daughter cells start to grow in a radial direction. Next, cell walls thicken during the thickening phase (TP) with the overlap of the deposition and the lignification of a secondary cell fibre wall. The lignification results from a chemical change in the composition of the cell wall through lignin deposition [12]. The differentiation ends with the programmed cell death, i.e. mature xylem cells, where cells are empty (i.e. absence of cytoplasm). In the present paper, we consider cells in the second and third xylogenesis phases of the differentiation (enlargement and thickening) as part of the so-called immature xylem. When cambial activity is detected, the cambial zone (CZ) includes both the initial cells as well as the cell in division phase which are indistinguishable from each other.

Determination of cambial activity stage

For each microcore, we then determined the cambial activity stages by the number of xylogenesis phases that are present. Also referred to as cambial dormancy, cambial activity stage 0 corresponds to the absence of any xylogenesis phase, which is characterised by a distinct boundary between the cambial zone, consisting only of a narrow layer of meristem (non-lignified cells), i.e. the cambial zone (CZ) and the adjacent mature xylem (MX) (composed of fully lignified secondary fibre cell walls) [14, 51]. Cambial activity stage 1 corresponds to the presence of cells in DP (division phase); cambial activity stage 2 corresponds to the presence of cells in DP and EP (enlarging phase); cambial activity stage 3 corresponds to the presence of cells in DP, EP and TP (thickening phase which includes deposition of a second cell wall and its lignification); cambial activity stage 4 corresponds to the presence of cells in DP, EP, TP and MX (mature xylem). Mature xylem is considered when secondary cell walls

in fibre cells are complete and entirely lignified, and the intercellular spaces are lignified. We also defined the "lignification front line", which marks the boundary between non-lignified cells, whatever their stage of development, and lignified cells, identified with epifluorescence microscopy.

We further conducted a second cambial analysis by repeating three measurements of the radial width of the different phases of wood formation for each individual, species, and census. To address the challenge of distinguishing these phases, we defined the measurement range as extending from the first immature cells in the cambial zone (either xylem or phloem) to the first fully mature cells. However, the fragility and variability in the quality of the samples resulted in an incomplete dataset. All the measurements have been conducted with the Image J software.

Results

Inter- and intraspecific variability in cambial phenology

Half of the monitored species and 45% of the monitored individuals show cambial activity (Fig. 3). All individuals of *P. macrocarpus* and *S. subcordatum* had dormant cambium throughout the study period, while all individuals of *T. madagascariense* and *L. thompsonii* had active cambium. *P. oleosa* and *S. zenkeri* display intraspecific variability with active cambium in only 1 of the 3 individuals monitored for each species.

Trees of *Synsepalum subcordatum* (Supplementary Fig. 2) and *Petersianthus macrocarpus* (Supplementary Fig. 3) present predominantly cambial dormancy (cambial activity stage 0) throughout the monitoring period. Their cambial zones were characterized by a few tangential lines of narrow and unlignified meristematic cells, in stark contrast to the immediately adjacent xylem cells, which have thick and fully lignified secondary cell walls and strongly lignified middle lamella (Fig. 4). The average width of the cambial zone remains constant over time for both species but is wider in *P. macrocarpus* (~73µm) compared to *S. subcordatum* (~52µm) (Supplementary Table 2). The absence of division plates and any enlargement phase of the cambial zone for both species confirms the cambial dormancy (Supplementary Table 2).

Individuals of *Scorodophloeus zenkeri* (Supplementary Fig. 4) and *Panda oleosa* (Supplementary Fig. 5) show intraspecific variability in cambial activity. Two of the three monitored individuals of both *P. oleosa* and two individuals of *S. zenkeri* displayed cambial dormancy (cambial activity stage 0) throughout the monitoring period (Fig. 3 and Fig. 4). In individuals showing cambial dormancy, no cells in differentiation and no division plates were found between the cambial zone (CZ) and the mature xylem (MX). In contrast, cambial activity stage 4 was detected in one individual of each of these two species (Fig. 3). In these individuals, all phases of xylogenesis were observed throughout the monitoring period: division phase (DP), enlarging phase (EP), cell wall thickening phase (TP) and mature xylem (MX) (Fig. 5). New vessel elements were produced within the cambial zone and division plates were sometimes observed (Supplementary

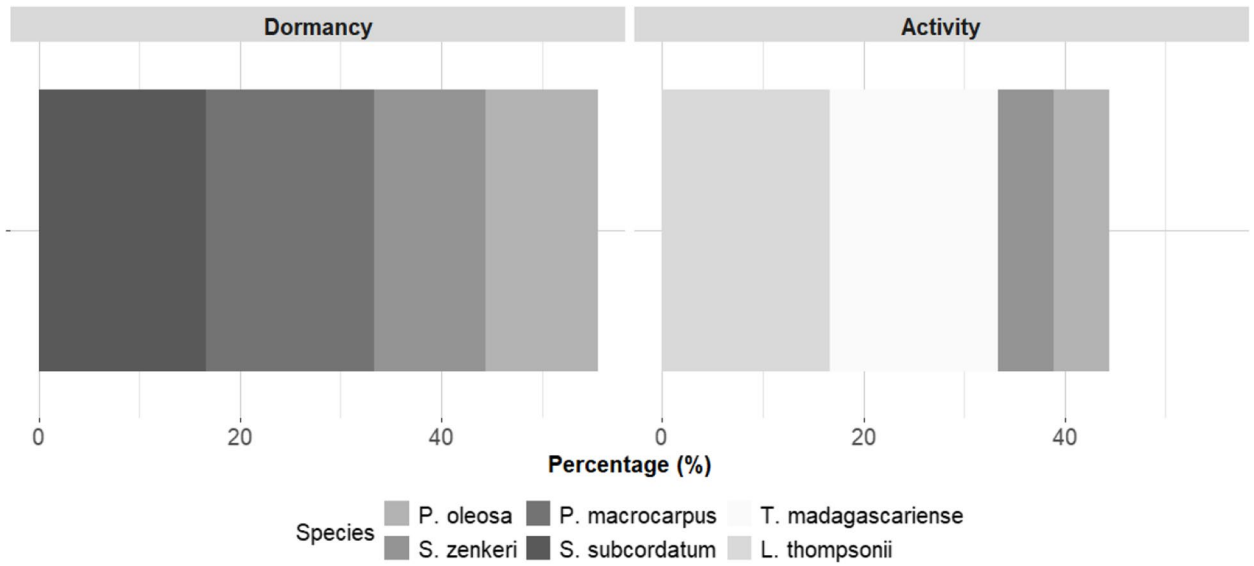

Fig. 3 Percentage of active or dormant cambium over the study period between 05/03/2022 to 15/04/2022. The percentage of active and dormant cambium for the different species is assessed as the relative number of individuals showing cambial activity or dormancy respectively (grey gradient stacked bar)

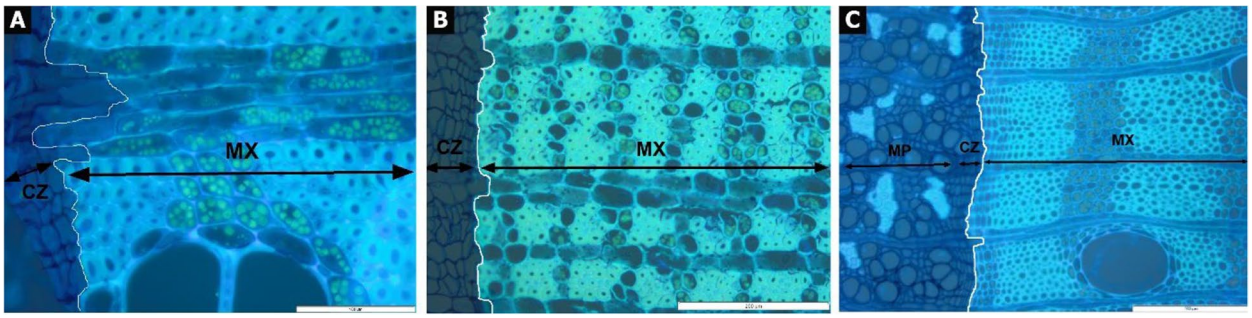

Fig. 4 Cambial dormancy (cambial activity stage 0) observed in three different species using epifluorescence microscopy. **A** Cross section of individual 1 of *Petersianthus macrocarpus* on 18/03/2022. **B** Cross section of individual 3 of *Synsepalum subcordatum* on 16/04/2022. **C** Cross section of individual 3 of *Scorodoploeus zenkeri* on 15/04/2022. Cells in the cambial zone (left; CZ) are non-lignified and appear dark blue under epifluorescent light. Cells in the mature xylem (right; MX) are fully lignified and are light blue. In each panel, the lignification front is highlighted with a white line between the cambial zone (CZ) and the mature xylem (MX). There are no developing non-lignified cells in the cambial zone. Abundant starch deposits are detected within parenchyma cells, close to the cambial zone. Scale bars are 200µm

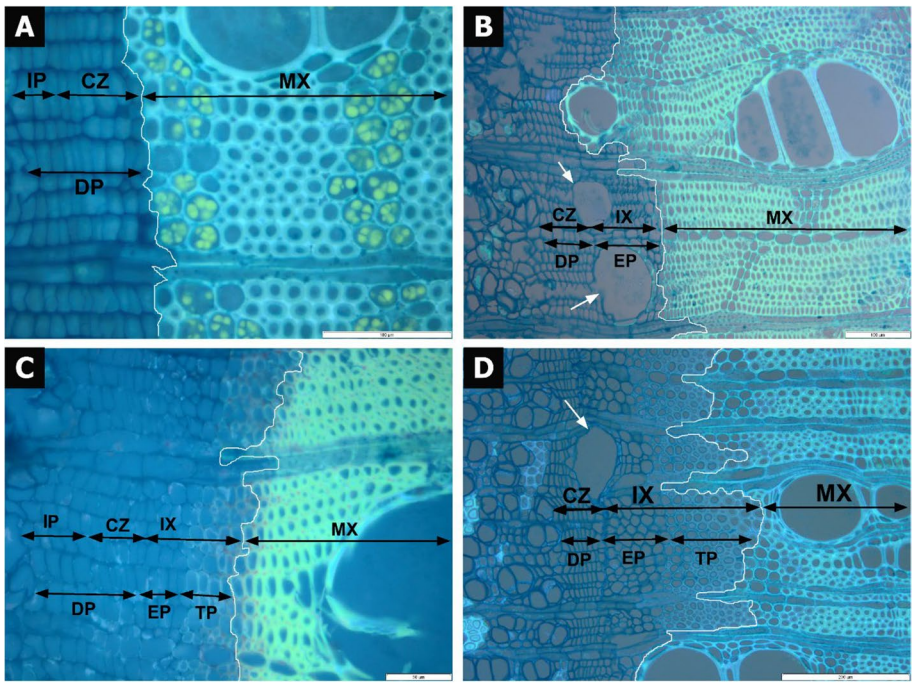

Fig. 5 Cambial activity (cambial activity stage (1–4) observed in different species using epifluorescence microscopy. **A** Cross section of the cambial zone (CZ) and the adjacent xylem (MX) of individual 2 of *Leplaea thompsonii* on 18/03/2022, in cambial activity stage 1 showing only the cell division phase (DP). **B** Cross section of individual 2 of *Trilepisium madagascariense* on 18/03/2022, in cambial activity stage 2 showing the DP and enlarging phase (EP). **C** Cross section of individual 1 of *Trilepisium madagascariense* on 22/04/2022, in cambial activity stage 3 showing the DP (including immature phloem (IP) and immature xylem (IX)), EP and cell wall thickening phase (TP). (D) Cross section of individual 2 of *Scorodophloeus zenkeri* on 05/03/2022, in cambial activity stage 4 showing the immature phloem (P), DP, EP, and mature xylem (MX). Cells in the cambial zone (left) are non-lignified and appear dark blue under epifluorescent light. Cells in the mature xylem (right) are fully lignified and appear light blue. In each panel, the lignification front is highlighted with a white line between the immature xylem (IX) and the xylem (MX). Juvenile vessels are produced within the enlarging phase (white arrows) and longitudinal division of fibres is detected within the thickening phase. Abundant starch deposits are observed within mature xylem parenchyma cells in both panels, but not in developing cells. Scale bars are respectively 100, 100, 50 and 200µm wide

Figs. 4, and 6). The average total width of the immature zone did not fluctuate significantly for active individuals of *S. zenkeri* and all the dormant individuals of both species, while we observed an increase in radial growth for *P. oleosa* in April (Fig. 6).

Abundant starch deposits were observed in mature axial and ray parenchyma cells in each individual, but they were absent from developing cells in individuals showing cambial activity. In both *Scorodophloeus zenkeri* and *Panda oleosa,* new vessels appeared within the immature xylem surrounded by parenchyma cells which appear to be the first elements to lignify, preceding vessels, (ray) parenchyma and fibre cells (Fig. 5 and Supplementary Figs. 4 and 5). Moreover, lignification was observed in cells in the enlarging phase, overlapping with the emergence of secondary walls and the middle lamella.

Trees of *Leplaea thompsonii* (Supplementary Fig. 6) and *Trilepisium madagascariense* (Supplementary Fig. 7) show cambial activity throughout the observation period for all individuals (Fig. 3). All individuals of *Leplaea thompsonii* were in cambial activity stage 1, showing cells in the division phase (DP) and slightly enlarged cambial cells adjacent to the xylem (Fig. 5). Individuals of *Trilepisium madagascariense* were in cambial activity stage 2 or 3 (Fig. 5). This was the only species where individuals changed from one cambial activity stage to another throughout the monitoring period (Fig. 6, Supplementary Table 1). All microcores of *T. madagascariense* displayed

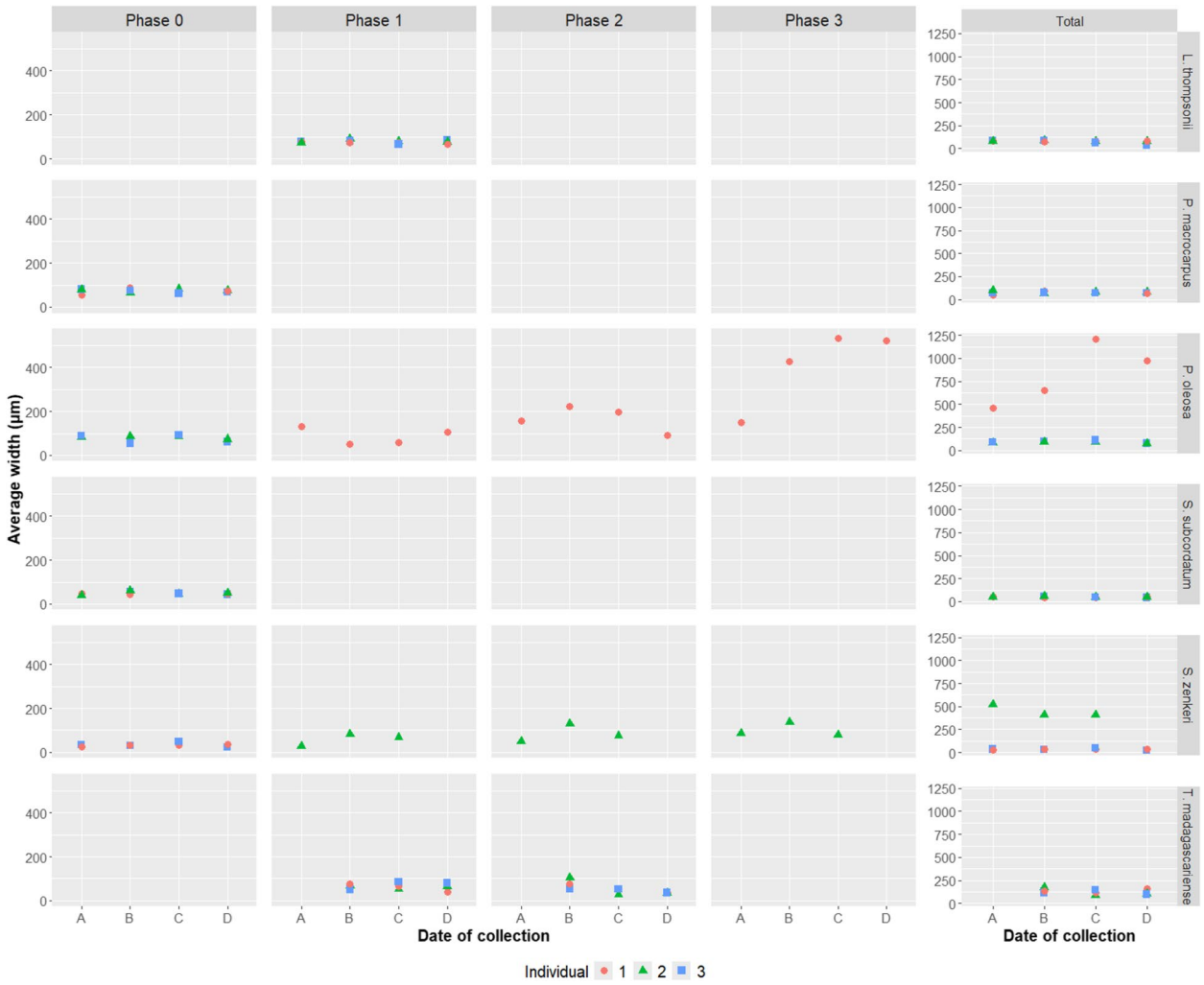

Fig. 6 Evolution of the average width (µm) of the different xylogenesis phases for the 6 monitored species. The average values have been calculated on the 3 radial width measurements for each monitored tree and each date of collection from 05/03/2022 to 16/04/2022. The average values only fluctuate slightly from one collection date to another for individuals of *L. thompsonii*, *P. oleosa, S. zenkeri,* and *S. subcordatum*., while measurements are less constant for individuals of *T. madagascariense* and *P. macrocarpus*. The first individual of *P. oleosa* and the third individual of *S. zenkeri* showed a higher average width

a very active cell division phase (DP), with the emergence of new vessels with non-lignified walls and substantial cambial cell divisions (Fig. 5). In both species, fibres close to the cambial zone often had thin but lignified walls. Starch deposition was visible within the parenchyma cells. Furthermore, in some individuals, cells close to the boundary between the cambial zone and the mature xylem started the radial enlarging phase (EP) and some initiated longitudinal division and cell thickening phase (TP). However, no variation in the average width of the different phases was observed for the individuals of *T. madagascariense* (~58µm) and *L. thompsonii* (~77µm) over time (Fig. 6, and Supplementary Table 2).

The width assessment of xylogenesis phases support the visual observations presented above (Fig. 6). While very little variation in the width of the cambial zone or mature xylem was observed in all species over the study period, significant interspecific variations were also detected (between 52µm and 163µm) (Fig. 6 and Supplementary Table 2).

Lack of relation between cambial and foliar phenology

All individuals and species, regardless of their cambial status, present predominantly old leaves (on average > 75% of the canopy) from the beginning of the rainy season (Fig. 7). The average proportion of old leaves is slightly higher for individuals with active cambium, while it tends to be higher over time for individuals with dormant cambium (Fig. 7). For most trees (15 out of 18), more than 75% of the canopy was covered with old leaves immediately after the dry season (Fig. 8). All individuals of *Petersianthus macrocarpus* and *Trilepisium madagascariense* retained more than 90% of their old leaves, while all individuals of *Synsepalum subcordatum* kept more than 80% of their old leaves just after the dry season (on 18/03/2022) and at the onset of rainy season (on 16/04/2022) (Fig. 8). However, all individuals of *P. macrocarpus* and *S. subcordatum* had dormant cambium throughout the study period, whereas all individuals of *T. madagascariense* presented active cambium.

Additionally, the canopy cover of individuals of the different species ranged from 5 and 20% (Fig. 8, Supplementary Table 1). The average leafless proportion tended to be slightly more important for individuals with dormant cambium while little variation over the onset of the rainy season were observed regardless of their cambial state (Fig. 7). The 3 trees with less than 75% old leaves are also the 3 individuals with more than 25% of their canopy covered with new leaves and a reduced leafless proportion, showing no cambial activity (*P. oleosa*) or only the first stage of xylogenesis (*L. thompsonii*). Leafless proportions reached 15% for an individual of *P. macrocarpus* with dormant cambium. During the study period, individuals of *S. zenkeri* (with dormant and active cambium) showed 30 and 20% of lost leaves. Yet, individuals of *P. oleosa* (with cambium dormant and active) presented 5 and 10% of lost leaves just after the dry season (Fig. 8).

The intra- and interspecific variability observed in cambial phenology during the study period did not appear to correspond to similar patterns in leaf phenology. Individuals of *Panda oleosa* and *Scorodophloeus zenkeri* exhibited variable cambial states, but little difference in the amount of old leaves retained. Dormant individuals of *P. oleosa* had about 65% of old leaves, while active individuals presented 80% of old leaves. Dormant individuals of *P. oleosa* had about 65% old leaves, while active individuals had 80% old leaves. In contrast, all *S. zenkeri* stems had

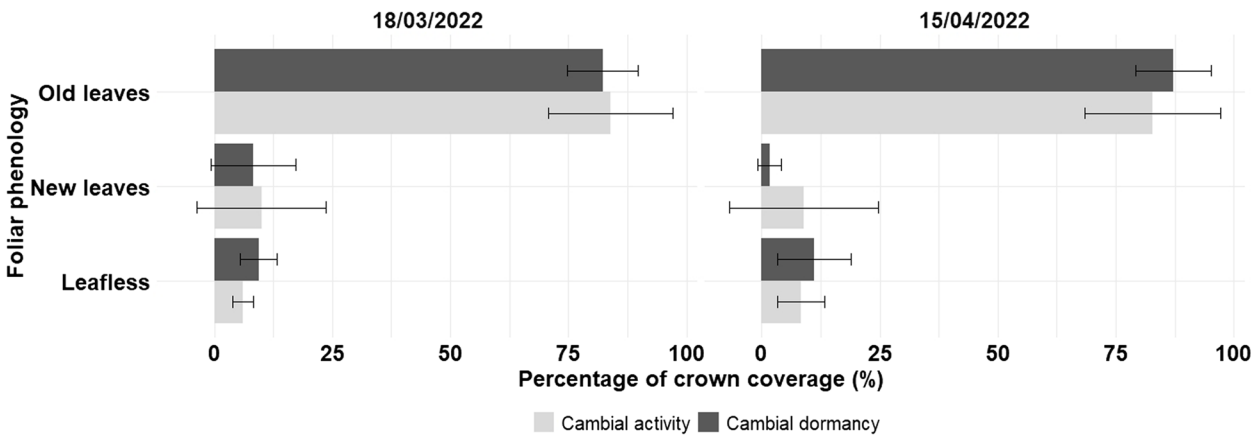

Fig. 7 Average percentage of old leaves, new leaves, and leafless canopy cover on 18 March and 15 April 2022. The averages values are obtained from the respective percentages of the different leaf categories for all species and for each census. The bars correspond to the standard deviations. We split the data in trees showing active cambium (light grey) versus trees showing dormant cambium (dark grey)

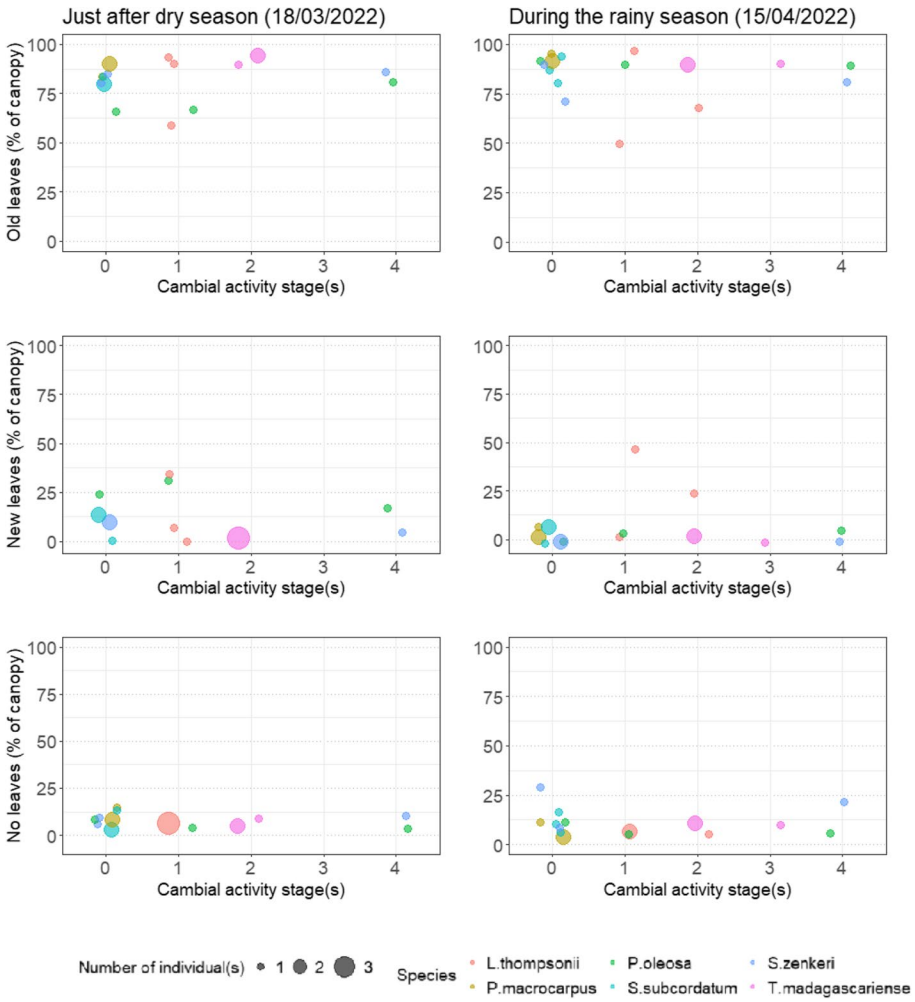

Fig. 8 Relation between cambial activity and foliar phenology (%) from 18/03/2022 to 15/04/2022 in Yangambi. The cambial activity stage is determined by the number of xylogenesis phases that are present. Cambial activity stage 0 corresponds to the absence of any xylogenesis phase (= cambial dormancy); cambial activity stage 1 corresponds to the presence of cells in division phase (DP); cambial activity stage 2 corresponds to the presence of cells in DP and enlarging phase (EP); cambial activity stage 3 corresponds to the presence of cells in DP, EP and thickening phase (TP); cambial activity stage 4 corresponds to the presence of cells in DP, EP, TP and mature xylem (MX). Each dot indicates an individual trees, dot sizes indicate the number of tree by stage and species, and colours refer to species

about 80% old leaves (Fig. 7). With the onset of the rainy season, the proportion of old leaves increased slightly in *P.oleosa* individuals, but decreased slightly in *S. zenkeri* individuals. Furthermore, the relationship between leaf production or loss and cambial phenology seemed to vary between species.

After the dry season, the average proportion of new leaves remains higher over time for individuals with an active cambium (Fig. 7). Stems of *S. zenkeri, P. oleosa*, *L. thompsonii* and *S. subcordatum* have produced new leaves, especially for those with a dormant cambium or a preliminary stage of cambial activity (division phase) (Fig. 8). The highest proportion of new leaves (25–35%) was found in individuals of *P. oleosa* and *L. thompsonii* with a dormant cambium or in the preliminary stages of cambial activity (division phase), corresponding to the presence of a lower proportion of old leaves (Fig. 8). During the study period, the proportion of new leaves decreased for all species, except for *L. thompsonii* of where the 2 individuals maintained 25 and 45% of new leaves, respectively. For *P. oleosa,* this decrease seems to be correlated with the increase of old leaves. To the contrary, no individuals of *P. macrocarpus* and *T. madagascariense* produced new leaves, despite a dormant and an active cambium, respectively (Fig. 8).

Discussion

Asynchrony of cambial activity between and within species

Based on our results, we reject our first hypothesis that trees display synchronous cambial activity between and within species, following the seasonality of rainfall.

Our observations indicate that some of the monitored species exhibit interspecific asynchronous cambial phenology during the study period in the Yangambi rainforest. All individual trees belonging to two species, *S. subcordatum* and *P. macrocarpus*, showed dormant cambium during the monitoring period. In contrast, all trees belonging to two other species, *L. thompsonii* and *T. madagascariense*, showed the division phases of xylogenesis (Fig. 3). This asynchrony among species could reflect variable response strategies to available resources, which has already been observed in different eco-climatic contexts [13, 55–57]. The asynchrony can be explained by the plasticity and sensitivity of species to their local environment [21, 38]. To coexist and share resources, species growing in a dense tropical rainforest adopt different reproductive and growth strategies [14, 56, 58, 59]. These are reflected in variable functional traits such as growth (timing and rate) [23, 60–63], stature, wood density, vessel density, light behaviour, leaf phenology or leaf area [16]. As xylogenesis takes time, the heterogeneity of cambial strategies could significantly influence the variation in annual carbon dynamics at the forest scale.

Two of the six species studied revealed intraspecific variability in cambial state during the study period. Specifically, two individuals of *S. zenkeri* and *P. oleosa* showed dormant cambium and one individual of each of these species showed cambial activity. Although cambial activity is often synchronous at the population level, intraspecific variability has also been observed in other tropical species such as *Parkia velutina* or *Prioria balsamifera* [13, 21]. This variability may result from resource competition [64, 65], environmental heterogeneity [55, 66, 67], heterogeneity in radial growth [57, 68], or differences in stature (height, diameter and crown dimensions) [26, 65]. Yet, most of these factors have been minimised through careful selection of individuals of similar stature and growing in similar environmental conditions. Other factors beyond our control are differences in functional traits [15, 16] or phenological events (fungal or insect attacks) [69], which could also play a role in both intra and interspecific variability [21, 26, 70]. *S. zenkeri* and *P. oleosa* together contribute to more than 20% to BA and more than 15% to biomass productivity in Yangambi (Table 1). *S. zenkeri* is also one of the biomass hyperdominant species in the Congo Basin [71]. Consequently, variability of the cambial phenology of these species could significantly determine the timing of carbon uptake at the local and regional scales.

Asynchrony between cambial phenology, foliar phenology and climate seasonality

Based on our results, we also reject our second hypothesis that there is synchrony between rainfall seasonality, leaf phenology and cambial phenology.

In temperate and other highly seasonal forests, climatic fluctuations affect the extent and timing of vegetation growth, with water availability being a critical driver of cell division and enlarging phases [10, 26, 56, 70, 72]. At Yangambi, where seasonality is more moderate (Supplementary Fig. 1), observations reveal great variability in cambial responses. Half of the individuals and species studied show the expected cambial dormancy during the dry season, followed by resumption of growth over the rainy season [13, 21, 26, 70, 73], consistent with findings from the Mayombe forests [23] or for *Pericopsis elata* in Yangambi [70]. The other half shows cambial dormancy extending at least two months into the rainy season. This highlights an asynchrony between cambial growth and intra-annual climatic variations, reflecting growth strategies specific to species or individuals.

The intermittent cambial activity affects the growing season structurally and temporally [21, 24, 26], leading to the homogenisation of anatomy. The sporadic appearance of annual growth rings, as observed in *S. zenkeri* and *P. oleosa,* challenges an understanding of the dynamics of wood formation, the dating of growing seasons and the identifying drivers. However, despite the lack of anatomical landmarks, significant periods of dormancy are revealed, underscoring the complexity of dendrochronological studies. It highlights the need for refined and detailed phenological studies to understand species-specific growth dynamics better. Furthermore, the low variation in the width of the cambial zone or the immature xylem between individuals suggests a limited reactivity to ecological variations, and/or slowed down cambial development.

Finally, leaf phenology is characterised by low defoliation and limited senescence (at least 75% of old leaves) (Table 1), as commonly observed for semi-deciduous species in Yangambi [45, 70]. This is consistent with previous findings for low climatic seasonal forests [38, 45, 74] and contrasts with patterns observed in highly seasonal forests [23, 24, 75], where both cambial and foliar phenology are closely linked to climate. In Yangambi, the relationships between these phenologies appear complex and variable, with no obvious link to climatic variations, particularly the dry season [21, 26]. This lack of synchronisation suggests that relationships are individual or species-specific, resulting in spatial and temporal shifts from the tree scale to the forest scale. The potential maintenance of photosynthesis, coupled with the absence of widespread annual

dormancy, is consistent with the significant contribution of tropical ecosystems to global estimates [76]. It also highlights the continuing uncertainties regarding the interactions between growth, carbon dynamics, and climate variations in these environments.

Uncertainties and recommendations

Phenological studies are essential to link tree-growth monitoring with flux tower measurements on intra-annual time scales. However, the high species richness and low species abundance in tropical forests complicate these analyses. In addition, the diverse cambial patterns identified in our study coupled with uncertain relationships with leaf phenology and climate, impede the understanding of growth dynamics and ecosystem processes. This limits a comprehensive view of carbon dynamics and the contributions of the underlying metabolic mechanisms.

While a few common species play a key role in forest dynamics and ecology [71], their phenology remains poorly understood. In this context, focusing on representative species, as in our selection of six species accounting for ~ 30% of the productivity and basal area of Yangambi forest, is a pragmatic approach to explore phenological diversity. To better capture spatio-temporal inter- and intraspecific variability, we recommend extending cambial and leaf phenology studies to a full year, with broader species and individual sampling. Accurate assessment of the cambial state is crucial for interpreting tree-level carbon dynamics and linking them to large-scale processes, although challenges remain in distinguishing xylogenesis stages due to overlapping phases, immature cell differentiation and the absence of annual growth rings. Advanced tools, such as wood density analysis, growth-ring boundaries detection or high-resolution X-ray tomography [20, 77, 78] could refine carbon allocation to xylogenesis.

These combined efforts will provide a better understanding of the mechanisms underlying carbon dynamics in tropical forests and improve ecosystem models, taking into account the complexity and richness of these ecosystems.

Conclusion and perspectives

The results reveal a consistent asynchronous cambial phenology within and between tree species in Yangambi forests regarding the dry season, with low foliar phenology variations. This suggests species-specific climate-growth relationships and genetic interspecific variability at intra-annual scales. This could lead to species- and individual-specific carbon dynamics at the tree level which may indicate the absence of vegetative dormancy at the forest scale. These findings give rise to questions regarding the interaction between carbon uptake, growth, and biomass production and the potential impact on the synchrony of processes from the tree to forest scales.

The diversity of cambial patterns and weak phenological relationships complicate the interpretation of the carbon cycle and challenge efforts to scale up from individual trees to whole stands. While the causes of phenological variability remain largely unresolved, linking wood formation, biomass production, and GPP estimates over time is crucial to refine forest dynamics models. Addressing the uncertainties requires a multidisciplinary approach that investigates the processes underlying carbon fluxes. To this end, the extension of cambial studies to larger sampling at the annual scale, using binary and xylogenesis phase-width analysis approaches, will help to refine a potential growing season and the impact of cambial asynchrony on forest carbon dynamics. With its wood laboratory, network of inventory plots and flux tower, the Yangambi site offers an ideal setting to advance this research. The integration of these approaches will provide deeper insights and contribute to more robust analyses of the carbon budgets in the Congo Basin.

Supplementary Information

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Supplementary Material 1

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Clinical trial number

Not applicable.

Authors' contributions

PH, BL and DM designed and conducted micro-coring process. HB conceptualized the project. HB, TDM, and WH supervised the project. PH and KL coordinated laboratory analysis and processes. PH, HB, TDM and PK analysed the anatomical features visible on sections. NL, FL, JM, and BI provided technical and organisational support for data collection. PH, HB, WH wrote the main text. TDM, JVB and MB reviewed and edited the main text. All the authors read and approved the final manuscript.

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Data availability

The main data generated or analysed during this study are included in this published article and its supplementary information files. Additional datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Wood Biology Service, Royal Museum for Central Africa, Leuvensesteenweg 13, 3080 Tervuren, Belgium. [2]UGent-Woodlab-Laboratory of Wood technology, Department of Environment, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium. [3]Forest Is Life, TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, University of Liège, Passage Des Déportés 2, 5300 Gembloux, Belgium. [4]Forest Products Laboratory, USDA Forest Service, One Gifford Pinchot Drive, Madison, WI 53726, USA. [5]Ghent University, 9000 Ghent, Belgium. [6]Department of Environment, Q-ForestLab, Ghent University, 9000 Ghent, Belgium. [7]Centre de Recherche de Yangambi, Institut National Pour L'Etude Et La Recherche Agronomiques, Yangambi, Democratic Republic of the Congo. [8]Faculty of Renewable Natural Resources Management, University of Kisangani, Kisangani, Democratic Republic of Congo. [9]Laboratoire de Biologie du Bois, Section de La Foresterie, Institut National Pour L'Etude Et La Recherche Agronomique, Yangambi, Democratic Republic of Congo. [10]Université Pédagogique Nationale, République Démocratique du Congo, B.P, 8815 Kinshasa-Ngaliema, Democratic Republic of Congo.

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