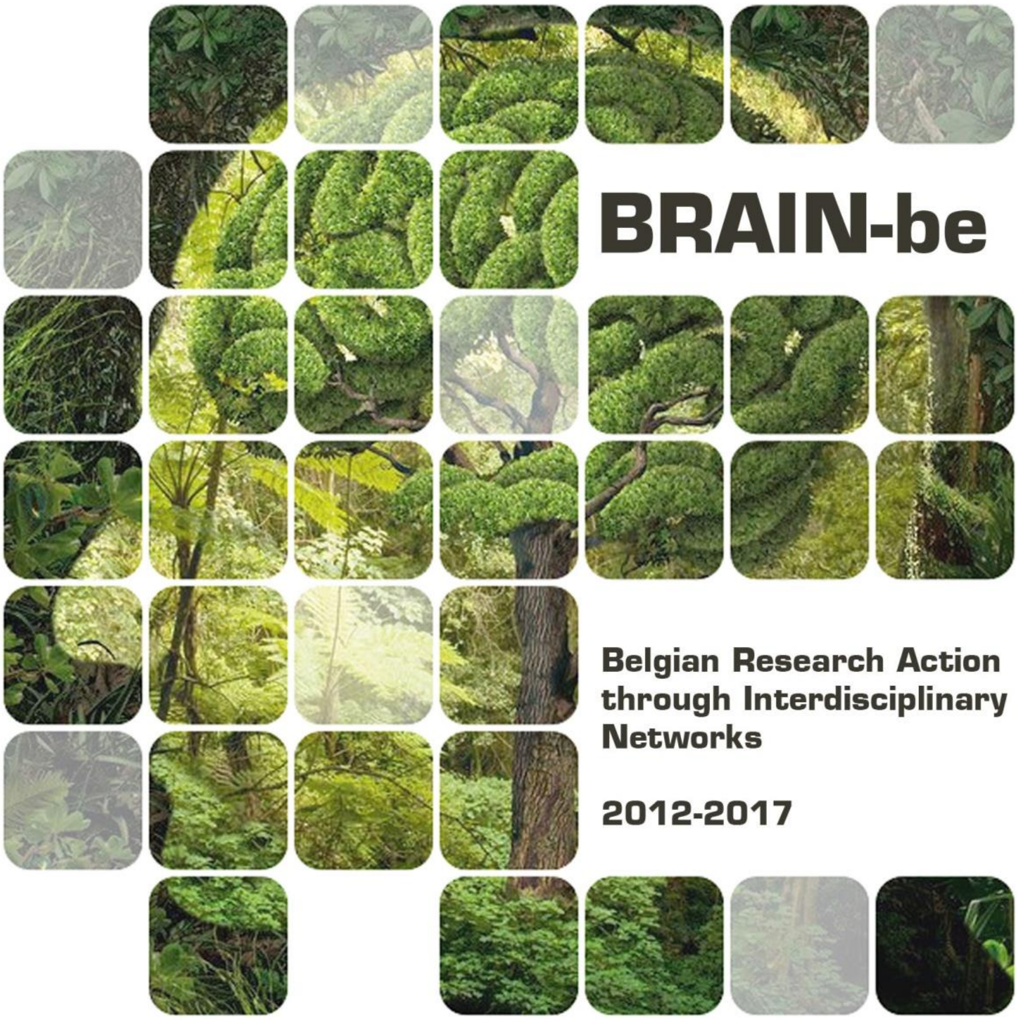

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Networks

2012-2017

HERBAXYLAREDD

Interdisciplinary exploitation of the federal Herbarium and Xylarium for tropical forest management

Victor Deklerck (Royal Museum for Central Africa, Ghent University) – Samuel Van den Abeele (Meise Botanic Garden, Université libre de Bruxelles) – Adeline Fayolle (Université de Liège, Gembloux Agro-Bio Tech) – Hans Beeckman (Royal Museum for Central Africa).

Axis 3: Cultural, historical and scientific heritage

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Interdisciplinary exploitation of the federal Herbarium and Xylarium for tropical forest management

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FINAL REPORT

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TABLE OF CONTENTS

ABSTRACT

Context

The Tervuren xylarium and the Meise herbarium are part of the Belgian scientific heritage. They include botanical collections from Central-Africa resulting of more than one century of fieldwork. They constitute by far the most important reference collections for Central-African plants. The actual scientific focus of the Tervuren xylarium is forest ecology and management, whereas for the Meise herbarium it is taxonomy and phylogeny. In de last decade, the demand for scientific support of policy actions in the domains of climate changes and illegal timber trade has been substantially rising. The collections have an important common role to play in this context.

Objectives

The Herbaxylaredd project aimed at strengthening the value of both collections for these actual societal needs. Fieldwork has been organized to calibrate the ecological information content of the collection material. Research has been done in the fields of wood biology, metabolomics, genomics, phenotype morphology and wood technology. This confirmed the importance of formally linking herbarium and xylarium specimens and of correct botanical identification of reference material.

Conclusions

The project showed the unique potential of the collections to understand material properties of timber and to underpin forensic research and combating illegal timber trade. Especially the opportunities of the collections to evaluate the resilience of tropical forests given the climate changes are matchless.

Keywords

Xylarium, Herbarium, tropical forest ecology,tropical forest management, wood technology, scientific heritage

1. INTRODUCTION

More information on tropical forests and specifically on tropical tree species is fundamental for all international efforts concerning climate change mitigation (Canadell and Raupach 2008; Saatchi *et al*., 2011) and biodiversity preservation (Cavanaugh *et al*., 2014; Day *et al*., 2014). Sustainable forest management, forest conservation and enforcement mechanisms need to be founded on reliable quantitative data (CITES 2013; De Ridder *et al*. 2013). Moreover, the international debates on gouvernance of the global climate change issues are based on models that need reliable data on plant traits controlling the carbon fluxes of the tropical rainforest belt. A focus on the tropical African vegetation, and specifically the area covered by moist forests, woodlands and savannas, is justified by the geographicalextent, covering the major part of the African continent, the relevance to the global climate, the biodiversity supported, and the ecosystem services provided and supporting the livelihood of many rural peoples, nearly a billion peoples in sub-Saharan Africa. The dense moist forests represent, through their huge stocks of organic carbon, a determining factor for how strong climate changes occur: disturbances result in significant sources of atmospheric carbon and enhanced greenhouse effects. The drier forests represent less carbon but are particularly sensitive for changes in temperature and precipitation. Illegal logging is an issue all over. Tropical forests comprise 55 % of the current carbon stock of the world’s forests (Pan *et al*., 2011) and nearly half of the world’s vegetation carbon. Intact tropical forests represent a carbon sink (Lewis *et al.*, 2009) (they sequester more carbon in their permanent plant structures than they emit through dying trees at a level higher than other vegetation types (tropical forests 1.9 Pg C yr-1, boreal forests 0.50 Pg C yr-1 and temperate forests 0.72 Pg C yr-1). Deforestation in the tropics signifies a carbon source of 2.9 Pg C yr-1 which is far from being compensated by spontaneous forest regrowth and afforestation (1.6 Pg C yr). Through the carbon sink of intact forests and the regrowth, tropical forests absorb 20-30 % of the world carbon emissions. The issues of tropical forest stocks, sinks and sources are therefore a central topic in international policy debates on climate changes. Scientific data underpinning the climate policy discussions like the International Panel on Climate Change (IPCC) are provided by models, like the Dynamic Global Vegetation Models. These models take into account both sensitivity and resilience of tropical forests. Resilience capacity of tropical forests remains largely unquantified. Such quantification typically relies on detailed information on species characteristics, including their intraspecific variability, that determine their performance through their influence on the so-called vital rates (regeneration, growth, mortality). An important drawback for models of climate changes is the uncertainty which is still large for the tropical carbon fluxes compared to other biomes. More detailed information on vital rates and traits of the many species is needed for accurate predictions. However, fieldwork in the tropics is difficult due to, e.g., high diversity and complex forest structures (Whitmore, 1990) and unstable political situations (Blaser *et al*., 2001). Existing botanical reference collections offer an excellent alternative for this fieldwork, especially for African moist forests, where data are most scarce (Ciais *et al*. 2011), specifically for the Congo Basin (Verbeeck *et al*., 2011). Collections have a high species diversity with a large geographical coverage and contain a vast amount of specimens, most of them being georeferenced.

Worldwide, the federal Xylarium (RMCA) and Herbarium (Meise Botanic Garden) are the most important reference collections for Central African forests (Beeckman, 2007).

In order to renew, complement and strengthen the reference value of both the Herbarium and Xylarium, HERBAXYLAREDD aimed at generating knowledge, through multidisciplinary and integrative analysis of specimens' traits and meta-data, on Central African forest ecology and management as well as on forest products, especially wood. The study of plant traits, wood anatomy and technology, molecular and genetic affinities of plant and wood samples allowed exploring species distribution and functional strategies, growth performance of trees, and technology of lesser used timber species, with implications for carbon stocks estimations of Central African forests, energy content of woody species and to combat the illegal timber trade.

2. STATE OF THE ART AND OBJECTIVES

Within HERBAXYLAREDD, two research lines are emphasized:

(1) The forest ecology line

Measuring **phenotypical traits** represents an added value for the understanding of the **functional strategies** of African tree species, as well as the relations between functional traits and growth, regeneration and mortality. Leaf and seed traits can be measured on herbarium specimens, such as those stored at the herbarium of Meise Botanic Garden (BR). Wood trait measurements on the other hand, can be obtained from wood samples stored in xylaria such as the one at the Royal Museum for Central Africa in Tervuren (Tw collection). To enable the combination of these different trait measurements from one individual tree, the herbarium (BR) and xylarium (Tw) collections have been linked and updated facilitating the use of metadata available from both databases.

Relations between functional trade-offs and growth variations in time and space were studied for specific tropical African tree species. Trait measurements from botanical collections have been calibrated through additional field work. Traits have been measured in the field: functional traits at the seedling stage (Mirabel *et al*., 2019) and architectural traits on larger trees (Loubota Panzou *et al*., 2018a). This has been done in several sites to optimally link trait data with forest structure and carbon stocks (Loubota Panzou *et al*., 2018b). The integrated framework, combining wood traits, leaf traits, and also ecophysiological measurements on branches of the same species, was applied to the *Erythrophleum* genus (Gorel *et al*., 2019a), for which georeferenced collections were first used to delineate species distribution and examine niche evolution (Gorel *et al*., 2019b). Georeferenced herbarium samples were also used to derive phenological data, and examine large-scale variation in flowering (Ouedraogo *et al*. in press). Herbarium derived phenology was first validated using field observations available for timber species (Ouédraogo *et al*., 2018), monitored in several sites across central Africa, mostly in logging concessions, but also from historical collections such as in Luki, DRC (Illondea *et al*. 2019 BFT). Extensive collections, archived scientific data and well directed field work allowed an informative analysis of 150 rainforest species, including their increment assessed by different means (Illondea *et al*. 2019 and in preparation).

Additionally, a large-scale dated phylogenetic tree of angiosperms was constructed. Such a framework can be used to assess the evolutionary relationships among species, but it also provides a useful tool to optimize the evolution of specific morphological, anatomical or physiological traits (e.g., vessel size of wood), resolve eco-evolutionary queries, or predict putative trait characteristics based on the position of a species within a certain lineage or group (most recent common ancestor approach).

(2) The forest products research line

Knowledge on the role of **lesser used tree species** is critical because without **reliable information on the wood characteristics**, logging companies will not take the risk of logging these species instead of the timber species under high pressure. However, highly valued timber species are under a consistent logging pressure, leading to exhaustion or even endangering the survival of the species (Deklerck, 2019). There might be interesting timber species that could be valid alternatives and that are currently being overlooked. Xylaria offer the unique opportunity to screen a large number of species for their wood technological characteristics (Deklerck, 2019). As such, we can search for ‘new’ and interesting timber species for local and international markets.

Finding new timber species is only one part of the solution to prevent exhaustion of certain species in the tropics. Halting the illegal timber trade is just as important. Xylaria have been used for timber identification questions for a long time, mainly in the form of reference material and wood microscopic slices (Deklerck, 2019). With new techniques being developed (for example DART TOFMS, Cody and Laramée (2005)) there are many opportunities for xylaria to provide reference material (Deklerck, 2019). Via DART TOFMS chemical fingerprints have been created, which are then the basis for the species identification (Deklerck *et al*., 2017; Deklerck *et al*., 2019a; Evans *et al*., 2017; Lancaster and Espinoza, 2012; McClure *et al*., 2015; Musah *et al*., 2015). Xylarium specimens are perfect to strengthen the reference database for this technique, since only a small wood sliver is needed to obtain the chemical fingerprint.
In addition to xylaria, herbaria contain valuable reference collections that can be used to verify the timber supply chain. By extracting and sequencing DNA from herbarium vouchers, a reliable DNA reference database has been created from curated and georeferenced specimens. Such a reference database can then be used for species identification, as well as to verify the origin of traded timber.

Because wood is used for 60 to 90% of the energy requirements in Central Africa, we paid attention to energetic properties of different species that will be used for energy production and that are occurring in regrowing forest around Yangambi. Knowledge on energetic properties of Central African species is currently lacking. However, wood is the main material used for energetic requirements in Central Africa. Within the HERBAXYLAREDD project, the calorific value and ash content has been determined for several species from the Congo Basin.

The HERBAXYLAREDD project was strongly involved in discussion about international policy towards forest conservaton and timber trade. Partners within the HERBAXYLAREDD project engaged in active discussions and meetings with the CITES department of the Department of Environment (FOD, Belgium). We hosted the annual meeting of the Congo Basin Forest Partnership in the Royal Museum for Central Africa. Victor Deklerck (Ghent University and RMCA) became active as a scientific advisor for the Global Timber Tracking Network and attended the Forest Legality Meeting in Washington D.C.(2017) and the 24th meeting of the CITES Plants Committee at the United Nations (2018, Geneva, Switzerland).

Democratic Republic of the Congo – The Luki Biosphere Reserve (© Victor DEKLERCK)

3. METHODOLOGY

3.0 Research material

Table I shows the subject and used material within HERBAXYLAREDD. Every one of these subjects will be handled separately throughout the report.

Table I: The different subjects researched within HERBAXYLAREDD and the number of species, xylarium specimens, archived material or material from fieldwork used in the study.

Subject	Number of species	Number of Xylarium specimens	Number of Herbarium specimens	Archived material	Material from fieldwork
Link between BR and Tw collections	c. 1890	6,933	10,199		
Validation of botanical identify	756	/	756		
Nkula park – trait atlas	158	/	/	/	Monitoring of 3642 individuals
Measurement of tree dimensions (height and crowns) to derive whole tree traits/architectural traits	30 in Luki DRC 45 in Loundoungou, Congo, 45 in Norther Congo	/	/	/	Forest inventory (plot data) and tree measurements, 30 trees per species
Intra-and inter-annual reproductive phenology	87			Phenological monitoring data from Luki Biosphere Reserve	
Cambium and leaf phenology of *Prioria balsamifera*	194	/	/	Leaf shedding data (194 trees) from Luki Biosphere Reserve	+ 13 individuals, time lapse cameras in Luki Biosphere Reserve
Aboveground biomass variation , effect of structural, composition and architectural attributes	/	2202	/	/	36 1 hectare plots
Temporal and spatial patterns in flowering phenology	8		322		7 sites across Central Africa for flowering phenology
Tree growth and mortality (carbon persistence)	>1500	/	/	/	565 forest plots (Amazonia and Africa)
Link between wood traits and wood anatomy	2 sister species (*Erythrophleum suaveolens* and *ivorense)*	/	/	/	/
Intraspecific variance within species + leaf chemical and isotope composition	7	/	/	/	21
Functional traits and species distribution (trait atlas)	158 (3642 individuals)	/	/	/	Phenology monitoring Nkula Park
Functional strategy of African tree species	15	/	/	/	10 seedlings per species

Tree growth analysis with no distinct growth rings for carbon budgets	/	/	/	1117 archived annual diameter measurements	7 permanent sampe plots Luki
Phenological traits and wood anatomy	13	/	/	/	358
Treering distinctness	103	342	/	/	/
DART-TOFMS (metabolites)	928	1821		/	/
Natural durability	32	246	/	/	/
Dimensional stability	53	421			/
Bioenergy and calorific value	153	/	/	/	496 samples (individuals)
Energy in aboveground biomass	/	/	/	/	43 plots
Wood density	>1000	9197	/	/	/
DNA barcoding and megaphylogeny	468	/	468	/	/
Khaya and *Entandrophragma* phylogeny using high-throughput sequencing	18	/	10	/	26
Population genetics and phylogeography	3	/	271	/	610 (silica-dried leaves and cambium slashes)
Plant performance and ecological strategy	1	/	/	/	5 individuals of *Pericopsis elata*
Relationship vessel size and growth of African tree species	5	/	/	/	10
Tree growth and mortality (carbon persistence)	>1500	/	/	/	565 forest plots (Amazonia and Africa)
Link between wood traits and wood anatomy	2	/	/	/	/
Dendrometrical data to assess the quantity of energy stored in the above-ground biomass	2	/	/	/	43 plots
Visual key	70	Images from anatomical slices (number not specified)			
Species distribution	>1000			Data gathered from literature: 455 forest sites (before HERBAXYLAREDD) + 298 savanna) sites (during HERBAXYLAREDD) providing information for > 1000 tropical tree species across the African continent, jointly analysed during the project.	

3.1 Linking the Tervuren xylarium and the Meise herbarium

The databases of the Xylarium (Tw) and Herbarium (BR) have their proper, unique structure but the xylarium catalogue gives information on which samples are backed by herbarium material in Meise Botanic Garden. The first task in the project was to **extract** this subcollection of Central African **wood samples that are backed by herbarium vouchers present at the Meise Herbarium**, to strengthen the reference value of the xylarium samples. The matching of such linked samples was done by **using the available metadata** from the Tw and the BR databases.

Two **protocols** were used:

1) Matching based on Tw-number (which is available for some BR herbarium vouchers)
2) Matching based on a unique key, using three variables: collector, country of origin and herbarium number.

Putative matches were always **verified with the given scientific species and/or genus names**. If needed, herbarium and wood records were corrected and/or completed. A protocol for data exchange was drawn up to allow the successful linking of future additions to both databases. In addition, the wood and herbarium samples that could be linked were ranked based on the following variables: country of origin, collection period, plant family and collector.

Furthermore, digitization efforts at Meise Botanic Garden allowed for a substantial expansion of the RAINBIO database (Dauby *et al.*, 2016). This served the subproject on the trait atlas (see 3.6.2 and 4.6.2 further in the report).

3.1.1 Validation of the botanical identity

Despite numerous publications on the importance of correct identifications and preparing proper vouchers (e.g., Carter *et al.*, 2007: Pleijel *et al.*, 2008), current practice remains problematic, especially in tropical regions. This is mainly caused by 1) the continuously decreasing number of botanists that are adequately trained in the identification of tropical plants, 2) a remaining lack of up-to-date flora treatments for many plant lineages, and 3) the current practice in research in general: when collecting material during field expeditions for scientific purposes, the identification of the specimens in the field often remains the final identification of the specimens used for scientific research afterwards (Eisenman *et al.*, 2012). Fayolle *et al*. (2013) also proved that differences in wood density between laboratory measurements and the DRYAD-database were often linked to misidentifications. Stoffelen et al. (pers. comm.) compared all field identifications in the DRC from the last 4 years with taxonomic revisions, flora treatments and, additionally, with herbarium specimens identified by specialists of the taxonomic group at hand, deposited in the herbarium of Meise Botanic Garden. They found more than 50% of the field identifications were wrong or lacking.

Within the frame of HERBAXYLAREDD, the same type of **comparison** was made **by experienced botanists** for all herbarium vouchers collected in the permanent plots of the

Yangambi Biosphere Reserve (Janssens *et al*., in preparation). In addition, species identification was further verified using **DNA barcoding**. Subsequently, the original and the corrected data were used in phylogenetic trait analyses and to estimate biodiversity indices, to assess the impact of species misidentifications. Diversity indices were calculated using the *vegan* package in R (R Development Core team, 2011), clustering analyses were performed using the *hclust* fuction, the phylogenetic signal was calculated using the *phytools* package, and evaluation of the evolutionary model selection was done using the *Geiger* and *OUwie* packages.

In addition, the link between the Xylarium and Herbarium established during HERBAXYLAREDD (Vanden Abeele *et al*., in preparation), including the digitizatione efforts at Meise Botanic Garden, allowed identifying the botanical identity for numerous wood samples.

3.2 Measurement of plant and wood traits

3.2.1 Leaf, seed and whole tree traits

(1) Bhély Angoboy Illondea gathered leaf, seed and flowering data out of the COFORTRAITS and TRY databases for the species described in the trait atlas (see further).

(2) Within the master thesis of Arthur Chantrain (Chantrain, 2018), eight one-hectare plots were surveyed for informing on forest structure and composition. These permanent plots were installed in 2009 and were inventoried in 2009 – 2016 and 2018. Every woody stem bigger than 10 cm in DBH was identified, tagged, mapped and its DBH was measured. For three plots, the tree height was also measured. On 30 selected species within these plots, additional tree measurements and crown obsevations were performed, following the protocol developped by Grace Jopaul Loubota Panzou in northern Congo (Loubota Panzou *et al*, 2018a, 2018b). For each individual, the stem DBH, tree height, height of the lowest foliage and four crown radii according to the four cardinal directions were measured. Crown depth was calculated and the crown's position in the canopy was estimated using the Dawkins index (CEI) on a five-point scale. Six structural forest attributes were computed at plot level (inventory data) for the 2018 inventory of the eight one-ha plots: stem density (N, ha^{-1}), density of trees bigger than 70 cm in diameter (N70, ha^{-1}), basal area (BA, m² ha^{-1}), the basal area weighed wood density (WMDBA, g cm^{-3}), Lorey's height (HLorey, m) and the quadratic mean diameter (QSD, cm). In addition to these structural attributes, AGB stocks were computed in 2009 and 2018 (see Chandrain, 2018). Species-specific modelling was used to derive a set of architectural traits including height, crown radius and crown depth at a 10 cm reference diameter, and at maximal size using the 98th percentile for computing maximum diameter. Three allometric models were built, the first compares the height of the tree to its diameter (scaling coefficient = bH). The two other models compare crown radius and crown depth to the height of the tree. These allometric models were used to quantify species specific architectural traits at the juvenilage and adult stage. Finally, these architectural traits were related to other key

functional traits of tropical tree species, the light requirement (regeneration guild, Hawthorne, 1995), CEljub (Poorter, 2006 and Sheil, 2006) and wood density (Chave, 2009) to study if multiple correlations between traits allowed to distinguish different ecological strategies among the 30 tropical tree species.

(3) To gain insight in how trait variance is structured within crown, individual, and species, Kafuti *et al*. (2019) and Bauters *et al*. (in prepation) performed a detailed stufy of the intra-specific trait variance for a few species. To assess stomatal traits, Kafuti et al. (2019) used samples of five species: *Pericopsis elata* (see chapter on wood biological traits), *Prioria balsamifera* (Vermoesen) Breteler (4 individuals), *Prioria oxyphylla* (Harms) Breteler (2 individuals), *Polyalthia suaveolens* Engl.&Diels (3 individuals)*, Trichilia gilgiana* Harms (3 individuals). For each individual 3 specimens were collected, one at the base of the crown, one in the middle and one at the top. Samples were collected in Luki, DRC in 2016 and in Biaro (*Pericopsis)*. For the leaf chemical and isotope composition Bauters *et al*. (in preparation) looked at intra-species variability for two species: *Gilbertiodendron dewevrei* (De Wild.) J.Leonard (4 trees) and *Mammea africana* Sabine (2 trees) sampled in 2013 in Yangambi. For each individual 3 samples were collected at three different levels in the canopy – i.e. the low, middle and upper level - and this was done on six different time points (considered replicates), spread over the year.

(4) From the work by Tarelkin *et al*. (2019): wood density profiles reveal significant differences in wood formation along a precipitation gradient in the Congo Basin. The leaf phenology and the pith-to-bark wood anatomical variability of 13 common tree species of the Democratic Republic of the Congo are compared. Wavelet analysis is applied on the pith-to-bark density profiles to obtain three descripto. These are used as proxies to describe and compare wood anatomical variability and they describe growth periodicity, regularity and the amplitude of variations of the anatomical patterns.

(5) Illondea *et al*. (2019) assessed the intra- and inter-annual reproductive phenology of trees in the Mayombe forests, using historical data on the Luki Reserve in the Democratic Republic of the Congo. The reproductive diameter was documented for the most abundant species in the dataset. Phenological monitoring of 3642 trees belonging to 158 species and 39 families was conducted in the Luki Reserve every 10 days from 1948 to 1957. Circular statistics were used to test the synchronicity of phenological events among trees, both at the community level, i.e. for the forest as a whole, and individually for 87 species, which included 35 well-represented species (n ≥ 20 trees), 16 commercial species and 36 other species. Logistic regressions were used to determine the diameter (minimum and regular) of these species on fruiting.

Furthermore, Illondea *et al*. (in preparation) investigate the effect of asynchronous growth on the resilience of tropical trees against climate change. Leaf shedding data was collected every 10 days from 1948 to 1957 on 3642 trees (same dataset as above), of which 194 belonged to *Prioria balsamifera*, the focus species in this study. Furthermore, timelapse cameras were installed to monitor the leaves from 14 December 2015 to 16 September 2016. Cores and wood samples from the cambial zone were collected in 2014 from the same 13 trees monitored for phenology through cambial woundings at regular intervals. Growth-ring series of 66 years since a dated nail mark in these 13 trees were measured. In addition, cambial pinning was performed on 10 trees at 20 months.

(6) The measurement of leaf traits was performed on seedlings grown in a nursery (Mirabel *et al*. 2019) and on herbarium samples and living collections (by Brechts and Dakis). Unfortunately the SLA measured on herbarium and living samples for the same species were hardly comparable, and this is the reason why SLA was not rountinely measured on herbarium, and efforts were rather concentrated on herbarium-derived phenology (Ouédraogo *et al*. in press).

In addition, in order to study the correlations between phenological, leaf and wood traits, we completed the measurements on collection material with trait data from relevant databases, essentially TRY and Cofortraits (see trait atlas further).

3.2.2 Genetic analysis

State-of-the-art genetic analyses were used to help the identification of reference collections (Janssens *et al*. 2006, 2007), to construct an evolutionary framework and to differentiate between populations of Central African trees.

3.2.2.1 DNA barcoding

DNA barcoding is a powerful molecular and bioinformatics tool that is especially interesting when traditional taxonomic identification is not feasible (e.g., when reproductive organs are not available) (Lahaye *et al*. 2008; Parmentier *et al*. 2013). It allows differentiating between species and discovering new species, based on the differences in short sections of the DNA from specific genes (also called “sequences”). These short standardized DNA sequences or “barcodes” can be matched to a reference database of DNA barcodes to identify an organism to species (similar concept as barcode scanning at a supermarket) (Hebert *et al.*, 2003). In plants, the search for a standardized barcode has been challenging, but based on assessments of recoverability, sequence quality and species discrimination success, two chloroplast DNA regions – the *rbc*L gene and *mat*K gene – were selected as the plant barcode (Hollingsworth *et al*., 2009). However, since chloroplast DNA barcodes (i.e. short sections of the DNA found in the chloroplast of an organismal cell) often have a lower mutation rate than nuclear DNA barcodes (i.e. short sections of the DNA found in the nucleus of an organismal cell), the chloroplast *rbc*L and *mat*K barcodes are often complemented with the internal transcribed spacers (ITS) of the nuclear ribosomal DNA (Hollingsworth *et al*., 2011). That way, even if the two chloroplast barcodes are the same for distinct species, the nuclear ITS region (more rapidly evolving) might still allow successful differentiation of the species.

Since the dawn of molecular genetic analyses (such as DNA barcoding), voucher specimens of African tree specimens in the herbarium of Meise Botanic Garden have often been accompanied by silica gel dried leaf samples for additional DNA analysis. This allows us to create a **reference collection of African tree species** (as curated herbarium vouchers)

linked with the main DNA barcode genes *matK* and *rbcL*. Such a reference database can serve as a comprehensive tool for rapid but thorough determination of specimens that are otherwise difficult to identify. Currently, a large amount of *rbcL* and *matK* sequence data is available online at GenBank and Barcode of Life Data (BOLD) Systems. However, such publicly available databases can contain large amounts of erroneous data, and sequence data is missing for many African tree species. Therefore, we generated new *rbcL* and *matK* sequences from curated herbarium vouchers stored at Meise Botanic Garden. Additionally, ***trnH-psbA* barcode sequences** were generated for the tropical African timber species *Staudtia kamerunensis*. Within this species, two varieties have been described for which morphological identification proves to be difficult. Hence, the newly generated DNA sequences could be **used to improve identifications at the intraspecific level within *Staudtia kamerunensis***.

3.2.2.2 Phylogenetic framework

Barcode sequence analyses of African tree collections not only help with the identification of specimens, the results can also be applied to construct an evolutionary framework in which each specimen is positioned within a known specific lineage. **Phylogenetic trees** aren't merely used anymore **to assess evolutionary relationships amongst species**, they also provide an interesting **instrument to optimize the evolution of specific morphological, anatomical or physiological traits** (e.g., vessel size of wood), **resolve eco-evolutionary queries, or predict putative trait characteristics based on the position of a species within a certain lineage or group** (most recent common ancestor approach). Therefore, we applied a total evidence methodology in which the newly generated barcode sequences were added to a large phylogeny of woody African rain forest taxa embedded in an evolutionary framework of angiosperms. This large phylogeny, constructed by Janssens *et al*. (2020) is based on *matK* and *rbcL* gene markers, and is therefore complementary with the barcoding sequences generated for WP2 (Task 2.2a).

3.2.2.3 Chloroplast genome sequencing

Although *matK* and *rbcL* barcoding genes provide interesting insights in the evolution of plants at the species level or higher, both gene markers evolve too slowly to provide resolution below the species level or in recently diverged species. At this taxonomic level, **well resolved phylogenies** can be obtained by conducting **whole chloroplast genome sequencing** using high-throughput sequencing (since more DNA characters can be compared between individuals). This approach has already proven its success in distinguishing varieties of wild rice (Waters *et al*. 2011) by generating a large amount of haploid chloroplast sequence data (usually many chloroplasts are present in a single plant cell, hence many chloroplast DNA copies are vailable) without the problem of possible recombination. Nowadays, high-throughput sequencing can cost-effectively sample a large number of informative DNA characters and hence dramatically increase the overall resolution to allow the delimitation of closely related lineages (Zhang *et al*. 2011). In addition, whole chloroplast genome sequencing without prior isolation or amplification (i.e. shallow shotgun sequencing of all available DNA from and individual followed by bioinformatic genome skimming to recover the chloroplast DNA) is relatively easy for plants nowadays (Nock *et al*., 2011).

Within Meliaceae, the genera *Entandrophragma* and *Khaya* (commonly known as African mahogany) comprise 11 and 9 species respectively, many of which are commercially important and renowned for their durability, beauty and coloration (Monthe *et al*., 2019). By analysing the genetic structure of these species through shotgun sequencing and genome skimming, the phylogenetic relationships within both genera have been investigated and morphology-based species delimitations have been verified with molecular phylogenetic evidence. Additionally, the unique 'chloroplast genome' fingerprint obtained for specimens of the same species but with a different geographic origin help to reveal the provenance of logs of *Entandrophragma,* thereby providing a tool against illegal logging (linked with WP5).

For all 11 species of *Entandrophragma* (28 specimens in total, representing different geographic regions) and eight of the nine species recognized in *Khaya* (Bouka, 2017), total genomic DNA was extracted from silica-dried leaves or from herbarium specimens (Meise Botanic Garden BR; National Herbarium of the Netherlands Wageningen, WAG; African Botanical Library of Université Libre de Bruxelles, BRLU and the Missouri Botanical Garden, MBG). Genomic libraries were prepared (see Monthe *et al*. 2019 for a detailed description) and sequencing was done on an Illumina NextSeq platform at GIGA (Liège, Belgium). Subsequently, the reference plastomes for each genus were extracted and constructed from the genomic sequence dataset. This approach is also known as **genome skimming** and is relatively time and cost efficient (compared to enriched sequencing approaches for example). Genome skimming also allows the extraction and reconstruction of the nuclear ribosomal genome. Comparison of both genomes is extremely valuable in species delimitation studies or to obtain reliable phylogenetic reconstructions.

3.2.2.4 Phylogeography and timber tracking

In order to gain more insight in the genetic diversity at lower taxonomic levels (e.g. populations, varieties), microsatellite markers are a valuable tool as well. Microsatellites, also referred to as simple sequence repeats (SSRs) or short tandem repeats (STRs), are short repetitive regions (1 to 6 bp) in the genome that mainly occur in non-coding DNA (Ellegren, 2004). Because of their high levels of polymorphism, co-dominance and reproducibility, **microsatellites** have proven their **utility in** many research areas such as **plant breeding, forensics, species conservation, population genetics, phylogeography and species delimitation** (Blanc-Jolivet and Degen, 2012; Degen *et al*., 2006, 2013; Hardy *et al*., 2013; Lissambou *et al*., 2019; Ma *et al*., 2019). Given this broad range of applications, we aimed to develop microsatellite markers for the tropical trees *Staudtia kamerunensis*, *Prioria balsamifera* and *Prioria oxyphylla* using high-throughput sequencing. These three timber species are commercially important but genetic information is lacking. However, such information is essential to prevent genetic erosion, to make proper assessments for conservation management and to verify the geographical origin of traded logs. Therefore, we extracted total genomic DNA from an herbarium voucher of each respective species. The prepared genomic libraries were sequenced on an Illumina high-throughput sequencing platform. Subsequently, the sequence data was analysed using the QDD bioinformatics pipeline and primers were developed for the chosen microsatellite regions (Vanden Abeele *et al*. 2018, 2019). The primers were tested and validated in *S. kamerunensis*, *P. balsamifera* and *P. oxyphylla* populations from the Luki Biosphere Reserve and the Yangambi Biosphere Reserve, two localities that were extensively samples as part of the HERBAXYLAREDD project. Additionally, amplification was tested in *Pycnantus angolensis* and other African tree species within the genus *Prioria*. Successful amplification would be beneficial since many of these species are commercially important and knowledge on their genetic composition is needed.

Our **newly developed microsatellite markers** were then used to (**1**) estimate the level of genetic diversity within populations of *Staudtia kamerunensis* and *Prioria balsamifera* with the software SPAGeDi (Hardy and Vekemans, 2002), (**2**) to identify distinct intraspecific clusters using the *structure* software (Pritchard *et al*. 2000), and (**3**) to assess the applicability of genetic assignment methods (Frequency, Bayesian and Nearest neighbour method) to determine the country of origin.

3.2.3 Wood biological traits

(1) Plant functional traits have shown to be relevant predictors of forest functional responses to climate change. However, the trait-based approach to study plant performances and ecological strategies has mostly been focused on trait comparisons at the interspecific and intraspecific levels. In the study by Kafuti *et al.* (2019), traits variation and association at the individual level was analyzed. Wood and leaf traits were measured at different height location within the crown of five individuals of *Pericopsis elata* (Harms) Meeuwen from the northern tropical forest of the Democratic Republic of the Congo. All the studied trees were emergent. Their crowns were above the canopy level and had a full access to sunlight. To test whether there was a height gradient of variation in wood and leaf traits, five leaf samples were collected at different locations within the crown. The sample location was selected on a regular basis within the crown at 0% (base of crown), 25%, 50% (middle of the crown), 75%, and 100% (top of the crown) crown height. From each leaf sample, five leaflets without obvious symptoms of pathogen or herbivore attacks as well as a wood segment from the twig directly supporting the leaf sample were also collected. In addition, a stem sector of about 3 cm^3 located on the trunk at 1.3 m height from the soil level was collected to estimate the xylem tapering ratio of *Pericopsis elata*. Wood microscopic analyses were performed on the complete transversal sections of twigs. Four wood traits were measured: Mean vessel diameter, and lumen area, vessel density, and fiber wall thickness. While vessel diameter, vessel area, and fiber wall thickness were measured on 30 vessels and fibers. Furthermore, the vessel lumen fraction, total number of vessels, theoretical specific hydraulic conductivity and hydraulic weighted vessel diameter were calculated. For the leaflets, the specific specific leaf area was obtained. The following was also obtained: stomatal length, width and density, pore length, stomatal zie and maximum stomatal conductance to water vapor.

(2) Pith-to-bark vessel profiles were collected from *Etandrophragma angolese* (5 specimens), *candollei* (5), *cylindricum* (5) and *utile* (5), *Polyalthia suaveolens* (3) and *Trilepisium madagascariensis* (3). For each vessel, the size and the distance from the pith was determined. For the 5 specimens of *Entandrophragma angolense*, 2 specimens

of *Polyalthia suaveolens* and 3 specimens of *Trilepisium madagascariense*, the growth rings were indicated and growth ring width, growth ring surface, total vessel area per ring, total vessel area percentage, vessel density and average vessel size were determined. These data were then used to determine difference in trends between species with a different regeneration guild (see Hawthorne, 1995).

(3) The phenomenon of distinct, absent or indistinct growth rings is a highly variable feature used for wood identification and a wide range of tree-ring studies. However, causes for its variability are not yet fully understood. Tarelkin *et al*. (2016) selected 103 Central African rainforest trees to analyse the anatomy of growth-ring boundaries and assessed the influence of climate, tree organ and leaf shedding behavior on growth-ring distinctness and anatomy. Species and specimens with high-quality microtome sections were collected from the Tervuren Wood Collection. For the full materials and methods, see Tarelkin *et al.* (2016).

(4) A literature review on plant traits allowed the preparation of an opinion paper on wood anatomy and trait-based ecology in (Beeckman, 2016). Within the opinion paper, some trait concepts are analysed aiming at optimally involving wood research in trait-based ecology. The value of the IAWA standard lists of wood anatomical features as starting points for trait databases is recognized. A summary of the functionality of wood is given to inform the trait-research community of basic aspects of tree performance. The time dimension is highlighted, as well as the foundations for understanding bio-hydraulics, bio-mechanics and metabolism of wood and relevant traits. Guidelines are given for sampling strategies and database concepts. Prospects of time axis construction and system integration are discussed, as well as the importance of standardizing for size.

Wood anatomical desciptions based on IAWA features have been used for the correlation analysis of the Nkula park species (see chapter on co-variation of traits). The species selection was limited to species from the Nkula park (see work by Bhély Angoboy Illondea) and over 5000 scans (3 anatomical planes) were taken.

3.2.4 Wood technological traits

Most of the time, only a very limited number of commercial species are tested on physical strength and other mechanical and technological properties. In addition, a very limited number of tropical species are highly valued as timber species (Deklerck, 2019). These species are used in several applications, ranging from musical instruments to furniture. However, they are becoming less and less abundant and we might evolve to a situation where trade in these species becomes heavily regulated or prohibited without knowledge on alternative species with similar desirable properties. There are several measurements for the physico-mechanical characterization within HERBAXYLAREDD:

(1) The wood density is determined at different scales:
   a. The average density (ovendry weight / ovendry volume) through immersion of xylarium specimens in water (see Maniatis *et al*., 2011),. Afterwards, the ovendry specific gravity is determined (ovendry weight/ovendry volume (-)*density of water) (Deklerck *et al*., 2019b)
   b. Pith-to-bark density profiles as the result of a state-of-the-art X-ray scanner (De Mil *et al*., 2019; Van den Bulcke *et al*., 2013; De Ridder *et al*., 2011)
(2) The dimensional stability or resistance against swelling and shrinkage *(Deklerck, et al*., 2019b).
(3) The natural durability or resistance against fungal decay. (Deklerck *et al*., under review)
(4) The calorific value and ash content resp. by use of a calorimeter and furnace.
(5) Modulus of elasticity (MOE)

The challenge to determine the dimensional stability and natural durability on is the non-standardised and limited format of the xylarium specimens. We will have to find new methodologies, or adapt old ones, to get the same results as with standardised or normalised tests. Once these methodologies are developed, a full screening of the xylarium is possible.

The species selection for the wood technological traits was dependent on several criteria (from Deklerck, 2019). The main criteria for the species choice were:

(1) The species has to be present in the xylarium
(2) The aim is to have at least 10 heartwood specimens of that species
(3) The species has to be present in the Congo Basin
(4)

A small subset of species was chosen to develop the different test methodologies (Table II). The focus was mainly on commercial species, as these have known wood technological properties. This way we can assess whether the newly developed methodologies produce realistic results. Other species were added based on unexpected behaviour (see paradixocal characteristics) and might prove to be interesting timber species.

Table II: The subset of species to develop the different test methodologies.

Reason	Species
Commercial importance	*Albizia adianthifolia* (Schum.) W.Wight,, *Albizia ferruginea* (Guill. & Perr.) Benth., *Amphimas pterocarpoides* Harms, *Antiaris toxicaria* Lesch., *Baillonella toxisperma* Pierre., *Canarium schweinfurthii* Engl., *Cynometra alexandri* C.H. Wright, *Entandrophragma angolense* (Welw.) C.DC., *Entandrophragma candollei* De Wild. & T.Durand, *Entandrophragma cylindricum* (Sprague) Sprague *Entandrophragma utile* (Dawe & Sprague), *Erythrophleum suaveolens* (Guill. & Perr.) Brenan, *Gilbertiodendron dewerei* (De Wild.) J.Leonard, *Hallea stipulosa* (DC.) J.-F.Leroy, *Irvinga grandifolia* (Engl) Engl., *Khaya anthotheca* (Welw.) C.DC., *Klainedoxa gabonensi* Pierre, *Lophira alata* Banks ex C.F. Gaertn., *Lovoa trichilioides* Harms, *Milicia excelsa* (Welw.) C.C.Berg,, *Millettia laurentii* De Wild., *Millettia stuhlmannii* Taub., *Nauclea diderrichii* (De Wild.) Merr., *Ongokea gore* (Hua) Pierre, *Pentaclethra macrophylla* Benth., *Pericopsis elata* (Harms) Meeuwen, *Petersianthus macrocarpus* (P.Beauv.) Liben,, *Piptadeniastrum africanum* (Hook.f.) Brenan, *Prioria balsamifera* (Vermoesen) Breteler, *Prioira oxyphylla* (Harms) Breteler, *Pterocarpus soyauxii* Taub., *Pterocarpus tinctorius* Welw., *Pycnanthus angolensis* (Welw.) Warb., *Staudtia kamerunensis* (Warb.), *Terminalia superba* Engl.&Diels, *Zanthoxylum gilletii* (De Wild.) P.G.Waterman
Paradoxical characteristics (1) Wood anatomy: low double wall fibre width but high wood density	*Dalium pachyphyllum* Harms, *Gilletiodendron mildbraedii* (Harms) Vermoesen
Paradoxical characteristics (1) Temperament: Pioneer/Non Pioneer Light Demanding species with high wood density	*Afzelia africana* Pers., *Afzelia bipindensis* Harms, *Afzelia pachyloba* Harms, *Klainedoxa gabonensis* Pierre, *Lophira alata* Banks ex. C.F.Gaertn., *Margaritaria discoidea* (Baill.) G.L.Webster, *Milicia excelsa* (Welw.) C.C.Berg, *Millettia laurentii* De Wild., *Parkia bicolor* A.Chev., *Zanthoxylum gilletii* (De Wild.) P.G.Waterman
Added extra species with known dimensional stability, high number of heartwood samples of species of which samples were already taken from the collection and sample were already prepared in a previous project (XYLAREDD).	*Carapa procera* D.C., *Guarea laurentii* De Wild., *Pterocarpus angolensis* D.C., *Albizia gummifera* (J.F. Gmel.) C.A.Sm, *Afzelia bella* Harms., *Afzelia parviflora* Harms., *Afzelia peturei* De Wild., *Afzelia quanzensis* Welw., *Afzelia rhomboidea* (Blanco) S. Vidal, *Afzelia xylocarpa* (Kurz) Craib.

Please note the following:

(1) There are more species matching these criteria in the xylarium. This species selection (53 species) serves as a first test for the newly developed methodologies in this dissertation.
(2) Some of the species are present in multiple criterias.
(3) Some additional species were added for timber identification but these are not explicitely mentioned here as they are only used in that specific chapter.
(4) Most of the commercial species also have a known dimensional stability.
(5) The commercial importance was retrieved from the *Liste des essences forestières du Zaïre* (SPIAF, 1987) and Ruiz Pérez *et al.* (2005).
(6) Fibre wall width (µm) and wood specific gravity (oven dry mass over fresh volume) data were retrieved from the DRYAD database (Zanne *et al*., 2009).
(7) The species temperament was retrieved from Biwolé (2015) and Meunier *et al*. (2015).

3.2.4.1 Dimensional stability

The measurements were performed at the Laboratory of Wood Technology of Ghent University (https://www.ugent.be/bw/dfwm/en/research/woodlab). All samples were collected from the Tervuren Wood Collection (Royal Museum for Central Africa) (https://www.africamuseum.be/nl/research/collections_libraries/biology/collections/xylarium).

One of the main wood technological characteristics that determines whether a species has a functional use is the dimensional stability (i.e. the resistance against volumetric swelling). A second wood technological trait that is of great interest is the wood density, which is related to other wood properties. However, to determine the dimensional stability of a certain species, normalised samples (50 x 50 x 5 mm) are necessary. On these samples the dimensional stability is determined by caliper measurements perpendicular (radial) and parallel (tangential) with the growth rings (Figure 1A). The volumetric swelling is then determined as the summation of the radial and tangential swelling.

Sample preparation and scanning

These normalised dimensions are not available using xylarium samples and as such the dimensional stability cannot be determined by caliper measurements. **A new technique based on Digital Image Correlation was developed that allows a full screening of the xylarium in terms of dimensional stability.**

Specimens, mainly book-shaped, were selected from the Tervuren xylarium (RMCA). Special care was taken to focus on heartwood specimens only. For each specimen, the transversal plane was sanded and a slice of 0.5 cm was cut off. In total 421 samples from 53 species were prepared this way (see Table S.3 in Supporting materials in Deklerck *et al*., 2019b). We also included 89 reference samples from 10 wood species, further referred to as reference samples, measuring 5.0 x 5.0 x 0.5 cm (Rd, Tg, Tv) (see Table S.4 in Supporting materials in Deklerck *et al*., 2019b). These 10 species represent a range in dimensional stability behaviour, wood density and wood anatomical structure. On these reference samples, dimensional stability was determined by both calliper (Absolute digimatic calliper, 0.01 mm, Mitutoyo Corporation, Kawasaki, Japan) measurements and DIC (see further) to validate the DIC approach. The samples were conditioned at 60 % relative humidity (RH) and 20°C in a WK111180 climate test chamber (Weiss Technik, Germany). After two months, when the samples were in equilibrium, the sanded surfaces were scanned using a flatbed scanner (SilverFast SE Plus 8, LaserSoft Imaging AG, Kiel, Germany) at 2400 dpi and stored as 48-bit colour images. Samples were then conditioned at 90% RH and 20°C and, when equilibrium was achieved, the samples were scanned again.

Digital Image Correlation

All images were preprocessed in ImageJ software (Schneider *et al*., 2012) to ensure that images at 60% RH and 90% RH are correctly aligned. The DIC algorithm used in Ncorr is based on Pan's RG-DIC framework (Blaber *et al*. 2015; Pan *et al*., 2009). The dimensional changes are calculated based on registered pixel displacements between two images, both in x - and y – direction (Figure 1B). To obtain radial and tangential measurements, the displacement respectively perpendicular and parallel to the growth rings should be calculated. A MATLAB interface was written (Laboratory of Wood Technology, Ghent University) that allows to manually indicate the tangential and radial direction on a set of images (yellow lines in Figure 1B). For each sample, 10 tangential and 10 radial directions are indicated and the average displacement and standard deviation per sample and species were calculated.

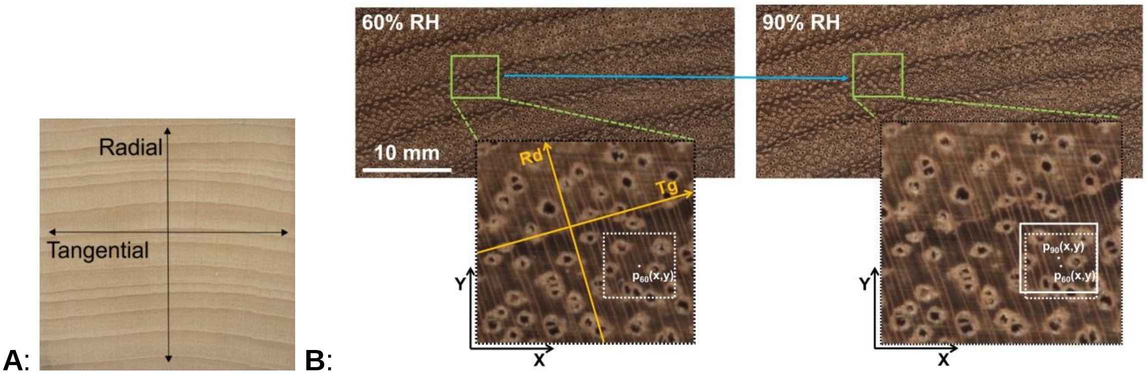


Figure 1: A: Indication of the radial (perpendicular on the growth rings) and tangential (parallel with the growth rings) direction on a normalised sample (50 x 50 x 5 mm). B: Representation of the tangential and radial direction for the displacement measures. Left (60% RH): scan of the crosscut section, right (90% RH): scan of the crosscut section on 90% RH. On the left image, $P_{60}(x,y)$ indicates the position of a pixel. On the right image, $P_{60}(x,y)$ indicates the old position of that pixel and $P_{90}(x,y)$ shows the new position of that same pixel. Notice the displacement of the pixel due to swelling ($P_{60}(x,y)$ to $P_{90}(x,y)$ on the right image). The tangential and radial swelling was determined using the displacement of pixel-groups in the x- and y- direction. From Deklerck et al. *(2019b).*

Wood anatomy

Next to determining the dimensional stability, we also want to assess the role of wood anatomical structure in dimensional stability and wood density. Anatomical cross-sections of 58 samples (see Table S.5 Supporting materials) were digitized at 10x magnification using Stream Image Analysis Software (StreamMotion, Olympus, Tokyo, Japan) with a scanning stage (Märzhäuser Wetzlar, Wetzlar, Germany) and a UC30 camera (Olympus, Tokyo, Japan) mounted on a light microscope (BX60, Olympus, Tokyo, Japan). On these samples the number of vessels per mm^2 (vessel density) and vessel lumen area per mm^2 (vessel fraction) were measured, for 28 to 120 vessels, following the IAWA guidelines (Wheeler *et al*., 1989) and depending on the number of vessels in the cross-section. On 24 samples (selected based on cross-section quality and visibility of anatomical features, see Table S.5 and Figure S.2 in Supporting Materials in Deklerck et al., 2019b), the percentage of vessel lumen, vessel walls, rays, axial parenchyma and fibres were also determined (Figure 2), based on manual delineation using the polygon selection tool in ImageJ (Schneider *et al*., 2012). On these 24 samples, the double fibre wall thickness was determined as well using SEM scans (jsm-6480 lv scanning electron microscope, JEOL, Tokyo, Japan).

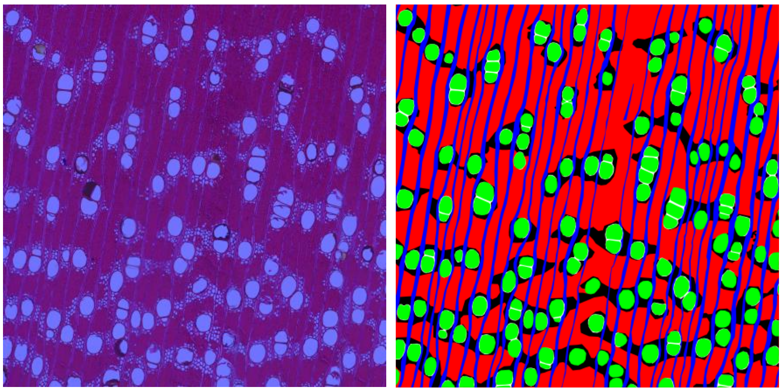


Figure 2: A Pericopsis elata anatomical slice (nine mm^2) before and after manual idntication of the different anatomical tissues (red = fibres, black = axial parenchyma, blue = rays, green = vessel lumen and white = vessel wall). From Deklerck et al. *(2019b).*

3.2.4.2 Natural durability

The measurements were performed at the Laboratory of Wood Technology of Ghent University (https://www.ugent.be/bw/dfwm/en/research/woodlab). All samples were collected from the Tervuren Wood Collection (Royal Museum for Central Africa) (https://www.africamuseum.be/nl/research/collections_libraries/biology/collections/xylarium).

The natural durability of wood determines its resistance against fungal decay and is an important determinant for the potential use of a species. The standardised sample dimensions to determine the natural durability are 50 x 25 x 15 mm (CEN/TS 15083 -1), however just as with the dimensional stability, this is impossible to obtain from xylarium samples. The Bravery (1979) mini-block test was initially developed as a screening test for preservatives. The main advantage of this test is the small required sample size (30 x 10 x 5 mm) compared to standardised tests. **In this part, we will adapt the time period of the mini-block test to allow determining the natural durability with xylarium specimens.**

577 heartwood mini-blocks (30 x 10 x 5 mm) were collected from 237 xylarium specimens from 31 species. 602 mini-blocks were collection from 11 species coming from store bought wood beams. The store-bought species were acquired from a commercial trade store and serve as comparison values in this test. All mini-blocks were oven-dried (18h to 24h at 103 °C) and weighed (m1) prior to gamma irradiation and fungal testing. Subsequently, the mini-blocks were sterilised with gamma irradiation and placed on a fungal mat of Trametes versicolor (strain CTB 863A – as listed in the CEN/TS 15083-1 standard, Centre Technique du Bois et de l'Ameublement Allée de Boutaut – BP 227, F 23 028 Bordeaux cedex) grown on malt-agar (40% malt, 2% agar). To avoid direct contact with the agar, mini-blocks were positioned on a metal support in the Petri dishes (Figure 3). Trametes versicolor was chosen as it is a white rot fungus, primarily able to degrade hardwood species, and an obligatory test fungus in standard CEN/TS 15083-1. *Fagus sylvatica* L. was included as reference species for assessing the virulence of the fungus.

Figure 3: Beech mini-blocks within a petri dish overgrown with Trametes versicolor. The mini-blocks are placed upon a metal grid, to avoid direct contact with the agar. From Deklerck et al. *(2019, under review).*

For a first set of store-bought samples (319 in total) mass loss was determined after 8 weeks. The beech wood already had on average 23% mass loss, however, this was still below the needed 30% threshold (EN 350, 2016). It was decided to prolong the mini-block test up to 12 weeks, to ensure the virulence of the test fungus. More tropical species can be found in higher natural durability classes compared to temperate species (EN 350, 2016). Since the xylarium samples are tropical, extending this period was expected to increase the likelihood of obtaining significant differences between species. After 12 weeks, mass loss was determined for the second set of commercial samples (283 total) and all xylarium samples (577 total). The mycelium was removed and all samples were weighed (m_2). Samples were then oven-dried (18h to 24h at 103 °C) and weighed (m_3) once more. The corresponding mass loss (ML) was determined as:

$$ML (\%) = ((m_1 - m_3)/m_1) \times 100 \qquad (1)$$

The final moisture content (MC) was determined as (see Table S.6 in Supporting Materials):

$$MC (\%) = ((m_2 - m_3)/m_3) \times 100 \qquad (2)$$

The durability class was determined based on the median of the percentage mass loss and the classifications given in EN 350 (2016). These classifications were compared to natural durability classes for the same species found in literature: Houtvademecum (Klaassen, 2018), Tropix 7 technical sheets (Cirad, 2017) and the EN 350 standard (2016). In what follows we use numbers to indicate the natural durability class even though some sources use Latin numerals. Normality and homoscedasticity of mass loss per species and time-period (8 weeks and 12 weeks) were assessed using the Shapiro-Wilk (Shapiro and Wilk, 1965) and Bartlett test (Snedecor and Cochran, 1989), respectively. Mann-Whitney U tests (Mann and Whitney, 1947) were performed to assess whether significant differences in mass loss are found between wood blocks exposed for 8 or 12 weeks and to determine whether there are significant differences in mass loss between store bought and xylarium species. To determine whether significant differences in mass loss between species changed from 8 weeks to 12 weeks, the Kruskal-Wallis Rank Sum test in combination with Dunn's test of multiple comparisons using rank sums (p-value adjustment via Benjamini-Hochberg method (Benjamini and Hochberg, 1995)) from the dunn.test package (Dinno, 2017) was used. These analyses were done in RStudio (RStudio Team, 2016) and graphs were produced with the ggplot2 package (Wickham, 2009).

The oven-dry wood density (kg/m³) was determined for 233 xylarium specimens (31 species) before sampling for the mini-block test. For the same specimens, chemical fingerprints were taken with DART-TOFMS (see 3.2.5)

3.2.4.3 Wood density at different scales

High-resolution density profiles based on X-ray Computed Tomography

The measurements were all performed at the Laboratory of Wood Technology at Ghent University. All analysis steps were performed at the Royal Museum for Central Africa. All sampled cores from the project have received a Tw number and can be consulted at the RMCA xylarium, and currently a density profile database is being developed.

Samples were cored in the field, and immediately stored. Via a field-to-desktop protocol (De Mil *et al*., 2016), the cores were stored in cardboard cylinders and dried in the oven for 24h at 103°C. Scanning was executed with the EMCT scanner (Dierick *et al*., 2014) at the UGCT (Ghent University Centre for X-ray Tomography, ww.ugct.ugent.be) (Figure 4). Depending on the length of the increment cores, up to six stacked scans were needed to visualize each cylinder; each of these scans (+-6.44 GB) took 15 minutes, where a total of 2000 projections were taken during a 360° rotation. A total scanning time of 18 h was necessary to scan all the samples, resulting in 0.5 TB raw data. 3-D volumes were reconstructed with the Octopus reconstruction software package (Vlassenbroeck *et al*., 2007) distributed by the company XRE (www.xre.be), and resulted in an approximate voxel pitch of 68 µm resolution. For each cylinder, the reconstructed stacked scans were then stitched, and the total data package is 1 TB.

Within HERBAXYLAREDD, 2047 density profiles (201 species) were collected with the X-ray CT methodology. An example for oil palm is given in Figure 5.

Figure 4: Setup of X-ray Computer Tomography toolchain at Woodlab, Ghent University

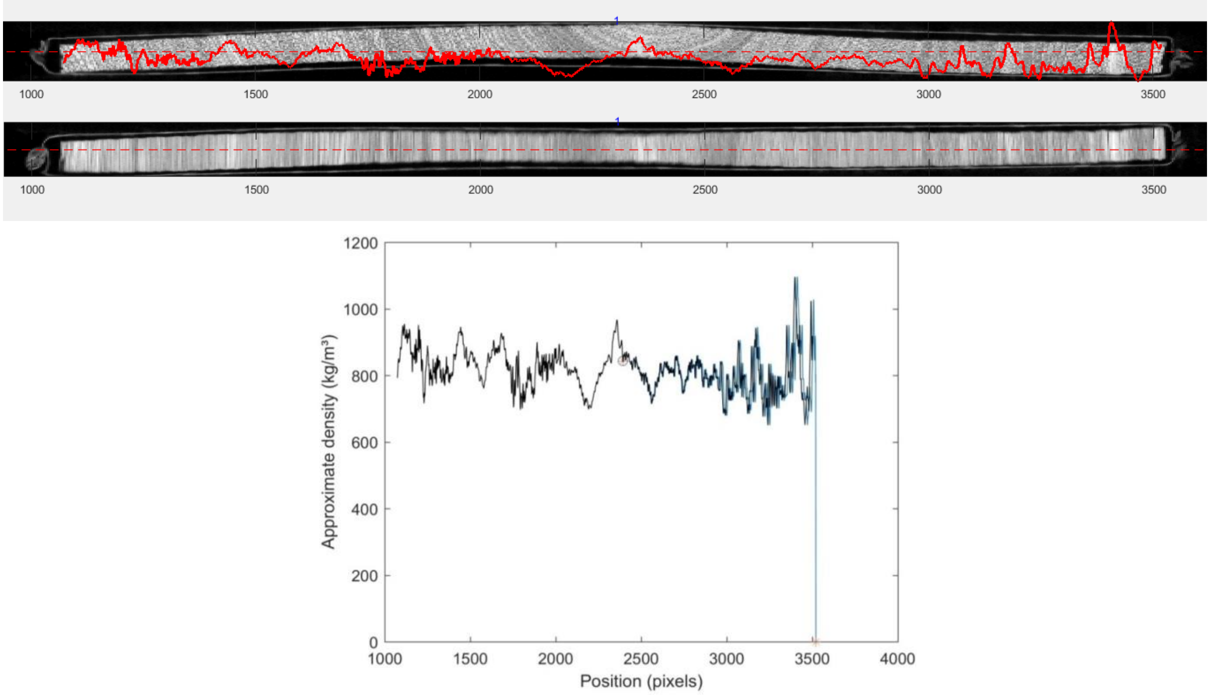


Figure 5: Top: Construction of X-ray CT wood core density profile of Elaeis guineensis. Bottom: X-ray CT wood core density profile corrected (blue) and uncorrected (black) of Elaeis guineensis.

Ovendry wood density measurements with water displacement method

Wood density (here defined as oven-dry weight/ oven-dry volume) was determined for every specimen (Figure 6). The specimens were oven dried at 103°C for 48h and weighed (depending on the size of the sample: Scaltec spb53, 0.01 g, Kern 572, 0.1 g, Germany). The volume of the specimens was determined based on the Archimedes principle, the proposed method for wood density estimates using xylarium specimens according to Maniatis *et al*. (2011).

Figure 6: Setup for the ovendry wood density measurements with the water displacement method at the Royal Museum for Central Africa (Service of Wood Biology). Left: ovens to ovendry the wood samples, Middle: scale and water displacement for small samples, Right: scale and water displacement for large samples.

Wood anatomical components of density

An example of the X-ray CT methodology can be found in De Mil *et al*. (2018) (see also further in the report), which was published within HERBAXYLAREDD. In this publication, density profiles of tropical angiosperm trees were linked to anatomical tissue fractions. Cores of 8 tree species were scanned with X-ray Computed Tomography to calculate density profiles. Then, cores were sanded and the outermost 3 cm were used to semi-automatically measure vessel lumen, parenchyma and fibre fractions. Furthermore, an assessment of density variation in function of growth ring boundary detection was done. A mixed regression model was used to estimate the relative contribution of each trait to the density, with a species effect on slope and intercept of the regression. Finally, position-dependent correlations were made between the fractions and the corresponding wood density profile.

3.2.4.4 Ash content and calorific value

Ash content

With a standard Retsch hammer mill, the tree cores were grinded to splinters of approximately 1 mm (Figure 7a). Samples were made ovendry for 24h at 103.5°C and put into platina and porcelain crucibles. Ash contents were determined with a Nabertherm muffle furnace at 580° for 3 h, preceded by a linear increase in temperature of 2 h (Figure 7b). We only partially applied the ASTM D 1102-84 standard, as we used 0.5 g to 1 g sample material, instead of 2 g, due to the limited sample size of tree cores. After cooling down, samples were put in a dessicator (Figure 7c) and the weight was noted (Figure 7d). The composition of ash was not examined in this study, but visual inspection shows a large variation in color and texture of the ashes between species (Figure 8).

Figure 7: Process of ash content determination

Figure 8: Ash residue after the muffle furnace, shows varying ash colors and textures between different species, which could affect burning processes and should be further explored in future studies.

Calorific value

All samples were grinded in a first stage with a hammermill. Then, to further grind the sample, an additional milling stage was applied (Figure 9a). The wood powder was completely dried at 103.5°C for 24 h, and then subjected to bomb calorimetry. A Parr 6200 isoperibole calorimeter was used for determination of the Higher Heating Value (HHV). Each sample was weighed with an analytical balance so that 0.36-0.6g sample could be loaded into a metal crucible (Figure 9b) that fits in the bomb (Figure 9c). Oxygen is added to the bomb (Figure 9d), and the magnesium ignition wick (Figure 9b above sample) burns the sample completely. Each measurement takes about 20 minutes. The obtained value is expressed in MJ/kg and is the Higher Heating Value of the dry sample.

Figure 9: process of calorific measurements.

3.2.4.5 Modulus of elasticity (MOE)

In the initial proposal of HERBAXYLAREDD, determination of the MOE was also included. However, the initial focus was on the other wood technological traits. Some proposals were made to determine the MOE on xylarium specimens but these were not further investigated during the project due to time constraints and its lesser importance compared to the other dicussed traits.

3.2.5 Metabolites in wood

Next to determining alternative species, we need to be able to identify the current highly valued timber species in trade. The chemical compounds that are metabolised in the heartwood can also be used for identification of wood (Deklerck, 2019). Depending on the natural variation in different taxa, and the relative degree of chemical change over time (based on use and processing), identification of different taxonomic levels could be possible (Dormontt *et al*., 2015).

The chemical spectrum of > 900 specimens (55 species) was collected with DART-TOFMS (see Cody and Laramée (2005)). This in collaboration with Ed Espinoza who is Deputy Director at the **U.S. Fish and Wildlife Forensic Laboratory in Ashland, Oregon (international partner 2)**. With DART TOFMS, wood slivers are placed in a heated helium gas stream, resulting in thermal desorption and ionization of the molecules. The relative abundance of these ions in the sample represents the chemical fingerprint of that sample. Multiple sample chemical fingerprints are then the basis for the species fingerprint (for a more detailed explanation see Deklerck (2019)). This is the basis for species identification with DART TOFMS. Species of interest included commercial timber species from the Congo Basin, for example *Pterocarpus* spp, *Pericopsis* spp and *Entandrophragma* spp. For every specimen, a sliver of wood was taken from the xylarium block and shipped to the US lab where Victor Deklerck (Woodlab-UGent) obtained the chemical fingerprint using DART-TOFMS (Figure 10 and Figure 11).

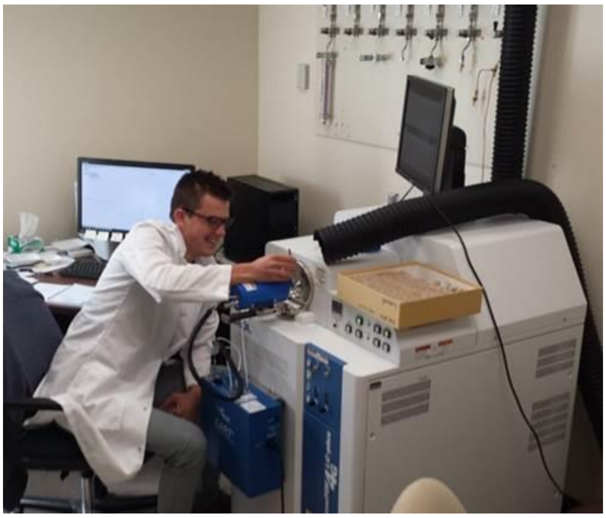


Figure 10: Victor Deklerck working on the DART-TOFMS at the U.S. Fish and Wildlife Forensics Laboratory.

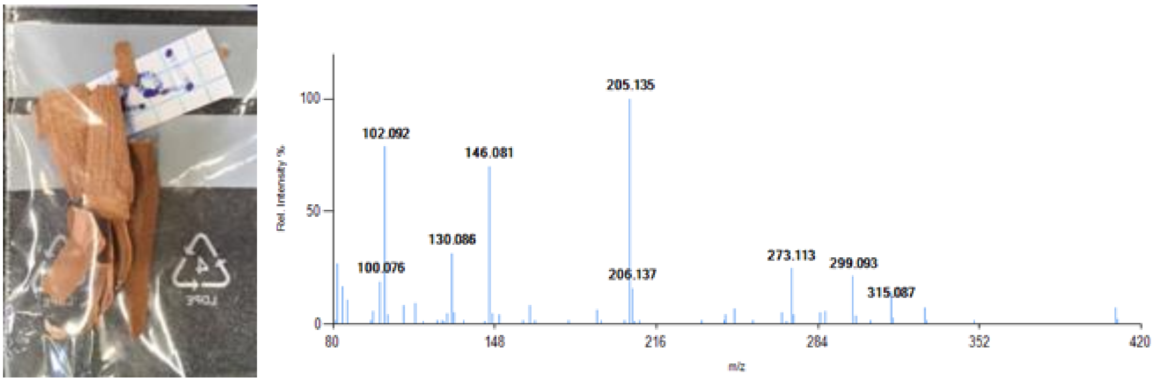


Figure 11: Left: Wood slivers obtained from a xylarium specimen. The sliver-size is around 2 to 4 cm long and less than 0.5 cm wide. Right: chemical spectra obtained using DART TOFMS on a wood sliver.

In total four missions were planned to the U.S. Fish and Wildlife Forensic lab, resulting in multiple publications. Ed Espinoza also visited the Royal Museum for Central Africa and was part of the jury for the PhD defense of Victor Deklerck. For the first publication (Deklerck *et al*., 2017) the focus is on the highly valued timber species *Pericopsis elata* (afrormosia). The main goal of this study is to determine if *Pericopsis elata* could be distinguished from the following species using DART TOFMS: *P*. *angolensis*, *P*. *laxiflora*, *P*. *mooniana*, *M*. *excelsa*, *H*. *monophylla*, and *D*. *melanoxylon*. A second goal was to determine: (1) which classification technique, Kernel Discriminant Analysis (KDA) or random forest, performs better to separate these species; (2) if by using the variable (ions) importance lists retrieved from the random forest, the KDA could be improved; and (3) the lowest number of ions needed to separate the species. In the second publication (Deklerck *et al*., 2019a) we take a closer look to the effect of varying the (1) mDa binning and (2) abundance cut-off threshold setting in the dataframe construction process on the classification accuracy. For, the third publication the chemical spectra of stem disks from two species were obtained (Figure 12). Over 1600 growth rings from *Pericopsis elata* and *Terminalia superba* were obtained to determine within individual variability and to determine whether samples could be allocated to the country of exploitation.

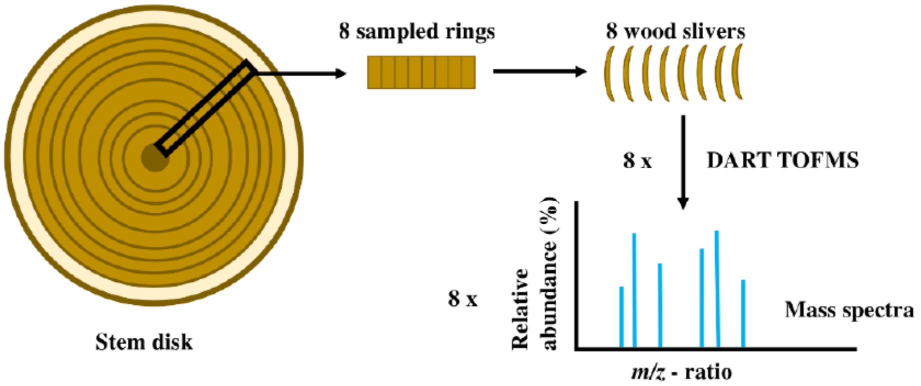


Figure 12: Schematic illustration of the procedure. For every sampled wood sliver, a mass spectrum is obtained (Deklerck et al.*, 2020).*

3.3 Distribution mapping

Georeferenced data from different sources were used for the species distribution mapping across tropical Africa, and even at a larger and continental scale. The African Plant Database (http://www.ville-ge.ch/musinfo/bd/cjb/africa/recherche.php) was first used to study the distribution for the *Erythrophleum* species across Africa using a species modelling approach (Gorel *et al*., 2019a). Then, the African floristics data assembled by Adeline Fayolle and Michael D. Swaine (U. Aberdeen, UK) and which is a combined data set of coexisting tree species in forests (Fayolle *et al*., 2014) and savanna (Fayolle *et al*., 2019),. Were used to identify bioregions and floristics clusters, using classical approaches in community ecology (ordinations, clustering), separetely for forest (Fayolle *et al*., 2014) and savanna (Fayolle *et al*. 2019) sites. These data of tree species composition in specific forest and savanna sites were then merged, and used to determine climatic envelopes of the forest and savanna biomes across Africa, and map the bistability area (see also Aleman *et al*., unpublished). These data were also used to identify forest and savanna specialist species, and generalist species, present in the two biomes. The RAINBIO (https://gdauby.github.io/rainbio/index.html, Dauby *et al*. (2016)) data became available during HERBAXYLAREDD and were also used for different purposes:

(1) To examine the coverage of herbarium derived phenological data (based on Ouédraogo *et al*., in press)

Temporal and spatial patterns in flowering phenology were assessed for eight tropical African timber species: *Entandrophragma cylindricum* (Sprague) Sprague, *Erythrophleum suaveolens* (Guill. & Perr.) Brenan, *Lophira alata* Banks ex C.F.Gaertn., Mansonia altissima (A.Chev.) A.Chev., *Milicia excelsa* (Welw.) C.C.Berg, *Pterocarpus soyauxii* Taub., *Terminalia superba* Engl. & Diels and *Triplochiton scleroxylon* K.Schum. Phenology data from direct observations of trees in seven sites were collected and monitoring was conducted monthly in Cameroon and in the north of the Republic of the Congo. For the eight listed species, Ouédraogo *et al*. (in press) examined herbarium collections of Meise Botanic Garden, the herbarium of Naturalis Biodiversity Center, the online collections of the French National Museum of Natural History and the Royal Botanical Gardens Kew. For all fertile specimens, the flowering status, the collection date, and geographical coordinates were recorded. Specimens with flowers just before anthesis, open flowers, or old flowers were categorized as “flowering”. Remote-sensing-based monthly rainfall estimates were computed from the CHIRPS for Africa (Funk *et al*., 2015) mean monthly rainfall over the period 1981-2000. Mean monthly rainfall values were then extracted for a large area which includes the seven study sites for which field observations of tree phenology were available, as well as all geographical locations covered by the herbarium collections (and thus including non-forested areas). For the specific data analysis we refer to Ouédraogo *et al*. (in press)

Specifically, (1) the frequency and seasonality of flowering a seven sites in central Africa were determined using field data, graphical analysis and circular statistics, and between-species and between-site differences were identified; (2) the latitudinal variation in the seasonality of flowering across species range was investigated using herbarium data; and (3) the correlation between the spatial variation in the seasonality of flowering and the seasonality of rainfall was tested.

(2) To map the distribution of forest and savanna species across the African continent (see Aleman *et al*. (submitted) and in section 4). The distribution of tropical tree species was mapped from RAINBIO, after having classified species into three groups: forest specialists, generalists species occurring both in forest and savanna, and savanna specialists (Figure 13).

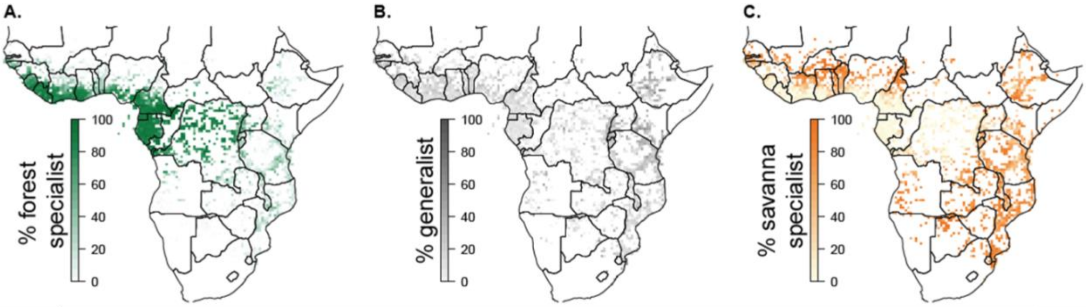


Figure 13:.Forest and savanna tree species distribution in Africa. For each 0.5° pixel containing herbarium records for at least 5 of our 1707 species, the percentage of (A) forest specialists, (B) generalists and (C) savanna specialists was computed and mapped

(3) Work of Bhély Angoboy Illondea (see also 3.2.1 Leaf and whole tree traits): The occurance data of the different species, obtained from the RAINBIO database, completed with herbarium vouchers from the Meise botanical garden was superimposed on the Worldclim data. This made it possible to collect 19 climatic variables corresponding to the location of the species. The following variables were included in the study: annual mean temperature, annual precipitation and precipitation of the driest month. The analysis of the response of species as a function of environmental gradients was studied individually for 131 species using the R package eHOF (extended Huisman-Olff-Fresco). This R package classifies species according to 7 models. These different models provide information on the optimum of the species according to the environmental gradient considered and also on its niche. This was also done to determine whether the species present in Luki are in there optimum conditions, or on the boundary of their distribution and niche. This information was also included in 3.6.2 *Atlases with complete trait information*.

(4) For the phylogeographic analyses of *Scorodophloeus zenkeri*, *Staudtia kamerunensis* and *Prioria balsamifera*, distribution maps were created in QGIS v.3.4.5 for the respective species to visualize the **distribution of intraspecific genetic clusters**. Distribution mapping was done using the coordinates available from herbarium samples at Meise Botanic Garden and silica-dried samples at Université Libre de Bruxelles. Additionally, **distribution maps** were created in QGIS **for 121 tropical African tree and woody plant species** using coordinates available from the Meise herbarium database, GBIF (gbif.org) and the RAINBIO database (Dauby *et al*. 2016).

3.4 Functional strategy

3.4.1 Functional traits co-variation

Recent studies on tropical trees have raised the importance of the stem (wood) economic spectrum (Chave *et al*. 2009; Baraloto *et al*. 2010; Fortunel *et al*. 2012), and whether the wood/stem economic spectrum is correlated or orthogonal to the leaf economic spectrum is hotly debated. We thus need to deeper investigate the functional strategy of tropical woody species in Africa to examine the relationships between leaf and wood traits, and the correlation/independence of the leaf and stem economic spectrum. The functional strategies, and the links between leaf/stem/seed/root traits, are usually determined based on the results of multivariate analyses at species or individual levels. Within HERBAXYLAREDD, several of these strategies have been investigated further; the link between seed/leaf/wood traits was not researched into detail.

Within HERBAXYLAREDD, the following has been done within the initial scope:

(1) Tree measurements in the field were used to develop architectural traits.
   a. In a first study, Loubota Panzou *et al*. (2018a) examined how interspecific variation in architectural traits is related to the functional traits of 45 coexisting tree species in Central Africa. At the tree level, they measured tree diameter, total height and crown dimensions for an average of 30 trees per species (range: 14–72, total 968 trees) distributed over a large range of diameters (up to 162 cm). Using log–log models, we fitted species-specific allometric relationships between tree diameter, height and crown dimensions. At the species level, we derived architectural traits (height and crown dimensions) at 15 cm and maximum diameters from species-specific allometries. The architectural traits were then related to functional traits, including light requirements, wood density, leaf habit and dispersal mode.
   b. In a second study, Loubota Panzou *et al*. (2018b) explored the local-scale variation and determinants of plot-level AGB, between and within two types of forests, the *Celtis* and *Manilkara* forests, growing under the same climate but on different geological substrates in northern Republic of the Congo. They developed height-diameter and crown-diameter allometric relationships and tested whether they differed between the two sites. For each 1-ha plot, they further estimated the AGB and calculated structural attributes (stem density and basal area), composition attributes (wood density) and architectural attributes (tree height and crown size), the latter being derived from site-specific allometric relationships.
   c. We can also include the work by Arthur Chantrain here, who developped the exact same approaches in the Luki site, DRC, using allometric models to quantify species specific architectural traits at the juvenile and adult stage. Finally, these architectural traits were related to other key functional traits of tropical tree species. This explained more into detail in 3.2.1 *Leaf, seed and whole tree traits*.

(2) The co-variation of the traits of the Nkula species has been studied (see trait atlas).

Within HERBAXYLAREDD, the following has been done outside of the initial scope:

(1) Established the link between wood traits and wood anatomy on trees for the two forest species of *Erythrophleum* (*ivorense* and *suaveolens*). Wood anatomical traits, plant hydraulics and growth data from the natural habitat were used to compare the listed species. The following wood traits were measured on transverse sections: tangential vessel diameter, vessel density and vessel grouping index. The proportion of vessel lumen, fiber and axial and radial parenchyma was also quantified. Intervessel wall thickness and fiber wall thickness (measured as the double wall between two adjacent fibers) were also measured (see Goret *et al*., 2019).

3.4.2 Functional traits and growth

(1) Quantifying carbon dynamics in forests is critical for understanding their role in long-term climate regulation (Hubau *et al*., 2019). Yet little is known about tree longevity in tropical forests, a factor vital for estimating carbon persistence. The tree vital rates are (1) regeneration, (2) growth and (3) mortality. These rates are determined by the traits described above. Hubau *et al*. (2019) (***nature plants***) calculate mean carbon age in different strata of African tropical forests using (1) growthring records with a unique timestamp accuralty demarcating 66 years of growth in one site and (2) measurements of diameter increments form the African Tropical Rainforest Observation Network (23 sites). The unique timestamp stems from nail traces retrieved when sampling with wood cores. These nails were put into the tree, 66 years ago, when the trees in the Nkula park were tagged with a number. This resulted in a wound within the wood, which can be seen when looking at the wood core (see Figure 14). Based on this, Hubau *et al.* (2019) were able to accurately determine the tree growth in 66 years. For a full description, we refer to Hubau *et al*. (2019) in *nature plants*.

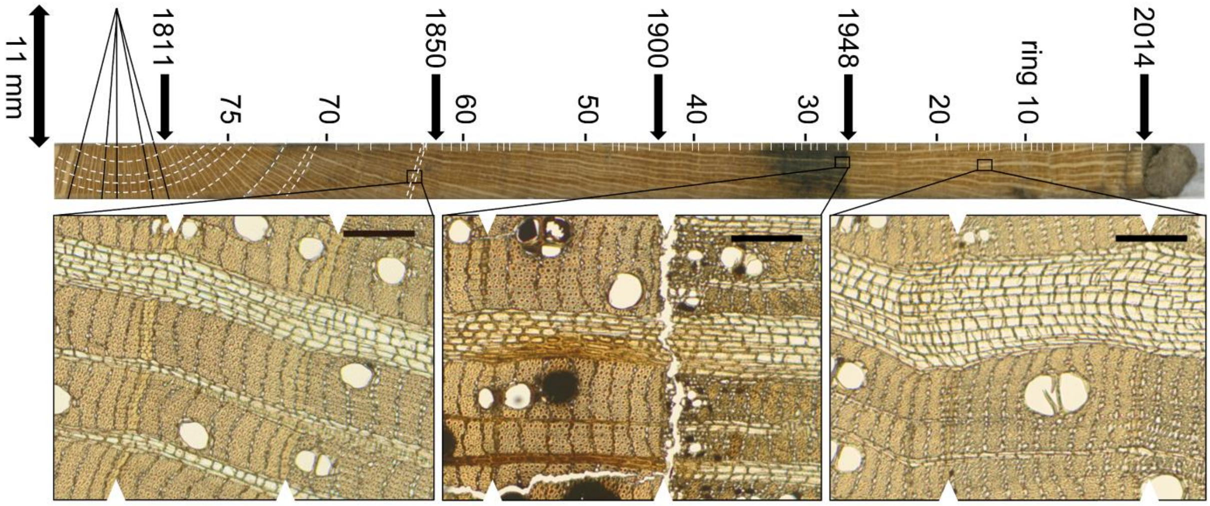


Figure 14: Example of a wood core (Greenwayodendron suaveolens, TreeID 765) showing the 1948 nail trace. The image at the top shows the full core. White lines indicate growth-ring boundaries, numbers indicate growth rings (counted from bark to pith), black arrows indicate important years. The bark to the right of the figure indicates the year of sampling (2014). The dark discoloration in growth rings 26 to 35 was caused by oxidates from the iron nail that were transported up and down in damaged vessels and fibres. The right border of the discoloration accurately marks the start of the year 1948. There are 25 rings between the bark and the 1948 nail trace, suggesting that this tree

needed on average 2.6 years to form a ring. Using this rate for the 53 rings that were formed before 1948, we find that the first ring in the core was probably formed around 1811. The location of the pith is indicated by the black lines to the left, which follow the direction of the wood rays41. This shows that the distance from the pith to the first ring boundary in the core is about 11 mm. When using the average ring width of rings 78 to 68, we estimate that 7 rings are missing. Therefore, this tree would be about 224 years old. The three close-ups at the bottom illustrate wood anatomical details used to identify growth-ring boundaries (indicated by white triangles). Ring boundaries in this species are demarcated by distended wood rays and flattening of the fibres. Black scale bars, 0.2 mm.

In the follow-up publication in **Nature**, Hubau *et al*. (2020) assess the trends in the carbon sink using 244 structurally intact African tropical forests, which are compared with 321 published plots from Amazonia. They also investigate the underlying drivers of the trends. Hubau et al. (2020) compiled and analysed data from structurally intact old-growth forests from the African Tropical Rainforest Observation Network27 (217 plots) and other sources (27 plots) spanning the period 1 January 1968 to 31 December 2014. In each plot (mean size, 1.1 ha), all trees ≥100 mm in stem diameter were identified, mapped and measured at least twice using standardized methods (135,625 trees monitored). Live biomass carbon stocks were estimated for each census date, with carbon gains and losses calculated for each interval.

(2) The work by Mirabel *et al.* (2019) (see also Mirabel, 2015): The functional strategy of 15 African tree species was assessed by (1) highlighting the structure of traits covariance and the underlying functional trade-offs, (2) inferring a whole-plant functional scheme and (3) testing the correlation of the functional scheme with plant performance for two early developmental stages (seedlings and saplings). The species were chosen widespread across the lowland mixed-moist forests of the Guineo-Congolian Region. They selected 10 seedlings for each of the 15 species studied from a nursery in south-eastern Cameroon and measured 18 functional traits, including leaf, stem and root traits, biomass allocation and stem anatomy. They assessed the height and diameter growth of the seedlings and the DBH growth and survival for the saplings of nearby plantations. Multivariate analyses highlighted the covariations among the functional traits of the leaf/stem/root, biomass allocation ratios and stem anatomy. Dated wood data are available from the same location thanks to nail tag traces that allowed to measure growth increments over a period of 66 years.

(3) The work by Illondea *et al*. (under review): They investigated whether a tree-growth analysis based on dated growth patterns, could produce additional information on carbon budgets in tropical tropical forests that are characterized by a high number of species and where anatomical distinct growth rings are barely present. Archived annual diameter measurements of 1117 trees from 1948-1957 were used in combination with census data for 2009 and 2018 in 7 permanent plots of 1 ha eac in the Biosphere Reserve of Luki (south-western margin of the Congo Basin). Dated wood data were available from the same location thanks to nail tag traces. These allowed to measure growth increments over a period of 66 years. A dated wood approach was proposed as the alternative, repeated measurements of stem diameter at DBH, require long periods of follow-up and are prone to some systematic errors.

3.4.3 Functional traits and species distribution

3642 trees belonging to 158 species, 123 genera and 39 botanical families were followed every 10 days for phenology in the Nkula park in the Luki Biosphere Reserve from 1947 to 1958 (Couralet *et al*, 2013, Illondea *et al* ., 2019). This constitutes a unique dataset for the forests of Central Africa. To date, it offers a huge opportunity to study species from the Congo Basin. The tree marking nails currently serve as cambial marking which dates from 1947. This is how these same monitored species were considered at the scale of the Congo Basin using the RAINBIO database to offer even more opportunities. The objective was to understand the response of species based on environmental gradients. Specifically, it involves: (i) determining the occurrence of the different species, (ii) knowing the optimum and the niche of the species (see also Distribution mapping and the trait atlas).

3.5 Wood technology of lesser used species

3.5.1 Paradoxical species

Mostly, wood density mediates a trade-off between strength and economy of construction. Rapid growth rates of light-demanding tree species are generally related to low wood quality (density, specific modulus, durability). However, few tropical rainforest tree species combine wood with good technological characteristics with high growth rates (Bossu *et al*. 2013). Because of the rather diversified target groups in our project, a lot of data will be generated that can easily be screened in the same way as mentioned. We will extract the ‘paradoxical’ species and analyse their mechanisms in order to offer indigenous alternatives for exotic tree plantation species. Thus, this will also generate valuable information for governments and forest logging companies (REDD+, sustainable forest management). First, in the start of the project the focus was on two paradoxical characteristics: (1) low double wall fibre width in combination with high wood density and (2) Pioneer/Non Pioneer Light Demanding (= Temperament) species with high wood density. The fibre wall width (µm) and wood specific gravity (oven dry mass over fresh volume) data were retrieved from the DRYAD database (Zanne *et al*., 2009). The species regeneration guild (Hawthorne, 1995) was retrieved from Biwolé (2015) and Meunier *et al*. (2015). This gave an initial list of 12 species that have one of the two paradoxical characteristics (see also Deklerck (2019)). These species were included in the test set-up for the dimensional stability and natural durability (see 3.2.4 Wood technological traits) to determine whether they also had interesting wood technological properties.

Second, anatomical information was collected for the species included in the dimensional stability test set-up as well. Based on the known relationship between the wood anatomical structure and the dimensional stability we can determine a third paradoxical characteristic. Several species were determined that can be labelled as “paradoxical” (see further).

Although the current set-up is rather limited, this is the first study that focusses on the African continent. The xylarium offers more wood species and specimen’s than we could screen at this time, and there might be other interesting paradoxical characteristics to look at.

3.5.2 High-value species for trade

A screening for paradoxical species and lesser used species with favourable properties is made, based on all measured properties within the list of target species. In this next step, a final selection will be made to distinguish which of these species with paradoxical and favourable properties are suitable for trade. Based on the results of the wood technological traits and paradoxical characteristics, a list of lesser used species with favourable properties is made. Several groups are also created with species that have a similar relationship between dimensional stability and wood density. A full screening of the xylarium will allow putting species in these groups and as such indicate their potential use (see further).

3.5.3 Species for bio-energy

Africa is the most important producer of fuelwood in the world (> 25 %). This is logical if you know that fuelwood accounts for 60 up to 90% of their primary energy consumption (Amous, 2013). There is a list available at the RMCA with species that are used but not always documented for bio-energy. Through collaboration with the ERAIFT, this list could be completed with local knowledge. The final list will be checked with the HERBAXYLAREDD target species to test the species in common on both lists and to formulate recommendations for future forest management plans. Determination of specific gravity, ash content and calorific value is of importance for firewood characterization (Ramos et al. 2008). Based on the measurements explained in “Ash content and calorific value” and in combination with local knowledge, we will be able to determine interesting species for bio-energy.

A key work here is the mission report “*Evaluation du potential énergétique de la biomasse aérienne ligneuse des anciennes plantation de l’INERA Yangambi”* by Esther Bustillo Vazquez and Laurent Raets. This report shows the results of the two missions by Esther Bustillo Vazquez and Laurent Raets in Yangambi and Ngazi during four months between October 2017 and March 2018. The objective was to develop a methodology allowing collecting dendrometrical data in plantations of *Hevea brasiliensis* and *Elaeis guineensis* in order to assess the quantity of energy stored in the above-ground biomass (AGB) in ligneous trees with allometric equations. One-hectare plots were created in plantations, plantations’ edges (0.08 ha) and agricultural resting fields. The following dendrometrical data was collected: diameter at breast height (1.3 m), trunk height, total height, bark thickness, wood carrots samples and Dawkins and Van Daalen indexes. The dendrometrical data were transformed in AGB values thanks to specific wood density data and calorific values. There was a stratified sampling according to years of plantation in order to extrapolate the datas to the whole plantations’ areas. 19 one-hectare plots were set in plantations, 21 plots in edges and 3 in agricultural resting fields. This lead to the following areas of plantations: 830.21 ha of *Hevea brasiliensis* in Ngazi, 675.68 ha in Yangambi, 1123.09 ha of *Elaeis guineensis* in Yangambi.

3.6 Identification keys/atlases

3.6.1 Visual keys

A visual identification key was constructed for 70 commercial species from the Democratic Republic of the Congo based on 22 macroscopic and microscopic characteristics. Based on this identification key, the observed characteristics can be compared with reference images. For a full description, the specific pathway for identification and the actual software with the visual key, please visit: http://woodbiology.africamuseum.be/identificationkey.

3.6.2 Atlases with complete trait information

This is closely related to the work by Bhély Angoboy Ilondea described in 3.2.1 Leaf, seed and trait analysis and 3.3 Distribution mapping. A trait atlas is being created based on the data retrieved in those two parts. This atlas gives per species, high-quality images and information on its plant and wood traits and its distribution. This way, the final results of HERBAXYLAREDD will be published in a book that will be useful for science and policy needs, in developed and developing countries. This type of publication can be consulted at any time, without the need for internet, a precondition that is often not fulfilled in Central Africa.

Taking tree cores in the Luki Biosphere Reserve (© Tom DE MIL, Victor DEKLERCK)

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

4.1 Linking the Tervuren xylarium and the Meise herbarium

4.1.1 More reliable reference collections made available *(Vanden Abeele et al., in prep.)*

The **Tervuren xylarium** (Tw) at the Royal Museum for Central Africa (RMCA) contains **80,937 wood specimens** (c. 13,534 species); of which approx. 40,000 specimens originate from sub-Sahara Africa. The metadata accompanying the wood collection (such as taxon names, commercial names, collector, geographical origin and microscopy pictures) is publicly available on the RMCA website. (https://www.africamuseum.be/nl/research/collections_libraries/biology/collections/xylarium) and the collections can be consulted on request. The herbarium (BR) at **MeiseBG** houses around **4 million collection items**, of which about 1.5 million have been digitized over the past few years (by staff of MeiseBG and as part of the DOE! Project). This means that images and associated data are now available for a large part of the collection, especially from tropical Africa. The newly digitized collections can be consulted via the virtual herbarium (http://www.botanicalcollections.be). The linking of samples from both databases was achieved by **(1)** directly matching the Tw-number (which was available for some BR samples) or by **(2)** matching a unique key comprised of three variables; the collector, country and herbarium number. Afterwards, matches were verified by comparing the scientific species names of both samples.

(1) Matching the **Tw-number**

In the MeiseBG herbarium database, 4,433 records were found for which a Tw-number was specified. These could be linked to 3,007 Tw-records in the Tervuren xylarium. This difference in numbers is due to the fact that each herbarium voucher receives a different herbarium number (even those collected from the same individual), while xylarium samples (blocs, sections, discs) from the same individual receive one unique Tw-number. Therefore, some Tw-numbers could be linked to multiple herbarium records.

Species identification was verified for all samples that could be linked based on Tw-number (Table III). The following categories were identified:

1. Species matching: same species for both sources, so the matching allowed updating the correct botanical identity of the Tervuren xylarium.
2. Genus matching: same genus, different species
3. Previously indet.: no species identification for the xylarium sample, updated with information from herbarium sample
4. Different – Indet.: different genus or no identification for both databses

Table III: Indication of Tw (Tervuren Wood Collection number) matching in the databases.

	Meise Herbarium	Tervuren Xylarium
Species matching	4,194	2,840
Genus matching	209	139
Previously indet.	10	9
Different - Indet.	22	19
Total	4,435	3,007

The species and/or genus identification matched for most of the linked samples. The small number of samples for which the species identification was lacking or incorrect, were updated accordingly.

(2) Matching through the **unique key**

Xylarium samples for which no Tw-number was found in the herbarium database were linked to the corresponding herbarium record(s) by matching the combination of collector, country and herbarium number.

First, collector, country and herbarium number were harmonised to allow the comparison of records from both databases. Previously, the xylarium database has been updated and completed with data from historical archives. By linking the xylarium and herbarium databases, the additional historical data could be used to provide the country of origin for 51 herbarium samples.

By using the unique key, 5,677 BR herbarium samples could be matched with 4,653 Tervuren wood samples, after checking the scientific species and/or genus names (Table IV).

Table IV: Indication of unique key matches in both databases.

	Meise Herbarium	Tervuren Xylarium
Species matching	4,254	3,502
Genus matching	647	509
Previously indet.	363	318
Different - Indet.	413	324
Total	5,677	4,653

Using both matching methods, a **total of 10,199 BR herbarium specimens** (Table V) **were linked to 6,933 Tw wood specimens** (Table VI).

Table V: Overview of all linked samples from the Meise Herbarium database.

	Linkage based on				
Meise Herbarium	**Tw in DB**	**Key 3 variabels**	**Key correct. Country**	**Key correct. alphanum.**	**Total**
Species matching	4,194	3,995	45	214	8,448
Genus matching	209	643	4	8	864
Species and Genus matching					
Species matching	31	8			39
Genus matching	9	30	1		40
Previously indet.	10	352	1	10	373
Different - Indet.	22	399		14	435
Total	4,475	5,427	51	246	10,199

Table VI: Overview of all linked samples from the Tervuren Xylarium database.

	Linkage based on			
Tervuren Xylarium	**Tw in DB**	**Tw in DB and Key 3 variables**	**Key 3 variables**	**Total**
Species matching	2,095	745	2,757	5,597
Genus matching	125	14	495	634
Species and Genus matching	6	25	2	33
Previously indet.	9	0	318	327
Different - Indet.	18	1	323	342
Total	2,253	785	3,895	6,933

Subsequently, the linked samples were ranked based on the following variables: country of origin (Table VII), collection period (Table VIII), plant family (Table IX) and collector (Table X). To avoid duplicate counts, only Tw-numbers for which the species (or species and genus) matched the corresponding BR herbarium vouchers were used.

Table VII: Number of links ranked per country.

Congo, Dem. Rep.	4,310
Angola	462
Congo	177
Rwanda	142
Uganda	124
Ivory Coast	118
Burundi	80
Benin	67
Comoros	57
Madagascar	44
Cameroon	41
Liberia	7
Guinea	1
Total	5,630

Table VIII: The number of links in a collection decade.

unknown	886
1900-1909	2
1910-1919	66
1920-1929	17
1930-1939	2,114
1940-1949	700
1950-1959	345
1960-1969	121
1970-1979	583
1980-1989	445
1990-1999	104
2000-2009	57
2010-2019	190
Total	5,630

More than 76% of the linked specimens were **collected in the Democratic Republic of the Congo** (DRC, Table VII). This is mainly the result of the intensive collecting efforts of (Belgian) researchers when the DRC was a Belgian colony. Most specimens with samples in both Meise and Tervuren were collected during the 1930's (Table VIII), a period during which the interest in Central African plants increased, as there was a general ambition to discover new botanical products that could be exploited in a semi-commercial framework.

The linked botanical samples belong to **148 different plant families** (Table IX), of which the **Fabaceae family is represented best** (886 specimens), followed by the Rubiaceae (446 specimens) and Euphorbiaceae (425). The linked samples were collected by **70 different collectors** (Table X), with **most samples collected by Louis** (2075 samples), followed by Donis (476 samples) and Dechamps (464).

Table IX: The number of links per family.

Fabaceae	886
Rubiaceae	446
Euphorbiaceae	425
Apocynaceae	265
Meliaceae	224
Annonaceae	193
Sapindaceae	165
Combretaceae	151
Sterculiaceae	141
Moraceae	122
Sapotaceae	102
Clusiaceae	101
Anacardiaceae	100
Dichapetalaceae	98
Ebenaceae	95
Flacourtiaceae	94
Verbenaceae	90
Connaraceae	88
Celastraceae	83
Tiliaceae	81
Subtotal	3,950
Total	5,630

Table X: Number of links per collector.

Louis, Jean Laurent Prosper	2,075
Donis, Camille Albert	476
Dechamp, Roger	464
Dechamp, Roger; Murta, Frederico; da Silva, Manuel	443
Malaisse, François	418
de Witte, Gaston-François	173
Bamps, Paul	124
de Briey, Jacques Antoine; Gobert, Charles	118
Wagemans, Jean	103
Maudoux, Emile	101
Subtotal	4,565
Total	5,630

By linking both the database of the herbarium of Meise Botanic Garden (BR) and the Tervuren wood collection (Tw), **more reliable reference data were made available**. A protocol for data exchange was drawn up to allow the successful linking of new samples that will be added to the databases.

Both databases are **publicly available**;
Tervuren xylarium (Tw) collection:
https://www.africamuseum.be/nl/research/collections_libraries/biology/collections/xylarium
MeiseBG herbarium (BR) collection: http://www.botanicalcollections.be (Virtual Herbarium) and https://doi.org/10.15468/wrthhx (GBIF)

4.1.2 Botanical field identifications need additional verification *(Janssens et al., in prep.)*

The **botanical species identification made in the field was verified for 762 woody plants** from the permanent monitoring plots in the Yangambi Biosphere Reserve (Annex I). At the **species level**, only **56%** of the individuals appeared to be **correctly identified**. Approximately 78% of the individuals were correctly identified at the genus level, while family identification was correct for approx. 90% of the individuals. This indicates that **caution is needed** when using specimens for which the botanical identity has not been validated after the initial identification in the field.

To verify the importance of correct botanical identification in the field on diversity assessments, the effect of the forest type has been verified with either original (‘Old’) and corrected identifications (‘New’). We found that the species richness (Figure 15) depends on the forest type, and not on the data verification. The same correlation was observed for the Shannon diversity index (Figure 16), Simpson diversity index (Figure 17) and the evenness (Figure 18), which all depended on the forest type and not on the data verification.

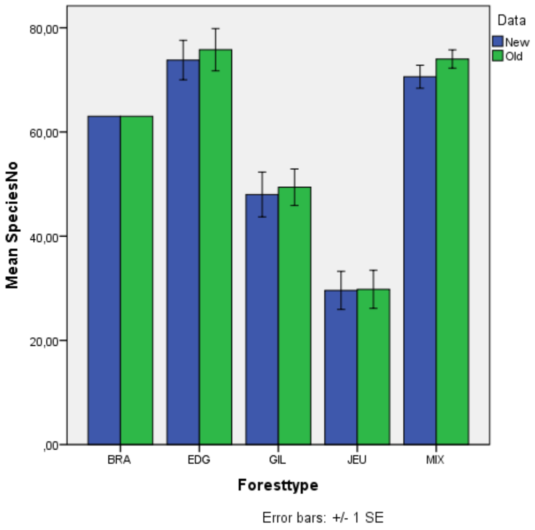


Figure 15: Species richness depends on forest type, not on data verification.

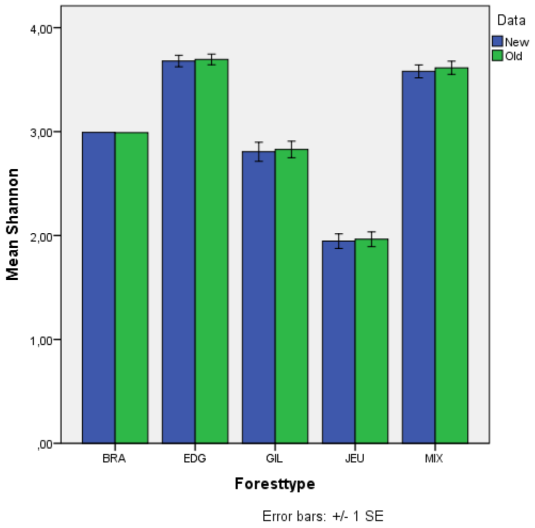
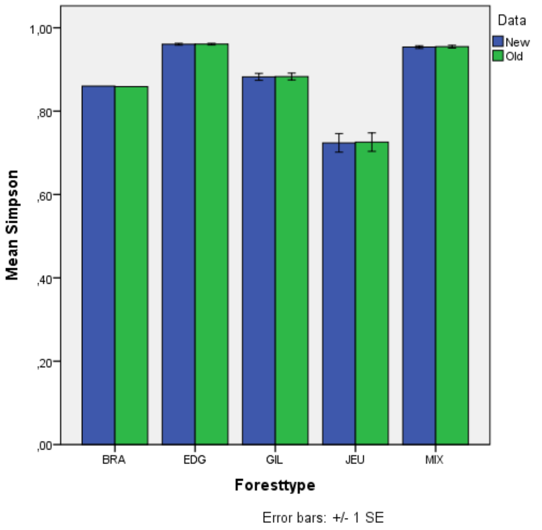


Figure 16: The Shannon diversity index depends on forest type, not on data verification.



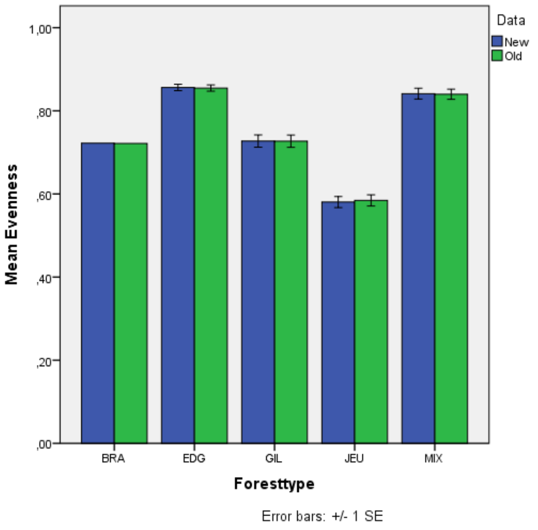
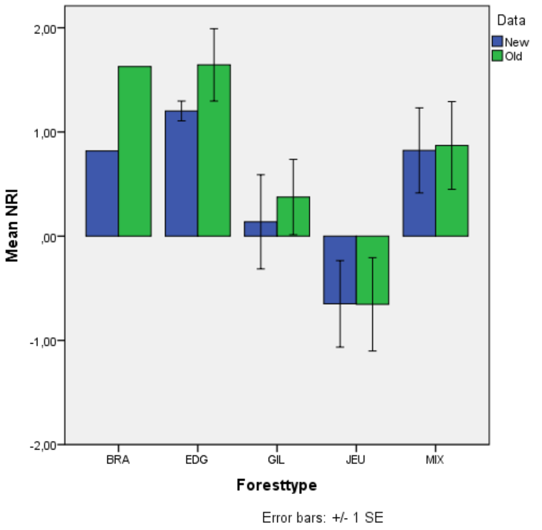


Figure 17: The Simpson diversity index depends on forest type, not on data verification.

Figure 18: Eveness depends on forest type, not on data verification.

Assessment of the phylogenetic community structure showed that both the Net Relatedness Index (Figure 19) and the Neatest Taxon Index (Figure 20) depended on the forest type and not on data verification.



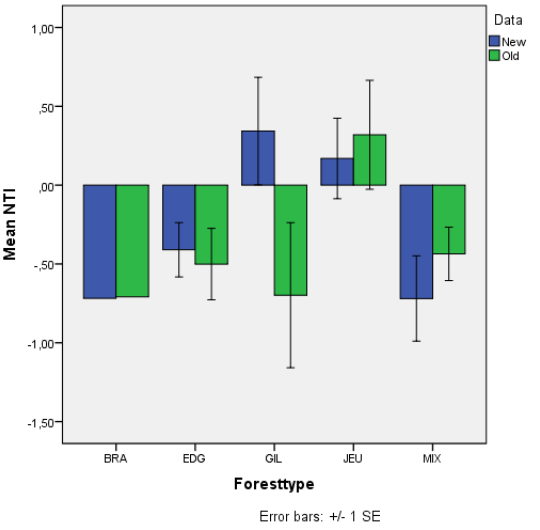


Figure 19: Net Relatedness Index (NRI) depends on forest type, not on data verification.

Figure 20: Nearest Taxon Index (NTI) depends on forest type, not on data verification.

Additionally, the clustering analysis based on tree frequency data of the different plots showed only minor differences when using verified data (Figure 21B) as compared to the original data (Figure 21A). In the clustering analysis based on the tree presence or absence data, more clear differences were observed when using verified data (Figure 22B) as compared to the original data (Figure 22A), although the main clusters remained similar.

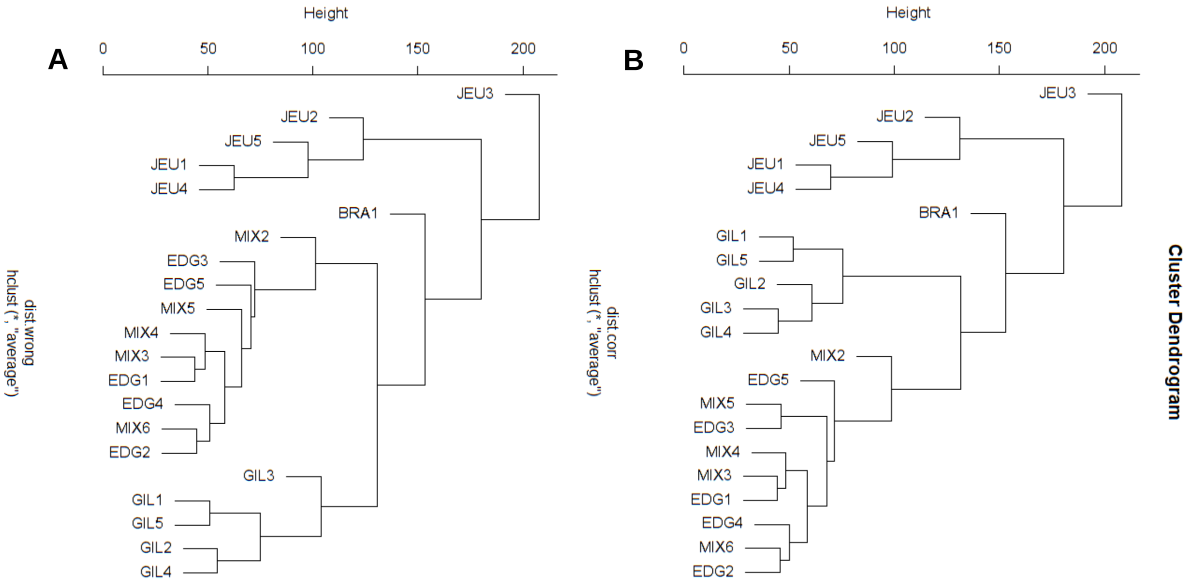


*Figure 21: The clustering analyses with frequency data for the inventory plots showed minor differences when using verified data (**B**) as compared to the original data (**A**).*

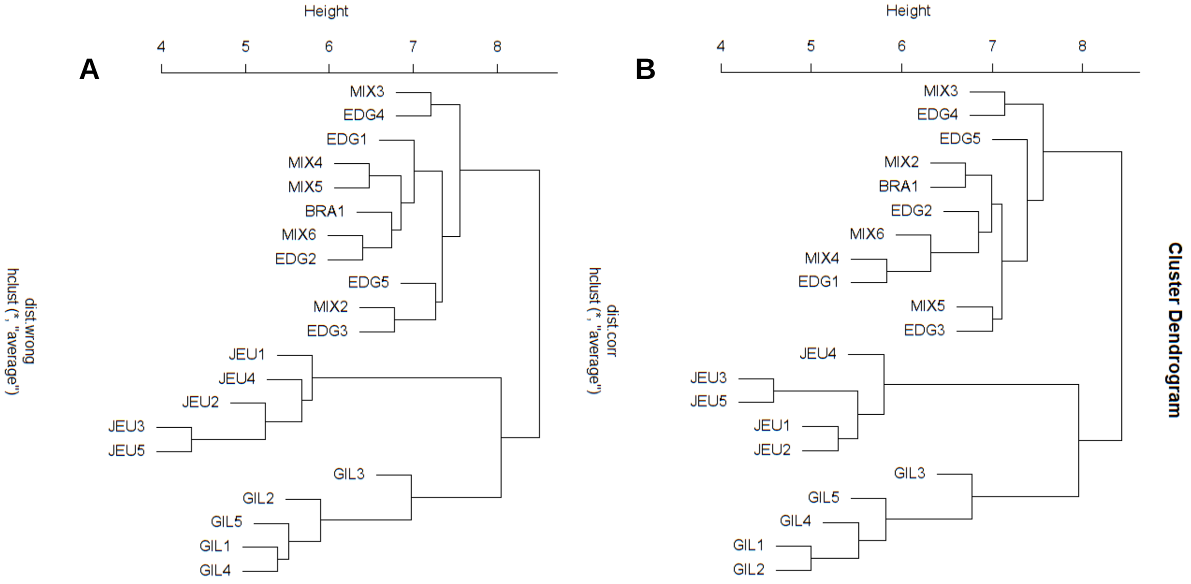


*Figure 22: The clustering analyses with presence/absence data for the inventory plots showed minor differences when using verified data (**B**) as compared to the original data (**A**).*

The calculated **phylogenetic signal** (lambda, ranging between 0 and 1) for different traits in the original and the verified dataset showed that **misidentifications have a clear impact** (Figure 23). When using a phylogeny with correctly identified species, the phylogenetic signal is generally higher (as expected) and error intervals are smaller. For dioecy, the phylogenetic signal was significantly higher when a phylogeny was used based on correctly identified species.

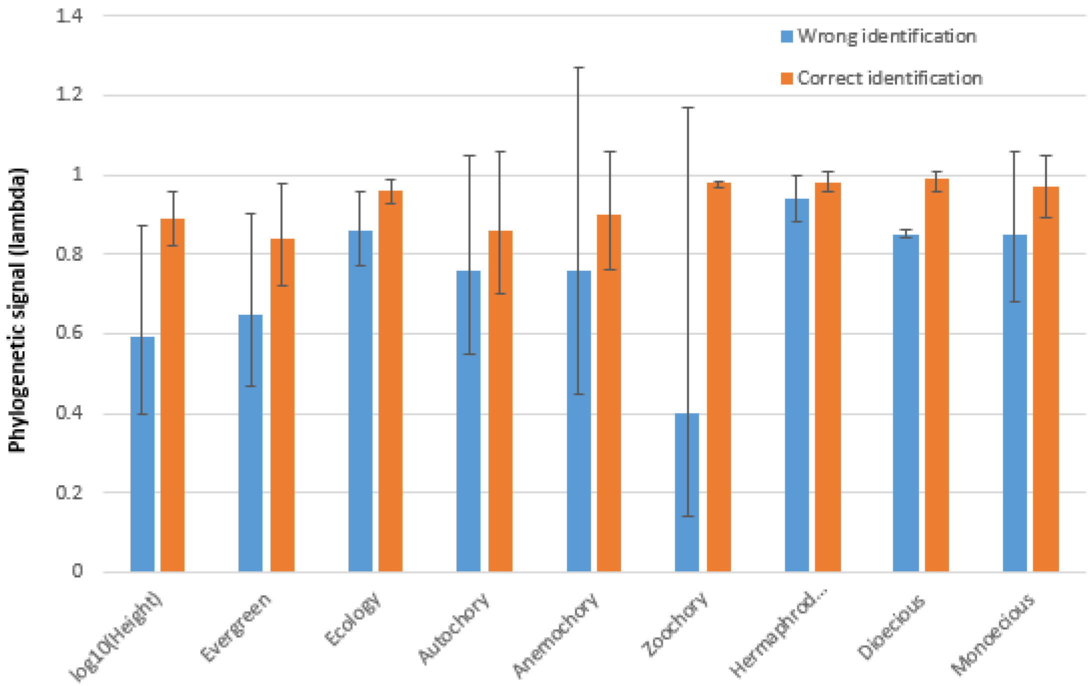


Figure 23: The bar plots show a clear effect of correct identification of species when creating a phylogeny and calculating the phylogenetic signal of traits.

In addition, **misidentifications** can **affect evolutionary model selection**, since it can alter the relevance of different evolutionary models to explain phylogenetic tree height evolution. The evolutionary model tests inferred a different optimal model for each dataset (Table XI). The lambda model performed best (lower AIC) for wrongly identified species, while the Ornstein Uhlenbeck model with stabilizing selection in function of deciduousness (OUM evergreen) performed best when a phylogeny with correctly identified species was used.

*Table XI: Species misidentifications have an impact on evolutionary model selection.**

Without verification

model log height		lnL	AIC	AICc	ntax
BM1		-128.485	260.969	261.0321	193
OU1		3.670043	-1.34009	-1.2131	193
Lambda		19.09115	**-32.1823**	**-32.0553**	193
nonphylo		2.919389	-1.83878	-1.77562	193
OUM	anemochory	8.005881	-8.01176	-7.799	193
OUM	evergreen	14.38369	-18.7674	-18.4465	193

After verification

model log height		lnL	AIC	AICc	ntax
BM1		-22.931	49.86196	49.9235	198
OU1		34.32788	-62.6558	-62.532	198
Lambda		34.25648	-62.513	-62.3893	198
nonphylo		-8.47702	20.95404	21.01557	198
OUM	anemochory	34.76353	-61.5271	-61.3198	198
OUM	evergreen	41.71571	**-73.4314**	**-73.1189**	198

**BM1* Brownian motion of trait evolution, *OU1* Ornstein Uhlenbeck model of trait evolution with stabilizing selection of tree height towards one optimum, *Lambda* model of trait evolution with branch lengths optimized in function of a maximum likelihood estimated lambde value, *nonphylo* with evolution according to one explosive radiation event, *OUM* Ornstein Uhlenbeck model with stabilizing selection in function of optima according to dispersal mode, or deciduousness

In summary, **species misidentifications** appeared to have **little impact on a general assessment of the diversity** of a forest stand. It should be noted that a classical floristic assessment aiming at a vegetation typology absolutely depend on correct distinction between the species. However, **phylogenetic studies and trait analyses** based on datasets **with wrongly identified specimens can lead to wrong conclusions**.

4.2 Measurements of plant and wood traits

4.2.1 Leaf, seed and whole tree traits

(1) The data obtained from COFORTRAITS is currently being gathered and analysed. These results will be discerned in a later stage.

(2) Work by Chantrain (2018): Species architecture was examined for 30 abundant species in the Luki Biosphere Reserve, both from the understory and canopy. Species-specific allometries were fitted using the power law model following Poorter *et al.* (2009). All allometric models reached the same overall goodness of fit, the average species-specific r^2 values obtained are 0.55, 0.44 and 0.56 for the height-diameter, crownradius-height and crown depth – height models respectively. The maximum height varies between 14.65m (*Tapura lujae*) and 66.15m (*Terminalia superba*) and the height at 10cm DBH ranges from 9.8 m (*Garcinia epunctata*) to 18.7m (*Pterocarpus tinctorius*) among the 30 species. The different models showed a significant difference between the species. In terms of crown shape, significant differences were found between the slope coefficients of the species-specific models. At small size (10cm in DBH), the smallest calculated crown radius was found for *Celtis mildbraedii* (1.90m), and the widest for *Monodora angolensis* (6.40m). Regarding the calculated crown depth (total height – height of the first branch), the vertical length of the crown goes from 2.10m for *Zanthoxylum gilletii* to 9.60m for *Strombosia glaucescens*. At maximal size, crownradius ranged from 4.83m for *Pseudospondias longifolia* to 9.60m for *Strombosia glaucescens*. Crown depth varied between 11.15 m for *Tapura lujae* to 31.60m for *Plagiostyles africanum.* Figure 24, shows the allometric relationships. These models were used to derive species-specific architectural traits at both minimal and maximal size.

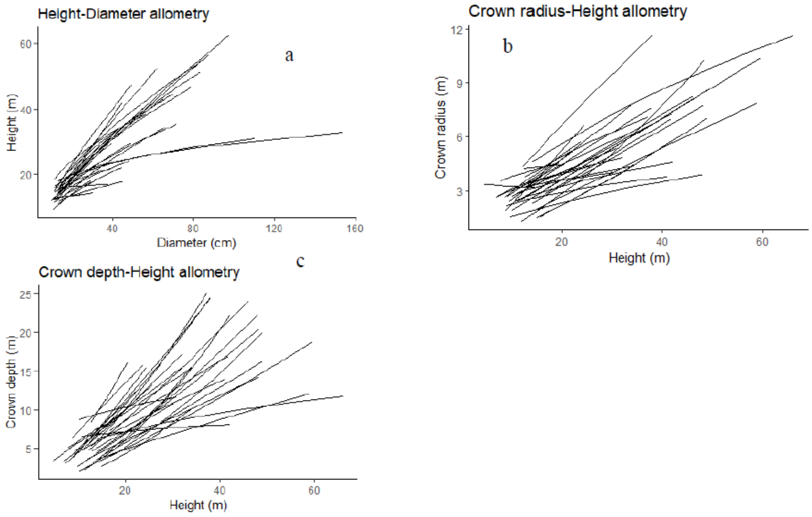


Figure 24 (a,b,c): Species-specific allometric relationships for 30 coexisting tree species. Power law models were fitted to depict: (a) height and diameter allometry, (b) crown radius and height allometry, and (c) crown depth and height allometry. Each species is represented by a different curve.

Figure 25 shows the significant Pearsons r-values for the correlation between the allometry derived traits that were studied. The firsts interesting observations are that Hmax is positively correlated to both the maximal crown radius (r=0.38, p<0.001) and the maximal crown depth (r=0.44, p = 0.0114) but negatively correlated to crown radius and crown depth at 10 cm of diameter (both r = -0.5, p crown radius<0.001 and p crown depth <0.003). Then Hmax is positively and strongly correlated to the slope coefficient of the crown radius – diameter model (r=0.52, p <0.0001) which means that when a species has a higher adult stature, the crown radius has a bigger relative increment in crown radius than in diameter. Hmax is also positively correlated to the juvenile light requirement with r = 0.48 and a p value of 0.0151. Wood density is negatively correlated to the maximum diameter (r = -0.46, p value < 0.0001) and with the maximal crown radius (r = -0.4, pval < 0.0001) but it is positively correlated to crown depth at a diameter of 10cm (r = 0.38, pvalue <0.01). Crown radius at maximal size is positively correlated with the height at 10 cm Dbh (r = 0.42, pvalue < 0.03), with crown depth at maximal size (r = 0.40, p-value < 0.05), and strongly positively correlated to Dmax (r = 0.85, p-value <0.001). But it is negatively correlated to wood density (r = -0.4, p-value < 0.002). Dmax is positively correlated to both h10 and hmax (r = 0.48 and 0.59, p-value < 0.02 and 0.003 respectively).

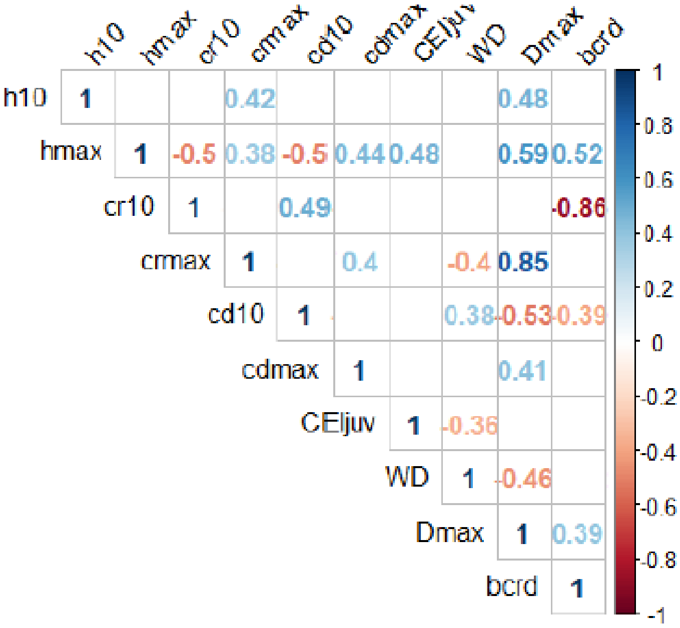


Figure 25: Bivariate relationships between architectural, functional traits and one scaling coefficient of the crown radius-diameter model of 30 coexisting tree species. Architectural traits were extracted from species specific allometric models at both small (crown radius, crown depth and height at 10 cm reference diameter: Cr10, Cd10, h10) and maximum sizes (Crmax (m), Cdmax (m), Hmax (m), Dmax (cm)). CEIjub is the juvenile light requirement. Functional traits were extracted from literature. Pearson's correlation coefficients are written only if the correlation was significant ($p < 0.05$) among architectural traits and between architectural traits, functional traits (species average wood density, WD (g cm-3, CEIjuv) and the scaling coefficient (bcrd).

An ordination was used to study the multivariate association between functional and architectural traits (Figure 26a). Axis were defined using only architectural and functional traits, then the allometry models scaling coefficients, the species-specific stem density and AGB were plotted on the graph as supplementary variables in order to see if the strategies were actually represented in the forest and had an impact on density and AGB. The first axis explains 40.8% of the total variability of the dataset and is stretched mainly by all architectural traits at maximum size. CEI juv, which lies just under "cdmax"is also found to explain the variation in axis one, confirming the negative correlation with wood density. These trends confirm the results coming from Figure 25. The second axis explains 15.8% of the total variation, and is mainly stretched by crown radius at minimal size. On its opposite side, the scaling coefficient of the height-diameter allometric model are exposed (bH and bHfut). This means that when a tree has a higher crown radius at its minimal size, it will show a low slope in its height-diameter curve. Thus, increment in diameter at high diameters is related to small increase in tree height, which is confirmed by the negative Pearson's correlation coefficient found in Figure 25. Most scaling coefficient and 22 species-specific AGB follow the trends of the architectural traits at maximal sizes, but they are opposed to wood density. Stem density seems quite unrelated to architectural traits at maximal sizes and wood density. Figure 26b shows how the species spread over these two axis based on their architectural characteristics. Large statured canopy tree species with eventually large crowns and great heights are found on the bottom right side of the panel, they are opposed to understory species with greater crown depth and crown radius at a 10 cm Dbh size and a higher wood density (negative end of axis 1).

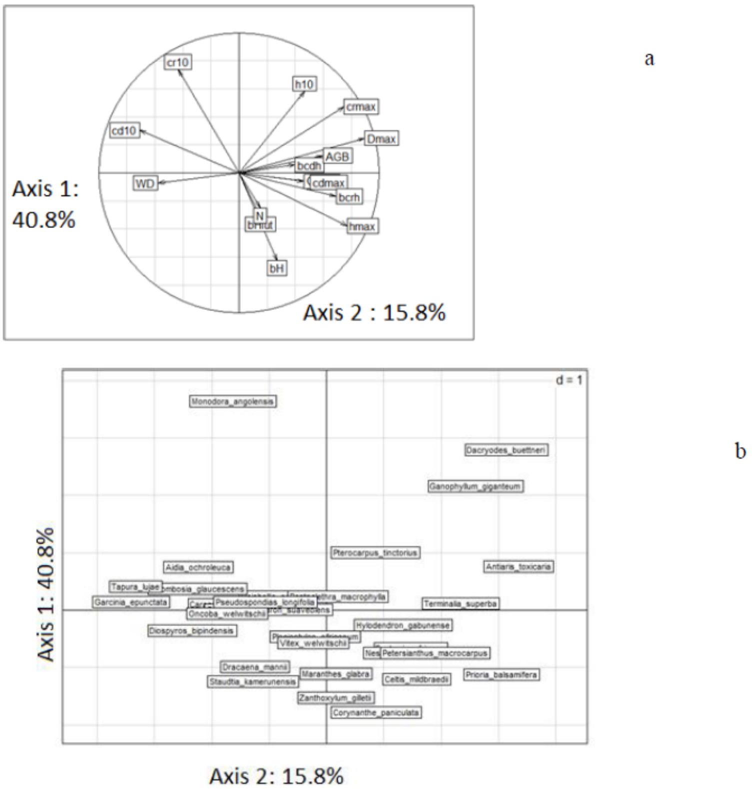


Figure 26 (a-b): Ordination of architectural traits at small size (h10, cr10, cd10 in m) and maximum size (hmax, crmax, cdmax in m and Dmx in cm), of functional traits (CEIjuv, WD in g cm-3). Species abundance (N in number of trees ha-1), AGB (in Mg of dry mass ha-1) and all scaling coefficient from allometry models (bH, bHfut both from H-D allometry, bcrh from crown radius- height, bcdh from crown depth-height allometry) were added as supplementary variables in the analysis. Axis 1 explains 41.8% of the total variability. Axis 2 accounts for 15.8% of the total variability.

In this study, strong variations of tree architecture among coexisting tree species were confirmed. The same trend was reported in the tropical forests of Liberia according to Poorter *et al*. (2003), Cameroon (Fayolle *et al*., 2016), Bolivia (Poorter *et al*., 2006), Malaysia (King, 1996; Sterck *et al*., 2001) and most recently with the tropical forests of Republic of the Congo (Loubota Pansou *et al*., 2018b). The height-diameter curves (Figure 23 a) allow us to see and distinguish tree species from the understory and tree species that will reach the canopy, as some curves level off at low height and others keep increasing. Thomas (1996) explains that the understory species stop their vertical growth to engage their reproduction. They reproduce much earlier in their ontogenetic trajectories, but are thus expelled of the race to the canopy as reproduction has a cost and requires a slowdown in height growth rate. The relations between height and crown size (crown radius – height and crown depth-height) allow us to see that multiple species have different strategies in regard of their crown development.

In contrast with Poorter *et al*. (2006), a stronger correlation was found between architectural traits and Hmax (the adult stature) of the trees while no correlations were found between architectural traits and CEIjuv which characterizes the light requirements at early stages. This is probably explained by that, the vertical light gradient is large and predictable while the horizontal light gradient is relatively small and unpredictable (Poorter *et al*., 2006). In this study we observed that Hmax is negatively correlated to both crown radius and crown depth at small sizes, but positively correlated to crown radius and crown depth at maximal sizes. This means that large statured species tend to have a smaller and shallower crown in their early ontogenetic stages, but that, once they reach the canopy, they begin to develop larger and deeper crowns. This can be explained by the need of large statured trees to attain large sizes before they commit to their reproduction (Poorter *et al*., 2003). They produce slender stems and their wood tends to be less dense to favorize their vertical growth, defeat the competition and reach the canopy as fast as possible to start reproducing. Small statured species have a lower light requirement during their early stages (positive correlation between Hmax and CEIjuv), because they develop larger and deeper crowns as early as possible in their ontogeny to maximize light capture (King, 1996; Poorter *et al*., 2003; Sterck *et al*., 2001). Unfortunately, these results do not allow us to confirm one hypothesis regarding the vertical light gradient partitioning among species. In fact, there seems to be a clear distinction between understory species and canopy species strategies as indicated by King (1996), but it could still be explained by a tradeoff between the will to reach the canopy as fast as possible and the will to reproduce as quickly as possible.

Dmax is positively correlated to all architectural traits at maximal sizes, with H10, and especially with crown radius max. The scaling coefficient corresponding to the slope of the crown radius – diameter allometry (bcrd) is strongly negatively correlated to Cr 10 wich means that the smaller the crown radius at a 10 cm diameter is, the bigger the relative increment in crown radius compared to the increase in diameter will be. Wood density is negatively correlated to crown radius at maximal size. It is also negatively correlated to Dmax and positively correlated to crown depth at small sizes. This may support that understory species tend to have a higher wood density (Poorter *et al*., 1999). *Carapa procera*, *Strombosia glaucescens* and *Maranthes glabra* were common to both our study and the study in Liberia (Poorter *et al*., 2003) and showed different trends between the two sites.

Regarding the different ecological strategies, the light requirement (regeneration guild according to Hawthorne, 1995) was related to the architectural traits and the continuum theoretical pattern was confirmed, according to Swaine and Whitmore (1988) and Chave *et al*. (2005). The left end of axis 1 (Figure 27) gathers small statured species with larger and deeper crowns at small sizes, which also have a denser wood. While on the opposite side, large statured species with wide and deep crowns at maximal sizes are found. The pioneer species were expected to differ from the two other light requirement groups but only three species were identified as pioneers and it could explain why they overlap on both shade bearers and non-pioneer light demanding species.

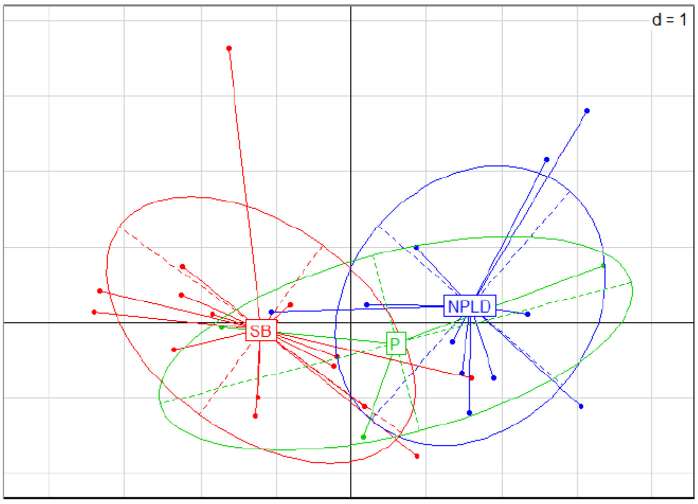


Figure 27: Ordination of architectural traits at small size (h10, cr10, cd10 in m) and maximum size (hmax, crmax, cdmax in m and Dmx in cm), of functional traits (CEljuv, WD in g cm^{-3}). Showing the species repartition depending on their light requirements.

(3) Work by Bauters *et al. (in preparation)*: **The structural variance associated with the sampling level in the crown was higher than the inter-individual variance for leaf N, P and N:P, while lower for all other measured variables** (two species: *Gilbertiodendron dewevrei* (De Wild.) J.Leonard and *Mammea africana* Sabine) (Figure 28). For leaf N and P, respectively 30% and 35% of the intraspecific variation was associated with the crown sampling level, and resulting in 29% for the leaf N:P stoichiometry. The isotope signatures were much less sensitive to crown sampling level with respectively 17% and 10% of the variation for $\delta^{13}C$ and $\delta^{18}O$. Especially stomatal density (SD), and leaf magnesium content exhibited high inter-individual variability, of respectively 45% and 23%, with crown level variance being 25% and 13%. The additional model fits including crown level sampling as a fixed effect revealed that sampling sun leaves positively affects $\delta^{13}C$ and leaf N, P and Mg content, and stomatal density, while it negatively affected leaf C:N, N:P and $\delta^{18}O$ signatures. The most important relative effects were for leaf P (46% higher in upper canopy vs. middle canopy), leaf N (12%) and leaf mass N:P (-17%), with all other variables exhibiting effects < 10% (Figure 28).

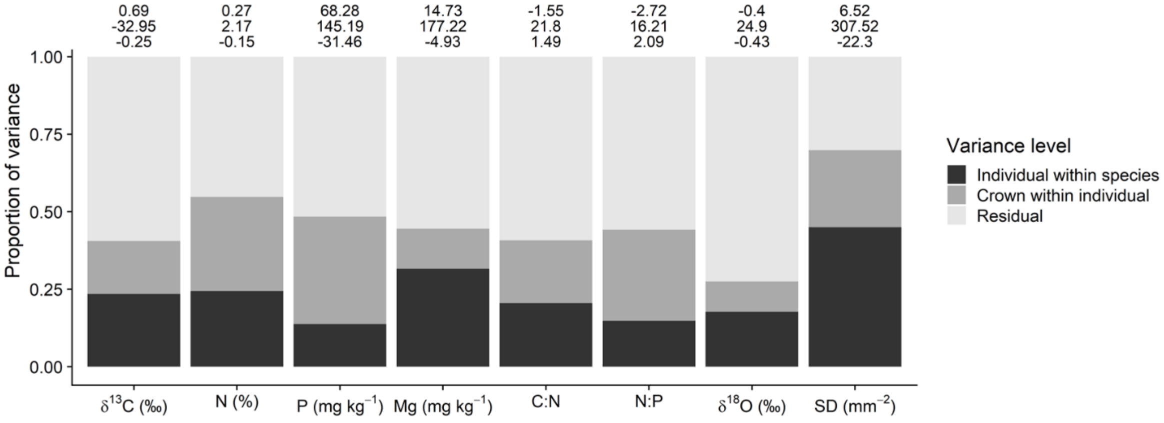


Figure 28: Intra-specific and intra-crown variability of the measured stable isotope signatures, nutrient concentrations in the leaf and stomatal density (SD) of the leaves based on two present-day sample sets from respectively Yangambi and Luki, in the Democratic Republic of the Congo. The bars indicate

the relative variance associated with each of the levels, the numbers above the plot give the specific effect of sampling in the upper or low canopy level (respectively highest and lowest line) versus the average canopy values in the center of the canopy (middle line).

(4) Work by Tarelkin *et al*. (2019): **Evergreen species tend to have significantly higher anatomical variability where rainfall seasonality is more pronounced. Deciduous species did not show significant differences in their anatomical variability.** The analyses of density profiles and phenology records suggest that the seasonality of precipitation influences both leaf phenology and cambial activity. The high intra-site variability in phenology and anatomy suggests that site-related micro-climate conditions also influence cambial activity.

(5) Work by Illondea *et al*. (2019): **Reproductive phenology for the majority of the trees and the species is largely seasonal, annual and regular (81.6%, 71 species)**. The peaks for flowering are more abrupt than the fruiting peaks and more spread out over time, although the timing of flowering and fruiting is significantly aggregated. Most of the trees and species bloom from December to February, during the short dry season, but flowers and fruits can be observed throughout the year within the community. Only 13 species showed a significant relationship between diameter and reproduction, including seven canopy species, five understory species and one light-demanding species. For these 13 species, the average minimum reproduction diameter was 17.3 cm.

Work by Illondea *et al*. (in preparation): **The cambium of *Prioria balsamifera* is dormant during the dry season and growth resumes at the beginning of the rainy season.** Defoliation and growth of *Prioria balsamifera* in the study region showed to be closely related to the annual precipitation. The defoliation peaks are observed annually at the end of the dry season and during the rainy season which starts in October in the Mayombe forest. However, defoliation is more pronounced during the dry season, which is usually characterized by reduced precipitation and temperature. The defoliation occurs only once a year in a very short period with strong intra-tree synchrony for the 13 trees monitored also for time series. **This strong intra-tree synchrony has highlighted defoliation peaks that correspond to the annual synchrony that is observed at the population level. However, inter-tree asynchrony of defoliation is observed as well. Although significantly aggregated around peak dates, tree defoliation peaks do not necessarily occur at the same date during the ten years of observations.** A synchronous pattern of defoliation is observed every year and yet asynchronicity is detected between years. The synchronous pattern is presumably among individual trees but it is not clear what the signal of asynchronicity means.

Leaf, seed and whole tree traits - added value of science in support of policy and development

Plant functional types are species grouped according to their traits or to similar response to the environment (Smith *et al*., 1993). The described species traits above are vital towards dynamic models of plant functional types since a small group of species with similar traits are at the core of most of these models. The lack of such information for species and regions, like tropical Africa, hampers model predictions. This also hampers monitoring of biomass and carbon stocks, since general allometric models are used to estimate biomass at the tree level and tree measurements are needed for this (diameter, height, and if possible wood density).

Furthermore, trait information for species is vital towards optimizing management practices. Growth, mortality rates and, for example, minimum cutting diameter determine forest administration and management practices.

4.2.2 Genetic analysis

4.2.2.1 Newly generated DNA barcoding sequences provide a valuable tool for species identification (Janssens *et al.*, 2020; Vanden Abeele, 2019)

In the frame of HERBAXYLAREDD, a **total of 906 DNA barcoding sequences** (*matK*, *rbcL* and *trnH-psbA*) were generated at the molecular lab of Meise Botanic Garden. For the *matK* gene, we obtained 366 sequences, while 454 sequences were obtained for *rbcL*. These sequences represent 468 different species for which barcoding sequences were previously lacking. All newly generated sequences were submitted to GenBank and are publicly available (accession numbers: *matK* MN370078 – MN370444, *rbcL* MN366472 – MN366928; for the species list, check 'Suppl. material 1' in Janssens *et al*. 2020). Since all these DNA barcoding sequences are accompanied by a curated herbarium voucher, they provide a **valuable tool for rapid and accurate species identifications**.

Additionally, 86 *trnH-psbA* barcode sequences were generated for the tropical African timber species *Staudtia kamerunensis*. These chloroplast sequences were then used to construct a median-joining haplotype network (Figure 31) to assess whether the two described varieties (var. *gabonensis* and var. *kamerunensis*) can be distinguished genetically. The haplotype network showed that the putative varieties mostly harbour distinct haplotypes. Hence the *trnH-psbA* barcode can be used to identify the intraspecific varieties within *S. kamerunensis*. The genetic break between both groups of individuals (the putative varieties) was confirmed by the clustering analysis (done with *structure* software) based on microsatellite markers (Figure 29) and by the haploweb (made with HaplowebMaker; https://eeg-ebe.github.io/HaplowebMaker/) constructed for the nuclear marker *Agt1* (Figure 30). However, additional field observations are needed to confirm whether both genetic groups truly correspond to the taxonomic varieties that have been described based on morphology. Nevertheless, given the relatively **strong genetic differentiation between both groups**, they **probably correspond to distinct species** that are unable to reproduce with each other. Since one group has a restricted distribution (mostly southwestern Cameroon), additional conservation efforts might be needed and the conservation status of *Staudtia kamerunensis* (including its varieties) should be reassessed. As species are the basic units of resource inventories and conservation management plans, it is advisable that the taxonomic status of *S. kamerunensis* var. *gabonensis* and *S. kamerunensis* var. *kamerunensis* is changed, and that they are considered distinct species.

More information on the methodology (e.g. clustering methods, haploweb construction) can be found in the PhD thesis of Samuel Vanden Abeele (Vanden Abeele, 2019).

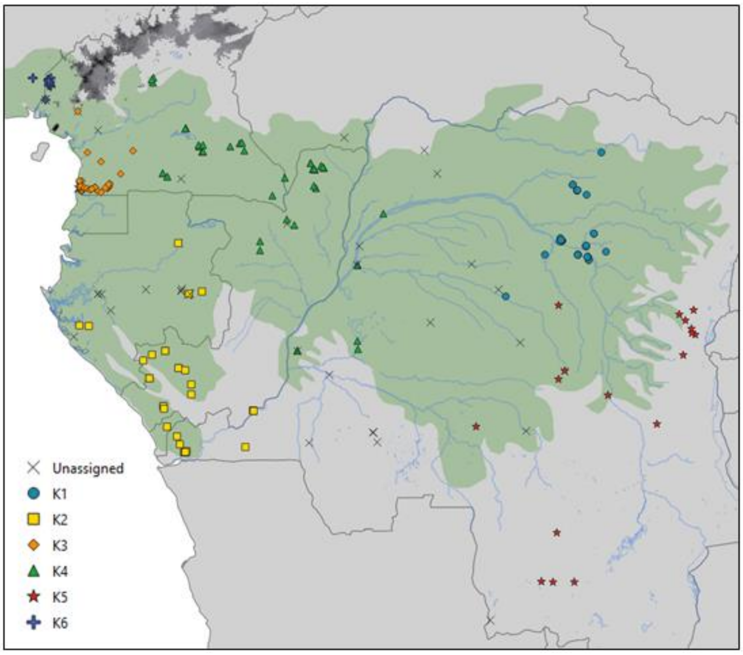


Figure 29: Distribution of genetic clusters in Staudtia kamerunensis as inferred with structure for the most likely scenario at K = 6. The green area depicts the natural distribution of rainforests in Central Africa. CVL Cameroon Volcanic Line.

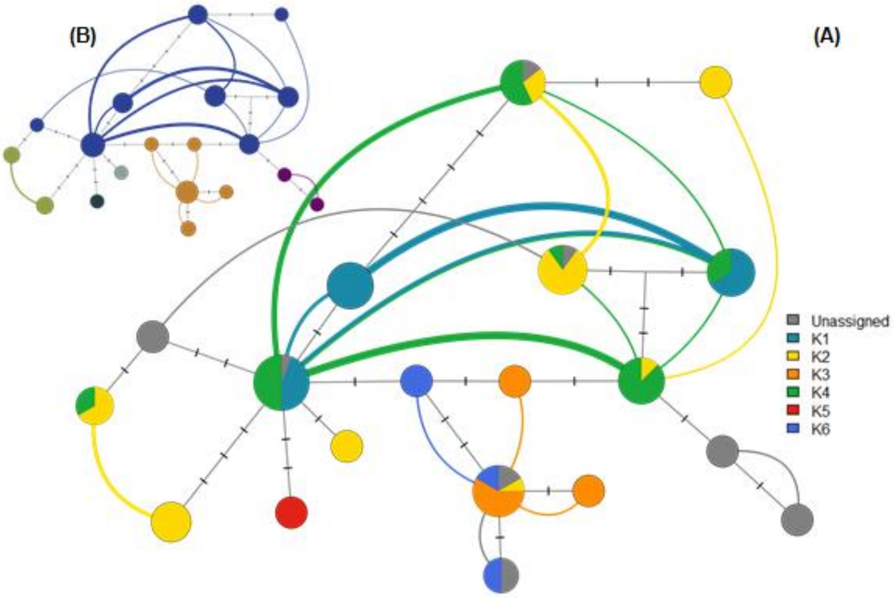


Figure 30: Haploweb for the nuclear marker Agt1 in Staudtia kamerunensis. Each circle corresponds to a haplotype and has a size proportional to the number of individuals. Curved lines connect haplotypes co-occurring in heterozygous individuals, with the thickness proportional to the frequency of the co-occurrence. Each crossing line corresponds to one mutated position in the alignment. (A) Colours correspond to the six genetic clusters inferred with structure. Individuals that appeared admixed in the SSR analysis (highest assignment probability q < 0.8) are coloured grey. (B) Colours correspond to the inferred single-locus fields for recombination (FFRs).

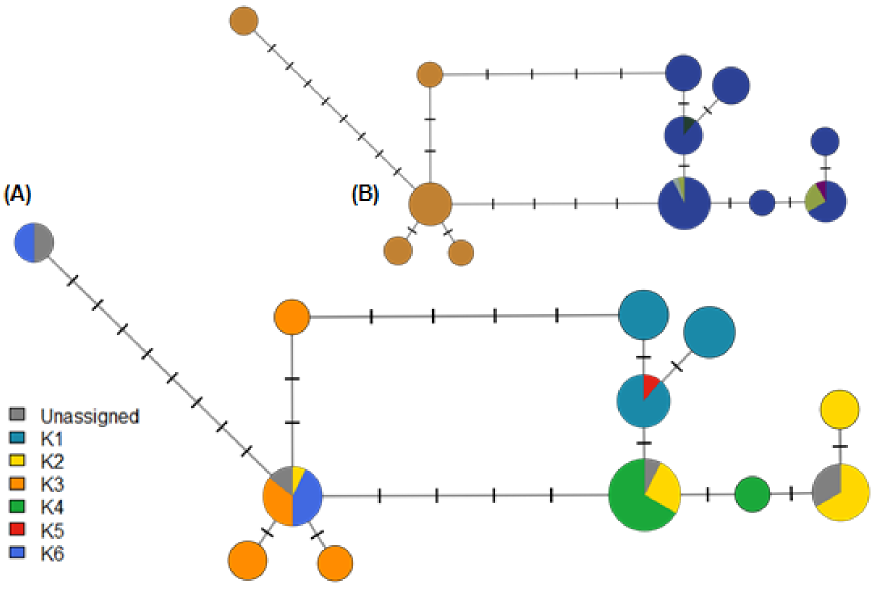


Figure 31: Haplotype network for the chloroplast marker psbA-trnH in Staudtia kamerunensis. Each circle corresponds to a haplotype and has a size proportional to the number of individuals. (A) Colours correspond to the six genetic clusters inferred with structure. Individuals that appeared admixed in the SSR analysis (highest assignment probability q smaller than 0.8) are coloured grey. (B) Colours correspond to the FFRs inferred from the Agt1 haploweb.

4.2.2.2 The largest dated angiosperm phylogeny yet (Janssens *et al.*, 2020)

The 820 newly generated *matK* (incl. *trnK)* and *rbcL* barcoding sequences (see previous section on DNA barcoding) were combined with angiosperm sequence data (same markers) extracted from GenBank. The resulting dataset was used to construct a large-scale dated angiosperm phylogeny, using 56 fossils as calibration points. Five gymnosperm genera were chosen as outgroup. The final aligned data matrix consisted of 36,101 angiosperm species. *matK* (incl. *trnK*) sequences were mined for 31,391 species (87%), whereas *rbcL* sequences were obtained for 26,811 (74%) species. The sequence dataset has an aligned length of 4,968 base pairs (bp) of which 4,285 (86%) belong to *matK* (incl. *trnK*) and 683 (14%) to *rbcL*. Within *rbcL,* all characters were variable (100%), whereas for *matK* (incl. *trnK*) 3,921 characters (91.5%) were variable. Based on the different studies that estimated the total number of flowering plants currently described (between 260,000 and 450,000 species) (Crane et al. 1995, Christenhusz and Byng 2016, Cronquist 1981, Lupia *et al*. 1999, Pimm and Joppa 2015, Prance *et al*. 2000, Thorne 2002), our phylogeny represented between 14% and 8% of the known flowering plants, respectively. In addition, the phylogenetic tree contained 54.6% (8,399) of all currently accepted angiosperm genera and 94.5% (426) of all families of flowering plants were included, as well as all currently known angiosperm orders. As such, the **current angiosperm tree** can be regarded as the **largest dated angiosperm phylogenetic framework that is generated by combining genuine sequence data and fossil calibration points, and will be useful for large-scale ecological and biogeographical studies**. The phylogenetic tree can also help to identify alternative wood species for trade purposes (complementary with WP5), by linking wood anatomical traits with genetic traits.

The phylogenetic tree, the species list and all other supporting information are publicly available from the website of the Biodiversity Data Journal (https://bdj.pensoft.net/article/39677/).

4.2.2.3 Chloroplast genome sequencing supports monophyly of the sections within *Entandrophragma*, but divergent topologies in *Khaya* (Monthe *et al*., 2019)

Shotgun sequencing followed by genome skimming to reconstruct the whole chloroplast genome resulted in reference plastomes with a length of 163,180 bp for *Entandrophragma* and 163,739 bp for *Khaya*. Both genomes are publicly available on GenBank (accession numbers MK058683 and MK058684). Read mapping to the reference plastomes resulted in the identification of 1480 SNPs and 380 indels for *Entandrophragma*, and 1086 SNPs and 177 indels for *Khaya*. The final chloroplast alignment for both genera combined was 169,732 bp long.

The **inferred phylogeny supported clear species delineation within the genus *Entandrophragma***, since the species represented by multiple samples appeared monophyletic. One exception was observed between the sister species *E. candollei* and *E. palustre*, which could be the result of a chloroplast capture in the past. The five phylogenetic clades that were identified within *Entandrophragma* correspond to the sections previously described based on morphological features, emphasizing the reliability of these features in species delimitation of *Entandrophragma*. However, *Entandrophragma congoense* and *E. angolense* were often put in synonymy, while the phylogenetic analysis showed that they are reciprocally monophyletic. The differentiation of both species was also confirmed by morphological and microsatellite markers (Monthe *et al*., 2018).

By contrast, **chloroplast DNA appeared unreliable to accurately identify *Khaya* species**, as tree topologies were not congruent between plastid and ribosomal genomes (Monthe *et al*. 2019) and plastid haplotypes can be shared between species (Bouka, 2017). To test whether this results from plastome captures and/or incomplete lineage sorting, more individuals per species and additional nuclear markers are needed.

For *Entandrophragma*, the sequenced chloroplast genomes of specimens from the same species but with different geographical origins **can be used in future provenancing studies** to combat illegal logging and trade of the valuable timber species within the genus.

4.2.2.4 Newly developed genetic markers show promise for estimating genetic diversity and timber tracking

The microsatellite primer development for *Staudtia kamerunensis* (Myristicaceae) (Vanden Abeele *et al*., 2018) **resulted in 16 polymorphic microsat loci, of which 14 loci were combined in four multiplex reactions and validated in 183 individuals of the species.** Cross-species amplification was successful in *Pycnanthus angolensis*, a multi-purpose tropical tree which is identified by the World Agroforestry Centre (ICRAF) as an important agroforestry species for a domestication programme. The **high levels of polymorphism** observed in the newly developed microsatellite markers demonstrate their usefulness to study gene flow, population structure and spatial distribution of genetic diversity in *S. kamerunensis, P. angolensis* and other taxa within the Myristicaceae family.

For ***Prioria balsamifera* and *Prioria oxyphylla***, sets of microsatellite primers were developed separately for each species, since the potential of cross-species transferability appeared to be very limited (Vanden Abeele *et al*., 2019). The final primer sets comprised **16 and 15 polymorphic markers per respective species**, and each set was combined into three multiplexed reactions. The cross-species amplification tests showed that multiple loci from both microsatellite sets are conserved across species, as various loci were successfully amplified in the different African *Prioria* species. Therefore, the primers developed in this study could be of **great value for genetic studies focused on other *Prioria* species as well**. Since *P. oxyphylla* and *P. buchholzii* are closely related sister-taxa (de la Estrella *et al*., 2018), cross-species amplification success was especially high. The low amplification success in *P. joveri* (both primer sets) and *P. balsamifera* (*P. oxyphylla* primer set) could result from higher species divergence.

In conclusion, the newly developed microsatellite markers proved to be useful for estimating genetic diversity at population level, and can thus be used to study gene flow and spatial genetic structure in *Prioria* species, which is needed to set up proper conservation guidelines and to prevent genetic erosion.

Our newly developed microsatellite markers were then used to estimate the level of genetic diversity within populations of *Staudtia kamerunensis* and *Prioria balsamifera* (Vanden Abeele, 2019).

The indices calculated for ***S. kamerunensis*** showed that the overall **genetic diversity of the species is relatively high**. Hence, this lesser used timber species could potentially be harvested as a substitute to highly pressured and/or endangered timber species. However, the genetic diversity is lower for the populations in southern DRC (miombo woodlands), possibly because they are little connected to northern populations and because population densities are expected to be low in the drier woodlands, where *S. kamerunensis* is mostly limited to gallery forests. This should be taken into account when management plans are drawn up.

By contract, allele fixation rates were high for the sixteen investigated microsatellite loci and the **genetic diversity in all populations of *Prioria balsamifera* appeared to be low**, even in the Mayombe region where the species occurs in high densities. Although the levels of inbreeding are low, the species could be prone to genetic erosion. Therefore, conservation efforts are necessary and sustainable management strategies should consider the preservation of the existing genetic diversity of exploited stands in logging concessions.
Intraspecific genetic diversity plays a crucial role in a species' resilience against changes in climate and other environmental variables. Even if a species does not go extinct, losing some of its differentiated populations can result in a net loss of genetic diversity and potentially interesting variants. Hence, conservation efforts should also consider the maintenance of the genetic diversity of a species.

The Bayesian **clustering analyses** based on microsatellite markers showed multiple intraspecific genetic discontinuities for both *S. kamerunensis* and *P. balsamifera* (Vanden Abeele, 2019). For *S. kamerunensis*, six differentiated genetic clusters were inferred (Figure 29 in proceding section 4.2.2.1). For *P. balsamifera*, four differentiated genetic clusters were inferred (Figure 32). However, additional intraspecific clusters might have remained undetected in the clustering analysis, especially in the underrepresented parts of the species' distribution range. In Gabon for instance, the high number of unassigned individuals in combination with the occurrence of some individuals assigned to cluster 'Southeast', leads us to believe that additional genetic clusters exist in Gabon and the surrounding countries.

Those differentiated populations occurring within ***S. kamerunensis* and *P. balsamifera*** are characterized by different levels of genetic diversity and distinctiveness. For example, those surrounding the CVL harbour many rare and unique genetic variants, which often concur with the oldest genetic lineages within a species. Moreover, **multiple patterns of differentiation are linked to ecological gradients**, thus distinct genetic clusters are most likely adapted to different soil types, amounts of rainfall, or other environmental variables. Hence, each gene pool contains valuable intraspecific diversity that is worth preserving in order to protect the species from environmental changes. Therefore, protected areas are needed in multiple countries and regions (coinciding with the differentiated gene pools). The distribution maps presented here can facilitate the designation of new protected areas. Additionally, it is advisable to use individuals from the local genetic cluster in reforestation and restoration projects, to ensure that the trees are adapted to the local habitat.

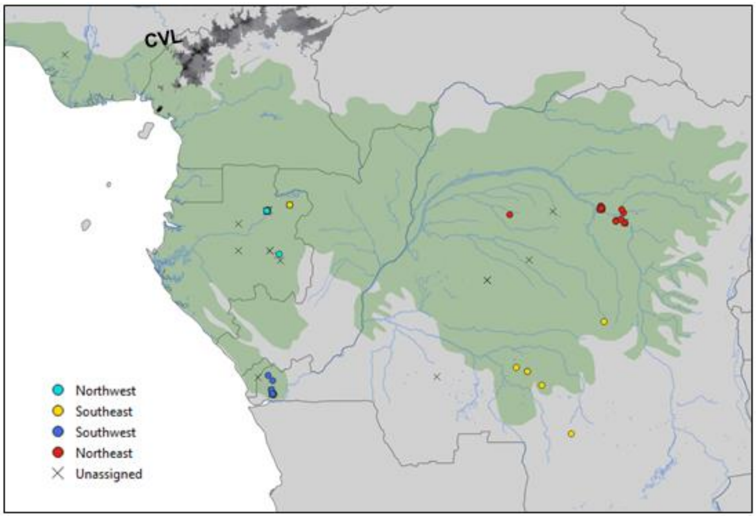


Figure 32: Distribution of the four genetic clusters (K = 4) inferred using the structure software, under the correlated allele frequencies model. CVL Cameroon Volcanic Line.

Since the differentiated clusters inferred for ***S. kamerunensis* and *P. balsamifera*** have a parapatric distribution and are usually restricted to smaller regions, the **origin can be determined at a regional scale for most individuals** from our sampling. This indicates that our microsat datasets can be used as a reference database for timber tracking of both species. However, our sampling currently doesn't cover the complete species' distribution range, so individuals from other regions should be added to create an applicable genetic reference database for origin determination.

Although Bayesian clustering approaches can be very useful for origin determination, some cases require different genetic tracking methods. For example, individuals with an unknown origin can show admixed genotypes, so that accurate cluster assignment is impossible. Additionally, inferred genetic clusters can be spread over multiple countries, thereby impeding the declaration of the country of origin (which is needed to comply with CITES, EU TR, FLEGT). In these cases, various statistical assignment methods can be used to determine the origin of an individual by comparing it to a genetic reference database.

In the frame of HERBAXYLAREDD, we tested three genetic assignment methods for the endangered timber species *P. balsamifera* (Vanden Abeele, 2019). The results showed that our **microsat dataset can be applied as a reference database for Gabon and DRC**, since the proportion of correctly assigned individuals was relatively high and similar to previously published genetic reference databases (Blanc-Jolivet and Degen, 2012; Chaves *et al*., 2018; Degen *et al*., 2013). The high accuracy of the nearest neighbour method for individuals from Gabon can be explained by the fact that this approach is less sensitive to overlapping sources of genetic differentiation and imperfect grouping of reference samples (Degen *et al*., 2017).

Genetic analysis - added value of science in support of policy and development

In the frame of HERBAXYLAREDD, a total of 906 DNA barcoding sequences (*matK*, *rbcL* and *trnH-psbA*) were generated at the molecular lab of Meise Botanic Garden. Since these DNA barcoding sequences are accompanied by a curated herbarium voucher, they provide a valuable tool for rapid and accurate **species identifications**. In addition, our barcoding analyses showed a clear genetic break between two groups of *Staudtia kamerunensis* individuals, which probably correspond to the taxonomic varieties that have been described in the species. However, additional field observations are needed to confirm these findings. Nevertheless, given the relatively strong genetic differentiation between both groups, they probably correspond to distinct species that are unable to reproduce with each other. Since one group has a restricted distribution (mostly southwestern Cameroon), additional conservation efforts might be needed and the conservation status of *Staudtia kamerunensis* (including its varieties) should be reassessed. As species are the basic units of resource inventories and conservation management plans, it is advisable that the taxonomic status of *S. kamerunensis* var. *gabonensis* and *S. kamerunensis* var. *kamerunensis* is changed, and that they are considered distinct species.

The genetic diversity indices calculated for *S. kamerunensis* showed that the overall genetic diversity of the species is relatively high. Hence, this lesser used timber species could potentially be harvested as a substitute to highly pressured and/or endangered timber species. However, the genetic diversity is lower for the populations in southern DRC (miombo woodlands), possibly because they are little connected to northern populations and because population densities are expected to be low in the drier woodlands, where *S. kamerunensis* is mostly limited to gallery forests. This should be taken into account when **management plans** are drawn up. By contract, allele fixation rates were high and the genetic diversity in all populations of *Prioria balsamifera* appeared to be low, even in the Mayombe region where the species occurs in high densities. Although the levels of inbreeding are low, the species could be prone to genetic erosion. Therefore, conservation efforts are necessary and sustainable management strategies should consider the preservation of the existing genetic diversity of exploited stands in logging concessions. The differentiated populations occurring within *S. kamerunensis* and *P. balsamifera* are characterized by different levels of genetic diversity and distinctiveness, and multiple patterns of differentiation are linked to ecological gradients. Hence, distinct genetic clusters are most likely adapted to different soil types, amounts of rainfall, or other environmental variables. Therefore, each gene pool contains valuable intraspecific diversity that is worth preserving in order to protect the species from environmental changes, and protected areas are needed in multiple countries and regions (coinciding with the differentiated gene pools). The distribution maps presented here can facilitate the designation of new protected areas.

Since the differentiated clusters inferred for *S. kamerunensis* and *P. balsamifera* have a parapatric distribution and are usually restricted to smaller regions, the origin can be determined at a regional scale for most individuals from our sampling. This indicates that our microsat datasets can be used as a reference database for **timber tracking** of both species. However, our sampling currently doesn't cover the complete species' distribution range, so individuals from other regions should be added to create an applicable genetic reference database for origin determination. In addition, the genetic assignment methods that we tested on the endangered timber species *P. balsamifera* (Vanden Abeele, 2019), showed that our microsat dataset can be applied as a reference database for Gabon and DRC, since the proportion of correctly assigned individuals was relatively high and similar to previously published genetic reference databases (Blanc-Jolivet and Degen, 2012; Chaves *et al*., 2018; Degen *et al*., 2013).

4.2.3 Wood biological traits

(1) From the opinion paper by Beeckman (2016), which serves as an introduction to "Wood biological traits":

> The largest part of forest biomass consists of wood. A global estimate of carbon stored in lignified tissues rises up to 400 Pg. Given these quantities, there is a growing interest of implementing wood research in diagnoses and evaluations of the carrying capacity of the global ecosystem and its forests. The question arises how disciplines like wood anatomy could reply to the increasing demands of a trait-based ecology, understood as a paradigmatic shift in addressing global changes. Dendrochronology and ecological wood anatomy, traditionally operating within the paradigm of species-based ecology, developed robust methods to address ecological questions. However, sampling strategies and database design will likely be different when wood traits are to be used to study individual tree performance, including responses to stress.
>
> Involving wood research in trait-based ecology should be based on a good understanding of the trait concept. The value of the IAWA standard lists of wood anatomical features as starting points for trait databases is to be recognized, but since traits per definition determine to some degree the tree performance, species mean values for wood anatomical features only have a limited significance in ecological research. They should be used only in studies where the environmental gradient is large enough. Smaller scale ecological analysis needs to make use of more sensitive features. Because of the key importance of the tree size in many wood anatomical features, trait values need to be calibrated with reliable measurements of tree size. Moreover, since trees are long lived organisms, it is important to evaluate there performance dynamics within the forest community through trait values ranked on a time axis and/or on a pith-to-bark or height axis.

The performance of trees changes along environmental gradients and through time. In trees and woody plants in general this variation is particularly high. High genetic and phenotypic variability of trees is a consequence of being long lived with high probabilities of mutations and high selection pressures (only a minor fraction of the produced seeds grow up to adult trees) (Petit and Hampe, 2006). Traits can be measured with binomial, categorical, ordinal or quantitative scales. Explaining variability among traits is a major issue. Intraspecific variation is interesting for short environmental gradients. Study of long gradients may use trait values that are typical for a species. A clear distinction should be made between variability within trees (between organs, and on a pith-bark or root-to-tree-top transect) and between individuals of the same species. Systematic information on the variability of most of the wood anatomical features is still lacking. The variability of measures (traits, properties or individual performance) within a population can be described quantitatively by a distribution function (Mohr and Schopfer, 1995). A normal distribution is theoretically obtained when many factors determine a measure independently, and allow a description with only two parameters: the mean and the standard deviation. Many of the wood traits are not distributed normally and cannot be described by mean and standard deviation alone. Characterizing asymmetrical distributions of traits is therefore difficult. Information on mode and median could be of help, but knowledge of the shape of the whole distribution functions is an absolute requirement for correct interpretation of trait measures (Mohr and Schopfer, 1995), possibly with the help of robust statistical analysis and modelling. (Kattge *et al*., 2011) characterize density distributions of trait data by skewness and kurtosis of raw and log-transformed data and they calculate the departure from normality. Conduit density, conduit area, vessel diameter, conduit lumen area per sapwood area and vessel element length show right tailed and acute peak distributions. Wood density is also right tailed, but shows a wider peak around the mean value. Wood density is the wood feature that is the closest to the normal distribution, with a mean value of approximately 600 mg mm-3 (Kattge *et al*., 2011). Choosing a relevant distribution for a response variable is the basis of modelling techniques such as generalised linear modelling and generalised additive modelling. Information on distribution shape of wood traits, upper limit of measures, whether there is overdispersion, zero truncation and mean-variance relationships is hard to find in the literature in an explicit form. Databases should systematically provide this type of statistics in order to optimally incorporate a wood anatomical approach in trait-based ecology.

A practical definition of stress effects on trees and wood formation is still lacking (Kranner *et al*., 2010). Nevertheless, wood traits can be seen as response variables to environmental impacts. Stress effects could ideally be described in terms of a numerical parameter like there are: tolerance, optimum, lower limit and upper limit of a probability distribution. It is therefore necessary to have information on typical response curves of responsive wood anatomical traits.

A consequence of the trait definition of Lachenbruch and McCulloh (2014) is that traits need to be quantifiable over a particular period of time. This means that the period of interest should be clearly defined. A trait measure is indeed supposed to comprise information on the performance of an individual plant, but only during a well-defined period. Juvenile features around the pith of tree stems can certainly provide information on the performance of these trees when they were young, but not necessarily on the performance of big trees. Mature features are more likely to be linked to the performance of adult trees, but it should not be overlooked that the performance of adult trees continue to depend on juvenile tissues in twigs and branches. Since secondary tissues of trees are the result of gradual accumulation of new cells, the construction of time axes with trait measures and, consequently, the reconstruction of the performance history of single trees is possible and appealing for trait-based ecology.

Modelling of ecosystem dynamics is an important issue for trait-based ecology. These models try to take into account the age-structure of separate populations. This is undoubtedly an area where conceptual and methodological breakthroughs are urgently needed (Violle *et al*., 2007) but where anatomical analysis of growth-ring patterns offers interesting perspectives.

Next to the possibility of construction of time series, there is also the integration topic that is of significance. Integration of components into a system of higher hierarchy signifies a classical challenge in ecology. Lachenbruch and McCulloh (2014) avoided this concept in their terminology, but it has certainly an attractive potential in system theory in general (Bertalanffy, 1968) and in biotic systems in particular (Allen and Hoekstra, 2015). A system of higher integration indeed shows other assets than the sum of the components of this system. The performance of an individual is more than the sum of single traits, it results from the integration of traits. Assembling or integrating traits adds information and gives typical properties. (Lachenbruch and McCulloh, 2014). A typical integrative feature that is frequently used in ecosystem models is wood density. Density of a piece of wood is a property that results from integrating trait measures like fibre cell-wall thickness, vessel sizes, parenchyma tissue and chemical extractives (mostly secondary metabolites deposited in the heartwood). The relevant level to which tree traits are to be integrated is the organism as a whole. It is indeed definitely the whole plant body which should be seen as an integrated functional unit. The separation of a plant into organs, tissues, cells and cell parts is largely conceptual. Accordingly, hierarchically organised living systems cannot be understood fully if only elements of them are analysed out of their context. Performance of trees is to a big extent controlled by different wood traits present at different levels of system integration: there are whole tree traits as well as anatomical traits. A tree can react to the environment through modifications of certain features of single cells, as is the case with reaction wood. A reaction is also possible through arranging tissues radially (e.g. vessels of vines are much bigger as soon as they reach a support) or vertically (e.g. spiral grain) (Lachenbruch and McCulloh 2014). The integration of wood anatomical elements within the higher unit is equally important as a thorough analysis of cell parts, cells and tissues. Cell biology is only one step and not a final goal of biological research of higher systems (Mohr and Schopfer, 1995). Integration of traits to the scale of a tree is essential, since natural selection acts on performance of whole plants. A plant’s viability is indeed determined by its performance, rather than its contributing traits and properties. Moreover there can be mutual compensations such that similar performance occurs with rather different traits (Lachenbruch and McCulloh 2014). Typical for trait research is the very complex interaction between different levels of integration and microscopic resolution, such as vessel networks and pit characteristics, as perceived by Lens *et al*. (2011). The integration issue makes it difficult to interpret traits independently from other traits.

Integration of traits needs to take into account the influence of tree size. Size influences nearly all the structural, functional and ecological characteristics of organisms in general and trees in particular. A tree with low density, weak wood can support impressive loading thanks to its size, like it is the case for some emergent rainforest trees like Ceiba pentandra and also colossal individuals of Adansonia. These trees consist of very light and weak wood.

Moreover there are the scaling laws where many wood anatomical features need to follow mathematical rules rather independent of their general ecophysiological function. This influence of size also confirms the need to standardize for stem size when structural attributes including wood anatomical traits are being compared between individuals (Olson and Rosell, 2013; Anfodillo *et al*., 2013)

Plants developed structures necessary for their functioning as an organism, but also as part of larger biotic systems and contribute as such to the functioning of populations and communities. The organismal performance of certain individuals could be considered as indicative for the population, community or ecosystem behaviour. Scaling-up from plant traits to higher biotic systems requires so-called integration functions (Violle *et al*., 2007). The standing biomass of a community system, like a forest stand, is a simple sum of the masses of individuals, but the mass of a few big trees might be sufficient to characterize the biomass of a stand (Bastin *et al*., 2015). Less simple integration functions are needed when the climatic response of a tree population is to be captured. The robust dendrochronological methodology is useful for that aim, where that part of the individual tree growth that can be crossdated with other individuals is extracted.

Since plant anatomy is understood as the research of tissue arrangements in plant organs, the number of wood traits logically also includes features like grain, sapwood width, tree-ring widths, density profiles, water content of fresh wood, stem sapwood cross-sectional area per supported leaf surface area (Huber value), stem circumference, plant height, plant growth rate, stem length, stem heartwood biomass, stem longevity, stem pith type, twig, bark and root characteristics,… These are not included in the IAWA feature lists, but are certainly relevant for tree performance.

Forests, woodlands and trees in open landscapes represent important stocks and sinks of carbon. As such they are essential to maintain levels of low entropy and to sustain the carrying capacity of the earth. Woody plants are a major component of forests and a large share of the organic carbon appears as wood. The function of wood in the performance of trees, the position of trees in the functioning of the ecosystem and the role of forest ecosystems in global interactions are reasons enough to give high research priority to woody tissues. When time and complexity is avoided by science in its classical definitions (Prigogine *et al*., 1985), it seems that a productive implication of wood research into diagnoses and evaluations of the carrying capacity of the global ecosystem implies the usage of models dealing with interactions between components and taking into account variability along time axes. Conceptual breakthroughs are expected in the domain of models for upscaling of wood and tree traits to systems of higher hierarchy of integration like populations, communities, ecosystems and biomes.

An interpretation of complexity of wood structures and functions needs to be based on correct terminology covering the wide spectrum of wood anatomical features. Understanding of the function of traits should be based on terminology that describes the variation of plants (Morris *et al*., 2016). Even if a wood anatomical glossary going beyond identification and taxonomy is actually lacking (Morris *et al*., 2016), the IAWA feature lists remain indispensable, thanks to the clear definitions and their comprehensiveness as checklists of potential traits.

There is a largely acknowledged need for global databases of wood anatomy (Anderegg and Meinzer, 2015) allowing quantifying the relative amount of intra- and interspecific variation, as well as variation within and between functional groups (Kattge *et al*., 2011). One of the major challenges of plant ecology is indeed defining consistent sets of measurable traits and developing databases allowing the quantification of ecological strategies of plants along gradients (Fournier *et al*., 2013). These databases should incorporate a time dimension since trees are long living organisms storing information on their performance into the traits of the lignified structures. Since measuring of wood traits is often laborious and does not necessarily fit into classical research projects, wood collection curators might reflect on a gradual establishment of collections of research material aiming at underpinning future or long-term projects in the domain of trait-based ecology. Wood collections are indeed to be considered as interesting archives of wood traits, provided that there are reliable and precise metadata. This would mean a systematic collection of stem discs, fragments of organs that are possibly less typical for taxon description, like juvenile and root tissues. Especially for the tropics such collections would certainly provide a wealth of study material helping investigations of carrying capacity of ecosystems. As such wood anatomy has excellent and firm foundations to contribute microscopically to global change research.

(2) Work by Kafuti *et al*. (2019): **A substantial variation of leaf and wood traits was found that was due to differences between (inter) and within (intra) individual trees of *Pericopsis elata*. These variations showed to be tissue and trait specific.** The inter-individual variation was more important for leaf traits (23%–48%) than for wood traits (<10%) where the intra-individual variation showed to be important (33%–39%). For leaf traits, the intra-individual variation showed to be negligible (<15%) for some traits (leaf area, stomatal size, and leaf dry mass) unlike for others (specific leaf area, stomatal

density, and maximum stomata density) where both inter- and intra-individual variation showed to be substantial.

Leaf area and specific leaf area were negatively correlated with sample location height, suggesting that leaves became smaller upward. Also, stomatal density was positively correlated with sample location height, suggesting that stomatal number per unit leaf area increased upward. Kafuti *et al*. (2019) suggest that these trends were due to the reduced water supply ability occurring upward. Further, they found a positive relationship between maximum stomatal conductance and sample location height, suggesting more potential water loss upward due to transpiration. This result was not consistent with previous findings as stomatal conductance has been reported to decrease with increasing tree total height and water deficit (Delzon *et al*., 2004; Hubbard *et al*., 2002; Whitehead *et al*., 1998; Zaehle *et al*., 2005).

In this study, we found no significant variation of the cross-sectional area of sapwood along the height gradient within the crown but a decreasing theoretical specific hydraulic conductivity, suggesting that the vessel lumina fraction decreased upward. As leaf area also decreased upward, we could presumably relate the negative relationship between maximum stomatal conductance and sample location height to the decrease in leaf number per unit sapwood area upward. This will be an adjustment maintaining the water supply per unit leaf area and allowing trees to continue growing taller despite the water stress occurring upward. This result shows how the trait-based approach can help to unravel the drought response of tropical tree species. However, up to now little is known about the water-use strategy of most tropical species, including *Pericopsis elata*.

A negative relationship between vessel size and vessel density was found. However, this relationship changed with the position within the crown. At lower canopy, a significant negative relationship between hydraulic conduit diameter and vessel density occurred, indicating that wood contains a small number of large vessels. At the top of the canopy, this relationship became fairly positive. Finally, no evidence was found was found of the trade off between hydraulic efficiency and mechanical strength. Theoretical specific hydraulic conductance increased significantly with vessel lumen fraction but decreased only fairly with fiber wall thickness, suggesting that high hydraulic conductivity may occur in twigs without significantly affecting their mechanical strength.

Kafuti *et al*. (2019) also found a significant negative relationship between stomatal size and stomatal density within *Pericopsis elata*, which became stronger in the canopy, suggesting that leaves at the tree top have many smaller stomata per unit leaf area. This result suggests that leaves at the tree top have higher operating stomatal conductance under standard conditions, a higher maximum rate of stomatal opening in response to light, and a faster stomatal response (Drake *et al*., 2013). These conditions have been associated with an enhanced water balance in dynamic light environments and a higher assimilation rate (Aasamaa *et al*., 2001). This means that trees of *Pericopsis elata* increase their stomatal density and decrease their stomatal size to be more flexible, especially at the top of the crown where the environment is more extreme and variable. No fiber-related traits showed significant relationships with leaf traits, suggesting that leaf–wood trade-off is more importantly functional than mechanical (Niinemets *et al.*, 2007).

To summarize, the results suggest that *Pericopsis elata* constantly adapts its water use with respect to its water-supply ability, more importantly at the top of the crown where the environment is more extreme and variable.

(3) For 5 specimens of *Entandrophragma angolense*, 2 specimens of *Polyalthia suaveolens* and 3 specimens of *Trilepisium madagascariense*, the growth rings were indicated on pith-to-bark profiles and growth ring width, growth ring surface, total vessel area per ring, total vessel area percentage, vessel density and average vessel size were determined (Kasongo Yakusu *et al.*, in preparation). Figure 33 shows the average vessel size (mm²) per ring for those specimens (colored per species). **The average vessel size (mm²) per ring for *Entandrophraghma angolense*, which is a light demanding species, appears to increase with increasing ring number. This in contrast with *Polyalthia suaveolens*, a shade-bearing species.** No conclusion could be drawn for *Trilepisium madagascariense* as the specimens were of limited age.

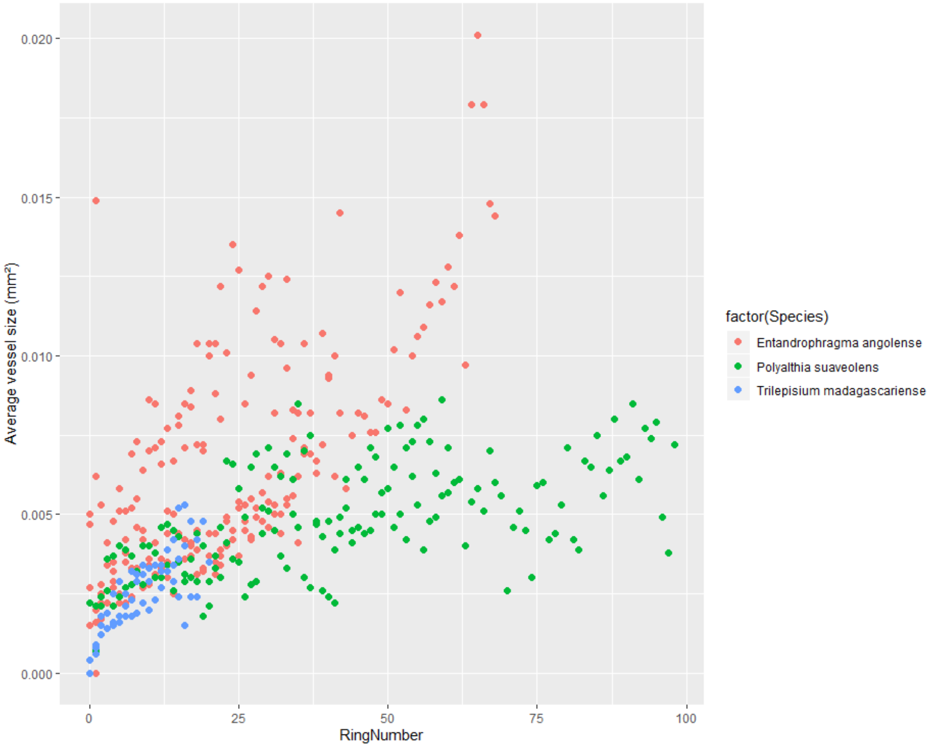


Figure 33: Average vessel size per ring for the three listed species. Entandrophragma angolense *includes five pith-to-bark samples,* Polyalthia suaveolens *includes two pith-to-bark samples and* Trilepisium madagascariense *includes three pith-to-bark samples.*

(4) Work by Tarelkin *et al*. (2016): **A high variability of tree-ring boundaries anatomy and distinctness within individuals and species was found. Although, for some semi-deciduous species, higher incidence of distinct growth rings appears to be related with a more pronounced seasonal climate, no general trends are observed for the assembly of studied species.** Growth rings are variable within individuals depending on the considered organ: trunks tend to show more distinct rings than branches. Growth-ring distinctness is difficult to implement as a trait to measure tree performance when only based on abrupt changes in fibre size and cell wall thickness. From the potential growth-ring markers identified in the IAWA list of hardwood features, those applying to vessel and parenchyma density and distended rays appear to be more useful in tropical trees than abruptly flattened latewood fibres or abrupt changes in vessel diameter.

Wood biological traits - added value of science in support of policy and development

Forests, woodlands and trees in open landscapes represent important stocks and sinks of carbon. As such they are essential to maintain levels of low entropy and to sustain the carrying capacity of the earth. Woody plants are a major component of forests and a large share of the organic carbon appears as wood. The function of wood in the performance of trees, the position of trees in the functioning of the ecosystem and the role of forest ecosystems in global interactions are reasons enough to give high research priority to woody tissues.

When time and complexity is avoided by science in its classical definitions (Prigogine et al., 1985), it seems that a productive implication of wood research into diagnoses and evaluations of the carrying capacity of the global ecosystem implies the usage of models dealing with interactions between components and taking into account variability along time axes. Conceptual breakthroughs are expected in the domain of models for upscaling of wood and tree traits to systems of higher hierarchy of integration like populations, communities, ecosystems and biomes. There is a largely acknowledged need for global databases of wood anatomy, allowing to quantify the relative amount of intra- and interspecific variation, as well as variation within and between functional groups. One of the major challenges of plant ecology is indeed defining consistent sets of measurable traits and developing databases allowing the quantification of ecological strategies of plants along gradients. These databases should incorporate a time dimension since trees are long living organisms storing information on their performance into the traits of the lignified structures. Since measuring of wood traits is often laborious and does not necessarily fit into classical research projects, wood collection curators might reflect on a gradual establishment of collections of research material aiming at underpinning future or long-term projects in the domain of climate change research and investigations of tropical forest resilience. Wood collections are indeed to be considered as interesting archives of wood traits which are crucial inputs for climate and vegetation models. This implies that wood collections should aim at a systematic collection of stem discs, fragments of organs that are possibly less typical for taxon description, like juvenile and root tissues. Especially for the tropics such collections would certainly provide a wealth of study material helping investigations of carrying capacity of ecosystems. As such wood anatomy has excellent and firm foundations to contribute microscopically to global change research and associated policy.

4.2.4 Wood technological traits

DIC allows determining the dimensional stability of un-standardized samples

The high correlation between the calliper measurements and the DIC method for the reference samples (Figure 34) justifies the latter as a valid technique for dimensional stability measurements. The R^2 and slope of the type II regression is 0.94 and 0.98 for the radial direction and 0.96 and 0.99 for the tangential direction. Slight deviations are noted, mostly related to the conventional approach of measuring. When preparing standard samples, it is often difficult to align perfectly with the tangential and radial direction, leading to a small offset using a calliper. Furthermore, small differences in measuring position with the calliper can lead to additional errors.

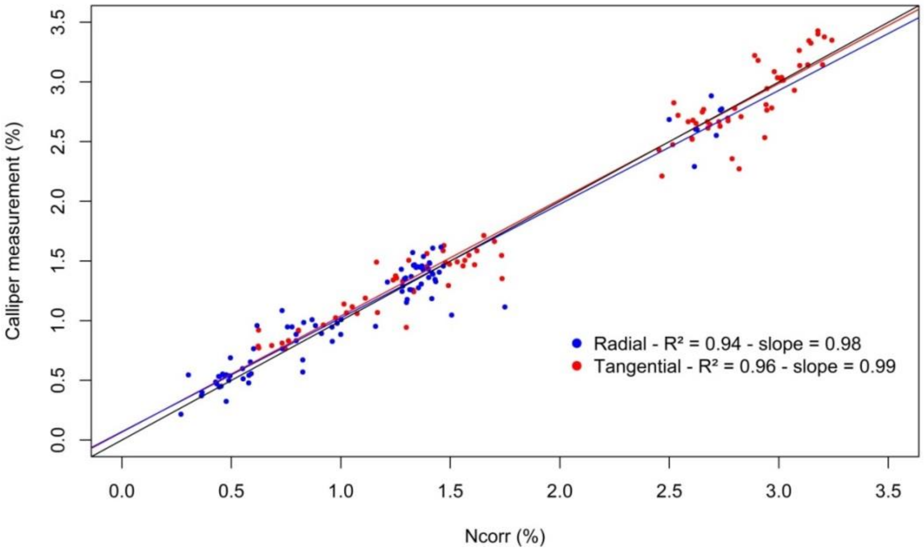


*Figure 34: Comparison between the swelling (%, 60- 90RH, 20°C) determined by calliper measurements and the DIC based measurements for every reference sample in the radial and tangential direction. Type II regression (MA – major axis): radial – intercept = 0.0374 and slope = 0.98, p-perm (1-tailed, 100 permutations) = 0.0099**. L Type II regression (MA – major axis): tangential – intercept 0.0299 and slope = 0.99, p-perm (1-tailed, 100 permutations) = 0.0099**. Significance levels: ns = p > 0.05, * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001.*

Several studies report on dimensional stability measurements, yet this is the first study to explore the potential of xylarium specimens in that regard. Mantanis *et al*. (1994) used linear variable displacement transformers on 25 x 25 x 5 mm pieces of several North American species. Shukla and Kamdem (2010) determined the swelling of nine tropical hardwoods from Cameroon by monitoring the swelling of wood samples (22 x 25 x 77 mm) immersed in water for 48h using linear voltage displacement transducers. In both studies, samples with correct tangential and radial directions were used, while the technique proposed in this study eliminates such sample requirement. Other, more advanced techniques have been used as well to study swelling or shrinkage on a more local and even microscopic scale. For example, high-resolution phase-contrast X-ray tomography has been used to determine the sorption and swelling behaviour of heterogeneous spruce wood (Patera *et al*., 2018). The shrinkage of oak was predicted using a model based on anatomical patterns and X-ray imaging (Badel and Perré, 2001). However, those studies do not cover the scale we are interested in to determine average swelling and shrinkage on the entire sample and do not cover the wide range of tropical species included here. Moreover, these studies also rely on advanced and often expensive techniques, contrary to the easy and low-cost flatbed approach proposed here. Given that so far we have only focussed on 53 species, this method now allows studying more species and specimens from the xylarium without the need for additional sampling, as such giving an additional value to more than a century of collecting.

Identifying several "use-groups" depending on the combination of wood density and dimensional stability

The relationship between wood density and dimensional stability has been previously described. Stamm (1964) (as cited by Mantanis *et al*., 1994) reports a clear correlation of shrinkage and swelling with specific gravity (cfr. IAWA list of microscopic features for hardwood identification: basic specific gravity = oven-dry weight/weight of water displaced by wood when fully swollen, Wheeler *et al*. (1989)). Mantanis *et al*. (1994) found a linear correlation and Shukla and Kamdem (2010) found a weak correlation (resp. 0.54, 0.56 and 0.44 for volumetric, radial and tangential swelling respectively) between swelling and wood density for nine hardwoods from Cameroon. Density has been correlated with shrinkage (see also Suchsland, 2004) but this correlation is a representation of a general trend for defect free wood of different species and is not particularly strong (Leonardon *et al*., 2010). In most cases, the limited number of samples and species diversity hampers a general conclusion.

Figure 35 illustrates the relation between average density and volumetric swelling for every species (421 samples, 53 species). **The linear model is weak (R^2 adjusted = 0.12), but with a significant slope ($p \leq 0.01$) indicating that changes in wood density are associated with changes in volumetric swelling.** Spearman correlation is significant at the 95%-level (r - Spearman = 0.34, $p \leq 0.05$) confirming this relationship, yet it is rather small. The polygons (I, II, III, IV and V) are the result of the K-means clustering of the individual samples and show the sample groups. Among the different available methods for determining the optimal number of clusters, we opted for the elbow method in combination with the derivative of the fitted spline.

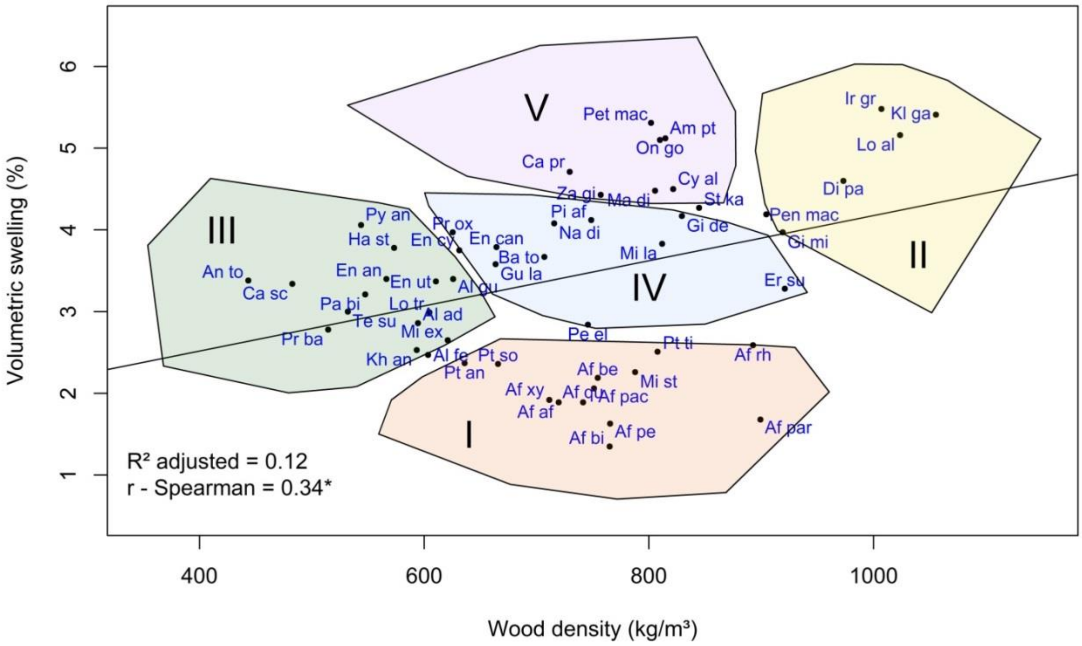


*Figure 35: Relation between wood density (kg m-3) and volumetric swelling (%, 60- 90RH, 20°C) per species. Linear model: p-value = 0.00737**, intercept p-value = 0.06048 and slope p-value = 0.00737**. Spearman correlation coefficient = r- Spearman. Significance levels: ns, p > 0.05, *, p ≤ 0.05, **, p ≤ 0.01, ***, p ≤ 0.001. Polygons indicate the results of the K-means clustering of the individual samples. Number of clusters was determined using the elbow method combined with the max value of the third derivative of the fitted spline.*

For 13 species all samples belonged to the same cluster, for 16 species the samples were present in two groups, for 22 species in three groups and for two species in four groups. This indicates variability within a species when considering the combination of volumetric swelling and wood density. We should note here that three of those 13 species only had a single sample. **Interesting timber species are dense yet stable (low volumetric swelling), which is the case for group I (Figure 35).** These species classify as paradoxical, in terms of the combination of both dimensional stability and wood density. Group I includes the well-known and highly-valued *Afzelia* species (Af spp, for averages and variability in wood density and volumetric swelling see Table S.3 Supporting materials in Deklerck (2019)), *Millettia stuhlmannii* (Mi st, 788 ± 88 kg m^{-3} and 2.3 ± 0.8%), *Pterocarpus tinctorius* (Pt ti, 808 ± 83 kg m^{-3} and 2.5 ± 1.3%), *Pterocarpus angolensis* (Pt an, 636 ± 75 kg m^{-3} and 2.4 ± 1.0%) and *Pterocarpus soyauxii* (666 ± 80 kg m^{-3} and 2.4 ± 0.4%). Group II is characterized by a high wood density and high volumetric swelling and includes *Irvingia grandifolia* (Ir gr, 1007 ± 49 kg m^{-3} and 5.5 ± 0.4 %), *Klainedoxa gabonensis* (Kl ga, 1056 ± 45 kg m^{-3} and 5.4 ± 0.4%) and *Lophira alata* (Lo al, 1024 ± 43 kg m^{-3} and 5.2 ± 0.3%). Group III, including species such as *Antiaris toxicaria* (An to, 443 ± 54 kg m^{-3} and 3.4 ± 0.4%) and *Canarium schweinfurthii* (Ca sc, 483 ± 119 kg m^{-3} and 3.3 ± 0.5%) contains species with a low wood density and rather high volumetric swelling. **Groups II and III are therefore less interesting for high-end applications. The wood density is too low and mechanical strength (related to density) is most probably insufficient, and / or volumetric swelling is too high for the wood to have any feasible use.** Group III can also be considered as paradoxical, yet opposite to what is required. Group IV contains species with medium wood density and medium swelling such as *Nauclea diderrichii* (Na di, 716 ± 57 kg m^{-3} and 4.1 ±

0.5%) and *Baillonella toxisperma* (Ba to, 707 ± 61 kg m^{-3} and 3.7 ± 0.6%). Finally, species with medium wood density but high swelling are found in group V. Examples are *Petersianthus macrocarpus* (Pet mac, 802 ± 44 kg m^{-3} and 5.3 ± 0.6%), *Ongokea gore* (On go, 810 ± 59 kg m^{-3} and 5.1 ± 0.9 %) and *Amphimas pterocarpoides* (Am pt, 815 ± 70 kg m^{-3} and 5.1 ± 0.6%). Multiple species groups in terms of volumetric swelling for the same range of wood density can be noted (polygon I, IV and V). Here, wood density cannot explain the differences in volumetric swelling and we need to take a closer look at the wood anatomy of the species, considering that the wood anatomical variation most probably does not support a straightforward relation. The role of wood anatomy is not further discussed in this report. For a detailed explanation we refer the reader to Deklerck *et al*. (2019b).

We do offer a short summary here:

Most species follow the general trend of high/low fibre wall thickness related to high/low wood density and high/low volumetric swelling respectively however there are clear exceptions on those combinations (see Figure 34 here and Figure 2.8 in the PhD Thesis by Deklerck (2019)). An intricate combination of anatomical characteristics affects swelling since a large range of volumetric swelling is found for a quite small range of wood density.

The natural durability of xylarium specimens can be determined using the mini-block test

The results and summary for the mini-block test on both xylarium (12 weeks) and commercial (8 and 12 weeks) samples can be found in Table S.7 (Supporting Materials) and Table S.8 (Supporting Materials) in Deklerck (2019) (PhD Thesis).

The mass loss of the beech reference (Figure 36) in mini-blocks increased significantly from 23.3% after 8 weeks to 33.3% after 12 weeks of degradation ($p \leq 0.001$). **When comparing the mass loss for the commercial species after 8 and 12 weeks of degradation it appears that all mass losses changed significantly, although most commercial species (European cherry, acajou d'Afrique, moabi, azobé, iroko, padouk d'Afrique and robinia) remain in the same natural durability class.** The additional four weeks of degradation did change the durability class of oak from class 1 to 2 ($p \leq 0.01$), sapeli from class 3 to 4 ($p \leq 0.001$) and iroko from class 2 to class 4 ($p \leq 0.01$).

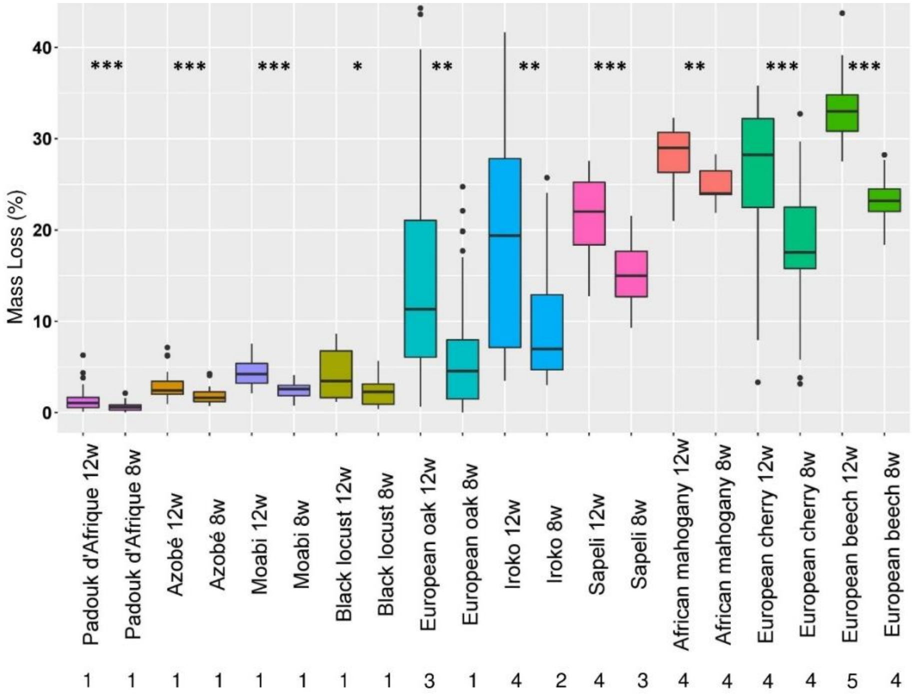


*Figure 36: Mass loss (%) for the commercial species, comparison between 8 weeks and 12 weeks of fungal decay. Significance levels were determined via the Mann Whitney U test or Welch's two-sample t-test depending on normality (ns = not significant, * = $p \leq 0.5$, ** = $p \leq 0.01$, *** = $p \leq 0.001$). The numbers at the bottom of the figure indicate the natural durability class.*

The results of the Dunn's test showed that after 8 weeks there were nine species comparisons with no significant difference in mean mass loss (azobé – moabi, azobé – black locust, European beech – European cherry, European beech – acajou d'Afrique, European cherry – acajou d'Afrique, European cherry – sapeli, iroko – sapeli, moabi – European oak and moabi – black locust). After 12 weeks, however, there was a significant difference between moabi and European oak but no significant difference between azobé – padouk d'Afrique, padouk d'Afrique – black locust, European cherry – iroko, iroko – acajou d'Afrique, acajou d'Afrique – sapeli and sapeli – European oak, in addition to the no significant differences after 8 weeks.

When comparing degradation after 12 weeks for xylarium specimens (Figure 37, Table S.7 in Supporting Materials in Deklerck (2019)) and commercial specimens originating from wood beams of the same species (Table S.8 - 12 weeks in Supporting Materials), we see that *Baillonella toxisperma* (moabi) specimens from the xylarium showed stronger decay (2 vs 1, with significant difference in mass loss(%)). Xylarium specimens from the species *Entandrophragma cylindricum* (sapeli), *Khaya anthotheca* (acajou d'Afrique)¸ *Lophira alata* (azobé) and *Pterocarpus soyauxii* (padouk d'Afrique) were in the same calculated durability class as the commercial ones (4, 4, 1 and 1). However, there is a significant difference in mass loss (%) between the xylarium and commercial specimens for *Khaya anthotheca* and *Pterocarpus soyauxii*. The natural durability class for *Milicia excelsa* (iroko) xylarium specimens was one class higher compared to the commercial specimens (3 vs 4, significant difference in mass loss (%)).

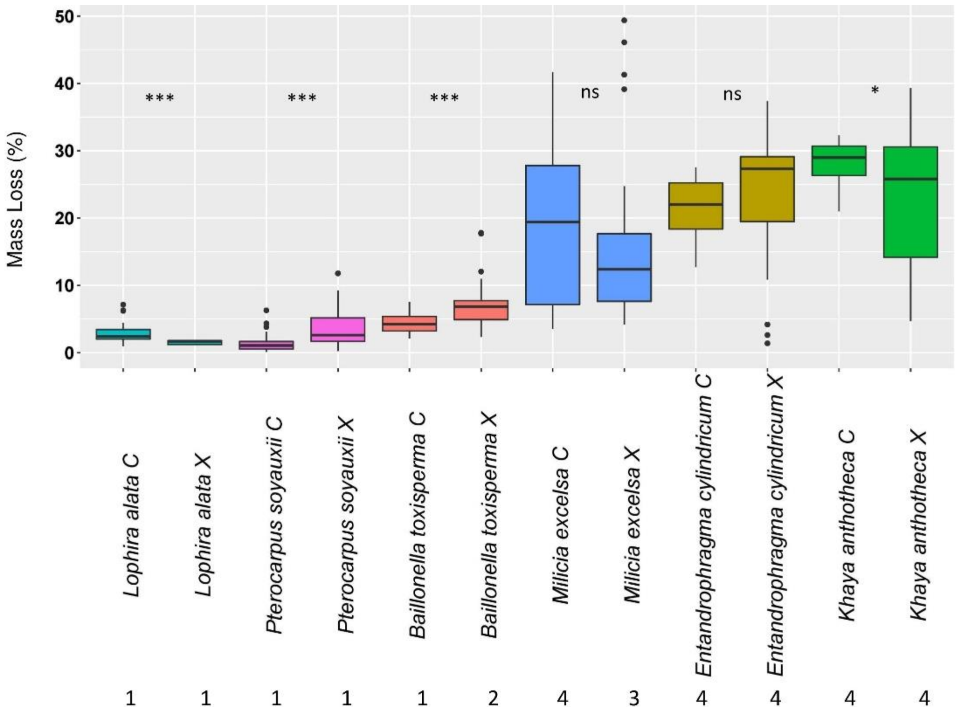


*Figure 37: Comparison of mass loss (%) after 12 weeks between the commercial specimens (C) and the xylarium specimens (X). Significance levels were determined via the Mann Whitney U test (ns = not significant, * = $p \le 0.5$, ** = $p \le 0.01$, *** = $p \le 0.001$). The natural durability class is shown at the bottom of the graph. The numbers at the bottom of the figure indicate the natural durability class.*

The most durable species (xylarium, class 1) are *Erythrophleum suaveolens* (tali), *Lophira alata* (azobé), *Milletia laurentii* (wengé), *Pterocarpus angolensis* (muninga), *soyauxi* (padouk d'Afrique) and *tinctorius* (padouk d'Afrique) while the least durable species (xylarium, class 5) are *Canarium schweinfurthii* (aiélé) and *Pycnanthus angolensis* (ilomba). Figure 2.13 (and Table S.7 in Supporting Materials) in Deklerck (2019) shows the results of the natural durability test for the xylarium species and the natural durability classes retrieved from literature (Houtvademecum, Klaassen (2018)), online repositories (Tropix 7, Cirad (2017)) as well as EN 350 (2016). Out of 31 species, 15 have a different classification compared to the literature classifications. 11 species have a lower durability and 4 have a better durability. Note the high within-species variability for *Khaya anthotheca* (Acajou d'Afrique), *Pycnanthus angolensis* (Ilomba), *Staudtia kamerunensis* (niové), *Zanthoxylum gilletii* (East African satin wood).

Mass loss is inversely related to wood density

Figure 38 shows the relationship between wood density (kg / m³) and mass loss (%) for 233 xylarium specimens. The R^2 adjusted of the linear models is 0.39 ($p \le 0.01$) and the Spearman rank correlation equals -0.65 ($p \le 0.01$). **A decrease in mass loss is linked with an increase in wood density.** For some specimens a range in mass loss of up to 20% can be noted.

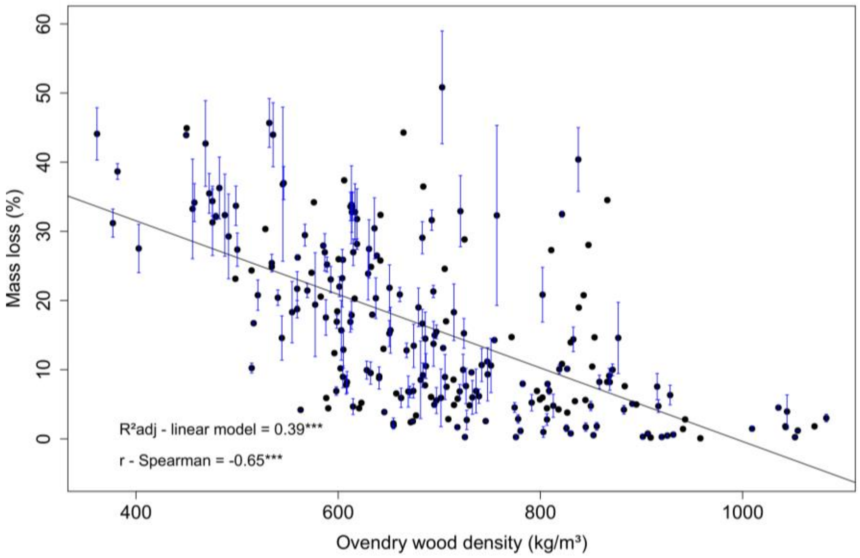


Figure 38: Mass loss (%) vs. oven-dry wood density (kg/m³) for 233 Tervuren Xylarium specimens (31 species). The mass loss for the xylarium specimens is the average mass loss from the samples from that specimen. The standard deviation is given by the blue bars and the black line gives the linear relationship (y = 52.80 – 0.05x).

Chemical compounds linked to natural durability and natural durability prediction

The PCA with Spearman Rank Correlation for ions that are present in more than 75% of the specimens showed which ions are related to higher mass loss and which are related to a lower mass loss. **Two chemical compounds could be identified that are linked to natural durability (or mass loss): 397.38 *m/z*, which is beta-sitosterol and is found in, for example, *Terminalia superba*; and 273.11 *m/z*, which is angolensin and can be found in *Pterocarpus angolensis.*** The identification of the other compounds was less straightforward. Figure 39 shows the results of a five-fold cross-validated PLS regression with five components and mass loss as response variable. There is a significant linear relationship between the true values of mass loss and the predicted ones (R² - adjusted = 0.40***).

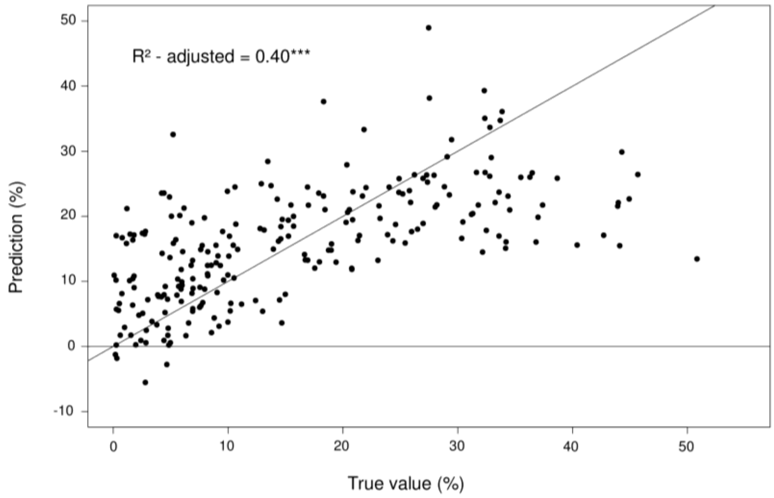


Figure 39: PLS regression with individual specimen numbers showing the original mass loss (%) on the x-axis and the predicted mass loss (%) on the y-axis. The black line shows the 1:1 line.

Wood density at different scales

High-resolution density profiles based on X-ray Computed Tomography

During HERBAXYLAREDD over 2047 tree cores were scanned at Ghent University. A full description of the database can be found on http://www.dendrochronomics.ugent.be/. These scans provide vital information on radial density patterns in trees (Figure 40). This density information can then be used to discern tree rings, to asses wood anatomical variability in height and on a pith-to-bark transect and in allometric equations to calculate carbon stocks.

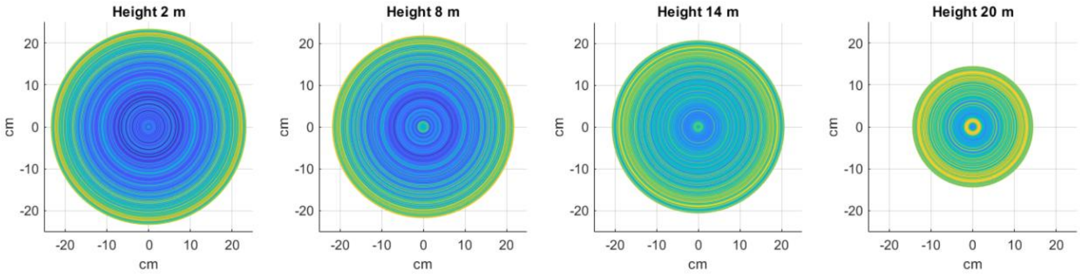


Figure 40: Axial and radial variation of a Terminalia superba tree from the Luki Reserve. One core per height was taken and the density profile was interpolated 360°. There is an increasing density trend from pith to bark, with highest density values in the upper stem.

Ovendry wood density measures with water displacement method

For over 9,197 specimens from the Tervuren Wood Collection, the ovendry wood density was determined based on the methodology described above. From these dataset, 4,517 wood density measurements (belonging to over 1,500 species) were added to the DRYAD repository database (see Zanne *et al*. (2009)) and is now publicly available (awaiting confirmation).

Wood anatomical components of density

From the work by De Mil *et al*. (2018): **On average, density profile variation mostly reflects variations in fibre lumen and wall fractions, but these are species- and position-dependent: on some positions, parenchyma and vessels have a more pronounced effect on density.** The model linking density to wood anatomical traits explains 92% of the variation, with 65% of the density profile variation attributed to the three measured traits. The remaining 27% is explained by species as a random effect. There is a clear variation between trees and within trees that have implications for interpreting density profiles in angiosperm trees: the exact driving anatomical fraction behind every density value will depend on the position within the core. The underlying function of density will thus vary accordingly.

Ash content and calorific values differ between tree parts

For 160 species, and different parts of the tree, the ash content and calorific value were determined. Figure 41 shows the calorific value and ash content respectively for the different parts of the tree (across species). For the calorific values, only the differences in mean between Branch-Stem and Regrowth-Stump are not significant (wilcoxon test). For the ash content, only the differences in mean between Stem-Stump and Regrowth-Stump are not significant (Wilcoxon test).

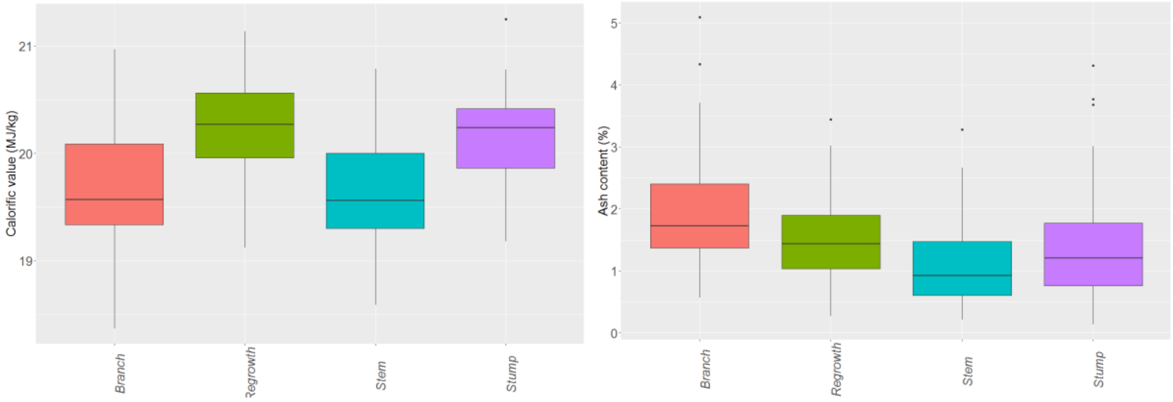


Figure 41: Left: Boxplots showing the calorific value for branches, regrowth, stem and stump across species. Right: Boxplots showing the ash content (%) for branches, regrowth, stem and stump across species.

Wood technological traits - added value of science in support of policy and development

The development of the digital image correlation technique for dimensional stability, and the adaptation of the mini-block test to determine the natural durability opens up opportunities to discover new and interesting timber species. The wood and timber industry can be seen as a conservative sector, with the same few species being used for a certain purpose. As species are disappearing and regulations are becoming more strict (see Deklerck (2019)), it is vital to have an idea about replacement species. Not only to sustain our constant need for timber, but also to allow a break for the current heavily exploited species. **With the described techniques a full screening of the xylarium is now possible. Over 80,000 specimens (13,000 species) can now be screened for their dimensional stability and natural durability. This in combination with the already available techniques to determine the wood density allows discovering alternative timber species. In addition, the described methodologies allow exploring the fit-for-purpose approach.** Figure 35 illustrates that multiple specimens and species have similar characteristics in terms of dimensional stability and wood density. This would suggest moving from a single species selection to a mix of timber species for the same purpose. Group I is the group with the highest potential for high-end applications, such as *Afzelia* and *Pterocarpus*, which are highly valued as timber species for joinery and furniture (Klaassen, 2018). As such, a mix of species (or specimens) from Group I can be used for the same high-end applications. Species for heavy construction (for example *Lophira alata*) are found in group II, characterized by high wood density. However, this means that the volumetric swelling is rather high (Figure 35), leading to potential problems. Contrary, group III contains species (for example *Canarium schweinfurthii* or *Prioria balsamifera*) with a combination of low wood density and medium swelling, which is unwanted in most applications. Apart from average values, taking into account variation is also needed, which is illustrated by the *Carapa procera* (Ca pr) species, of which the average is located in Group V but specimens are distributed over 4 different groups. The DIC approach presented in this study allows for screening of non-standardized specimens and can improve knowledge on lesser-known species. These species can be then be added to the current clusters of Figure 35 and their application potential can be indicated. This can be combined with the data retrieved on the natural durability and as such we can describe the usability of a species. Being able to mix timber species and the possible within species variation could ultimately lead towards a fit for purpose species and specimen approach. The species mix approach is already to some extent developed within the timber industry, e.d. as several tree species are used under one wood species denominator like DRM (dark red meranti) as material for window joinery. However, few examples are available and there is more potential for a fit-for-purpose approach. The specimen approach might require a higher dedication or investment of the timber industry and it is not certain if there is enough leverage for this and whether such an approach is feasible in the long run.

4.2.5 Chemical fingerprinting of wood allows for identification and gives towards geographical provenance

Over 2,000 DART TOFMS spectra (> 900 specimens, 55 species) were collected from Tervuren Wood Collection specimens, of which a part was added to the ForeST© database. This is the main database for DART TOFMS spectra for timber identification and is curated by the US Fish and Wildlife Forensics Laboratory. A first publication (Deklerck *et al*., 2017) discerned *Pericopsis elata* (CITES Appendix II) from its look-alikes based on these DART TOFMS spectra. There have been documented fraudulent imports of *Pericopsis elata* declared as *Milicia excelsa*, which is a non-CITES listed species. The second publication (Deklerck *et al*., 2019a) focussed on optimizing pre-process parameters to achieve the highest classification accuracy when determining the species identification of an unknown sample. This automated pathway was applied the Meliaceae, a family with high valued timber species. A final paper (Deklerck *et al*., 2020) shows a proof-of-concept to use chemical fingerprints of growth rings to differentiate between individuals and to determine the geographical provenance of an unknown sample.

Illegal timber trade concerns two main issues: (1) is the species being traded illegal (species identification) and (2) is the area where it was logged illegal (geographic provenancing)?

For the **first question**, several techniques are available. Traditional wood anatomy for example, focuses on the anatomical features, such as parenchyma, vessels, rays and fibres to identify a species. Although wood identification using conventional optical light microscopy is usually sufficient to identify a wood sample to genus level, the technique sometimes fails to determine the species (Dormontt *et al*., 2015; Gasson, 2011) and separating closely related taxa can be problematic as well (Deklerck *et al*., 2017). Automated classification techniques based on transverse cross-sections images are increasingly investigated and show promising results (Hermanson and Wiedenhoeft, 2011; Ravindran *et al*., 2018; Rosa da Silva *et al*., 2017). However, such techniques depend on the availability of wood transverse cross-sections and these are not always easy to obtain. The application of NIRS is less common but has shown success for discriminating between different species or even determining the provenance of a limited number of species (Brunner *et al*., 1996; Tsuchikawa *et al*., 2003; Pastore *et al*., 2011). DNA-analysis is successful in identifying the species, however at the expense of increased time (several days) and cost (Dormontt *et al*., 2015). Also, analysing DNA-sequences from wood samples for genetic differentiation is challenging due to the difficulties with isolating DNA from dried and processed wood (Höltken *et al*., 2012). Yet, DNA microsatellites have been used successfully for tropical timber tracing (Degen *et al*., 2013; Jolivet and Degen, 2012; Tnah *et al*., 2010). Alternatively, stable isotope-analysis can be used but is only capable, to a certain extent, to determine the origin of the traded timber, not the species (Dormontt *et al*., 2015; Kagawa and Leavitt, 2010). Although false claims of geographic origin (**second question**) are likely the most important form of illegal trade in tropical timber (Vlam *et al.*, 2018), the options to verify origin are limited. Until now, stable isotopes and DNA analysis remain among the most promising techniques. The use of stable isotopes has shown success for different species and regions (see Horacek *et al.*, 2009; Kagawa and Leavitt, 2010; Förstel *et al.*, 2011; Horacek, 2012), however there have been mixed results as well (Vlam *et al.*, 2018). The further use and practicality of stable isotopes for timber provenancing will be determinant on the development of databases for additional taxa and regions of interest (Dormontt *et al*., 2015). DNA analysis has shown potential in several studies both in determining the geographical provenance as in tracking individual logs (see Vanden Abeele 2019, Lowe *et al.*, 2010; Jolivet and Degen, 2012; Degen *et al.*, 2013; Hung *et al*., 2017; Vlam *et al.*, 2018). The main obstacles here are the development of discriminating markers, limited reference databases and the methodological difficulties to extract useful DNA out of wood (Dormontt *et al*., 2015).

The use of DART TOFMS is gaining momentum for identifying tropical timbers given that it is minimally destructive for finished, high-value wood products, is a fast analysis and no sample preparation is needed, which is essential for time-restrained enforcement policies. Moreover, it is rather inexpensive compared to genetic or stable isotope analysis. However, its potential for individual tracking or even individual distinction is currently unknown. With DART TOFMS, wood slivers are placed in a heated helium gas stream for an average of eight seconds, which leads to thermal desorption and ionization of the molecules. This results in a unique chemical pattern based on low molecular molecules (metabolites, metabolic or chemical fingerprint) which is used to identify the species in question. DART TOFMS has proven to be successful to discern between several timber species (Espinoza *et al*., 2015; Lancaster and Espinoza, 2012; McClure *et al*., 2015; Musah *et al*., 2015) or even keratin-types (Price *et al*., 2018). However, more research is needed on different species groups and little is known about the effect of parameter settings in the data processing on species classification accuracy. Finally, its potential for geographical provenancing has been tested, however, only limited case studies have been tested and this is mainly in an experimental stage.

Two master thesis students conducted their thesis on DART TOFMS data during HERBAXYLAREDD: Nathalie Goeders (2017-2018) and Bianca De Saedeleer (2019-2020). For the thesis of Bianca De Saedeleer, Near InfraRed data were collected of 219 specimens from the Tervuren Wood Collection for timber identification purposes, in combination with DART TOFMS data. This work is still in progress.

The case of *Pericopsis elata* (Deklerck *et al*., 2017)

Pericopsis elata, commonly known as Afrormosia, is an emblematic species of the African rainforests that has been protected by the Convention on International Trade in Endangered Species (CITES, UNEP-WCMC) since 1992. Its heartwood is characterized by high natural durability, mechanical strength, and dimensional stability. This combination makes it suitable for the most demanding applications of wood, especially for exterior joinery. The decorative value of the wood is also appreciated for the production of luxury furniture and parquetry. Because of law enforcement concerns, there is a need to distinguish *P*. *elata* from the other *Pericopsis* species and look-alike timbers. There have been documented fraudulent imports of *P*. *elata* declared as *Milicia excelsa*, a non-CITES listed species. The timber of *P*. *elata* can also be confused with *Dalbergia melanoxylon* (CITES App. II) from Africa. Traditional identification of wood has relied on anatomical features such as those in the extensive online database InsideWood (InsideWood, 2004-onwards). When searching InsideWood using standardized wood anatomical features of *P*. *elata*, the results indicate that several other species, such as *D*. *melanoxylon* and *Haplormosia monophylla*, have similar wood structures. *D*. *melanoxylon* is a timber species that also occurs in Central Africa. *H*. *monophylla*, which is taxonomically closely related to *P*. *elata*, also occurs in Africa and it is traded by the common name of Idewa. To a lesser extent, the timber of the three other *Pericopsis* species might also be sold or confused with *P*. *elata* (PROTA Foundation, 2008).

Within HERBAXYLAREDD, heartwood samples of all *Pericopsis* species, *M*. *excelsa*, *H*. *monophylla*, and *D*. *melanoxylon*, were collected and analysed at the U.S. Fish and Wildlife Forensics Laboratory (**international partner 2**). The table in Supporting Materials in Deklerck *et al*. (2017) lists the different specimens with their geographic provenance, country of origin, and the source and number of specimens. The main goal of this study is to determine if *Pericopsis elata* could be distinguished from the following species using DART TOFMS: *P*. *angolensis*, *P*. *laxiflora*, *P*. *mooniana*, *M*. *excelsa*, *H*. *monophylla*, and *D*. *melanoxylon*. A second goal was to determine: (1) which classification technique, Kernel Discriminant Analysis (KDA) or random forest, performs better to separate these species. KDA was performed with the Mass Mountaineer software package using a tolerance of 5 mDa and a 1% relative abundance threshold. For a full description on supplementary goals and the modelling methodologies we refer to Deklerck *et al*. (2017). For random forest, spectral data were exported from Mass Mountaineer (tolerance of 250 mDa and 1% threshold) to Microsoft Excel and imported into RStudio.

A heatmap, showing the intensity of each ion-mass (*m/z* – value) in the specimen (Figure 42), was created using the Mass Mountaineer software (RBC Software, Peabody, MA, USA). The black boxes indicate several diagnostic ions for a certain species. As shown in Figure 42, the chemotypes for *H*. *monophylla*, *M*. *excelsa*, and *D*. *melanoxylon* are different from those of the *Pericopsis* species. In addition, some differences appear among the chemotypes of the *Pericopsis* taxa. **The surprising finding that the chemotype of *P*. *laxiflora* is**

dissimilar to the chemotype of *P. angolensis* erodes the support for the hypothesis that *P. laxiflora* could be a subspecies of *P. angolensis* (PROTA Foundation, 2008). However, a larger sample size of *P. laxiflora* is needed to statistically test this observation. The main goal of this study was to separate the CITES-listed *P. elata* from the other species in its genus and from its look-alikes. **The LOOCV (KDA) was 95.79%, and the classification accuracy of the random forest was 96.05%, indicating that both KDA, with the empirically chosen ions, and random forest enabled us to correctly identify *P. elata* to a satisfactory level.** Next, we tried to differentiate between *Pericopsis* species. The LOOCV was 88.89% and the random forest accuracy was 93.75%. These results are based however on an unbalanced dataset, with only five samples for *P. laxiflora* compared with, for example, the 21 samples from *P. angolensis*. Although the final model performance is satisfactory, it might affect the model variability and the handling of misclassifications (Finch *et al.*, 2017). The overall classification accuracy is not significantly affected; however, this is often not an appropriate performance measure in learning extremely unbalanced data (Chen *et al.*, 2004). Using these small unbalanced datasets increases the risk of leaving a certain species out of the training dataset bootstrapping, skewing the model towards the more abundant species. Possible solutions are suggested by Chen *et al.* (2004). This was, however, outside the scope of the current study and should be investigated further.

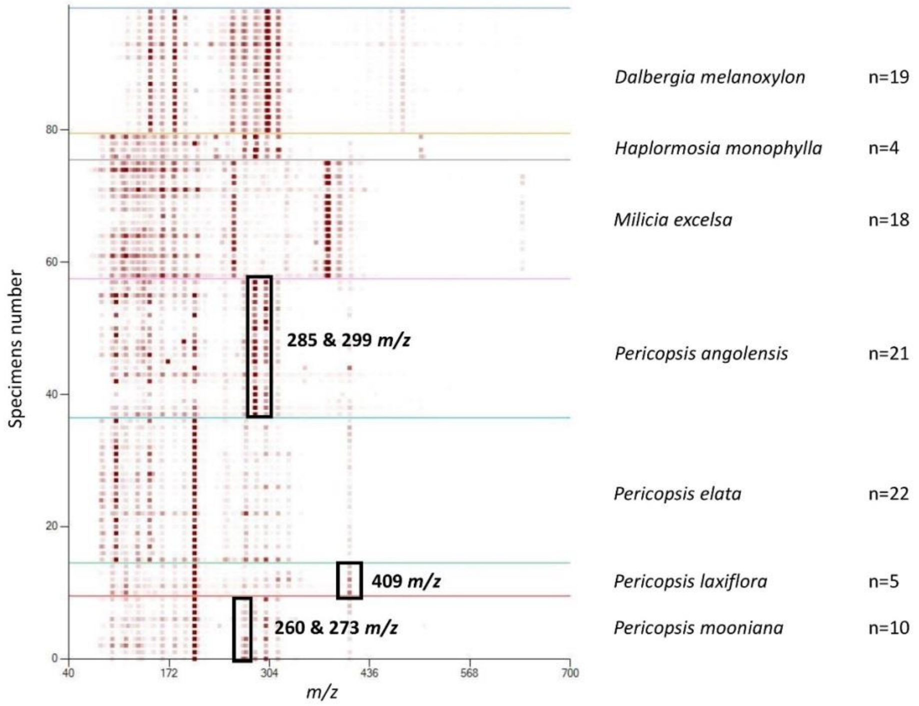


Figure 42: Heatmap of the ions present in the analysed specimens. The X-axis is the mass-to-charge ratio (m/z) of the ions detected. The Y-axis indicates specimen number with its chemotype grouped per species (n = the number of specimens for that species). The intensity of the red squares in the heatmap correlates with the abundance of the ions in the specimens. Diagonstic m/z-values for a species are indicated by black boxes.

Optimizing pre-processing parameters, a case study on the Meliaceae family (Deklerck *et al*., 2019a)

Using chemical fingerprints for timber species identification is a relatively new, but promising technique. However, little is known about the effect of pre-processing spectral data parameter settings on the timber species classification accuracy. **Therefore, we present an automated analysis method using the random forest machine learning algorithm on a set of highly valuable timber species from the Meliaceae family. This protocol could increase classification accuracies and as such improve the species identification of an unknown sample.** We focus on assessing the variability in classification accuracy using DART TOFMS and different parameter settings employing the random forest algorithm on species from the Meliaceae family: *Entandrophragma angolense* (Welw.) C.DC., *E. candollei* Harms, *E*. *cylindricum* (Sprague) Sprague, *E*. *utile* (Dawe & Sprague) Sprague), *Khaya anthotheca* (Welw.) C.DC., *K*. *ivorensis* A.Chec., *Swietenia macrophylla* King, *S*. *mahagonie* (L.) Jacq. And *S*. *humilis* Zucc. species, and *Lovoa trichilioides* Pierre ex Sprague. For more information on the specimens see Deklerck *et al*. (2019a). Two main objectives have been identified: (1) to provide an automated protocol to optimise timber identification using DART TOFMS by determining the optimal parameter settings combined with random forest analysis and, (2) to determine which species in the Meliaceae – family are most often misclassified by DART TOFMS.

Figure 43 shows the chemical fingerprint heatmap for the different samples grouped by species. The *Swietenia* species have a very different chemotype compared to the other species. The ion around 871 *m/z* is an identifier for this group. Clear differences between the *Swietenia* species are not that obvious. *L. trichilioides* has a clear presence of ions around 395, 409 and 427 *m/*z, similar to the *Swietenia* species but it is lacking the ion around 871 *m/z*. *K. ivorensis* differs by the high intensity of the ion around 338 *m/z*. It is more difficult to differentiate between the remaining species based on visual inspection of the heatmap only. *E. cylindricum* has a more or less clear presence of ions around 205, 713 and 829 *m/z* compared to the other species, while *E. utile* only has an ion signal around 829 *m/z*. There is a range of ions (429 – 574 *m/z*) that is present in *E. angolense*, but this is not consistent across all the samples of the species. *K. anthotheca* and *E. candollei* have a very similar chemotype and no immediate differences are visible.

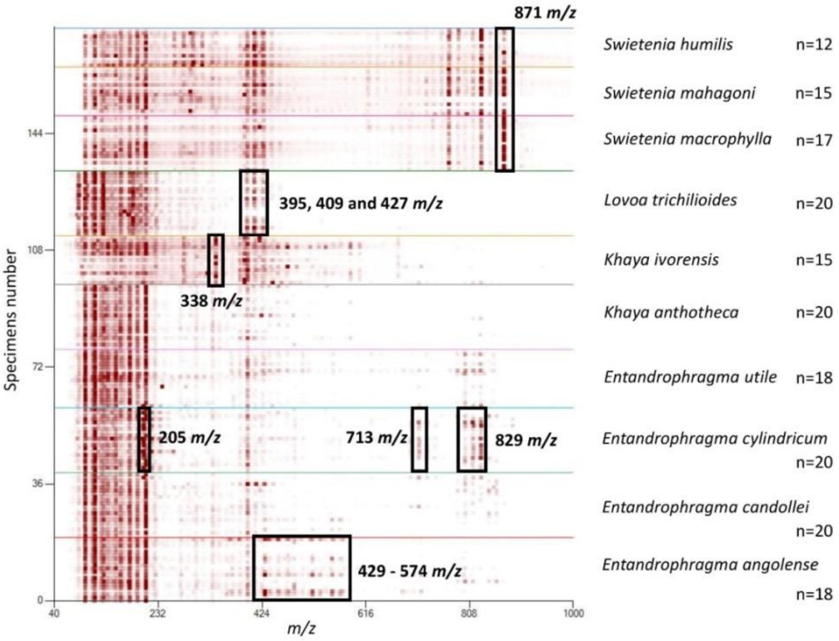


Figure 43: Heatmap showing the presence of the ions for the different specimens per species. Y-axis: specimen number with the chemotype grouped per species (n = the number of specimens for that species), x-axis: mass to charge ratio (m/z) for the detected ions. The color intensity of the squares is an indication of the abundance of the ions in the specimen. Diagnostic ions for each group of species are shown in black boxes.

Previous studies using DART TOFMS and the random forest machine learning algorithm (Deklerck *et al*., 2017; Finch *et al*., 2017; Paredes-villanueva *et al*., 2018) combined a 250 mDa (binning parameter) and 1% threshold (threshold parameter) for their classification. However, little is known about the effect of these parameters on the random forest classification accuracy.

The effect of the number of variables for the best binning and threshold combination on the accuracy and standard deviation can also be seen in Figure 44 Here, a 100 random forest models were run (with random number of variables) for the 40 mDa binning and 4% threshold combination. The blue line represents the LOESS fitted regression curve (local polynomial regression, fits a smoothed curve through scatter plot points) with 95% confidence intervals (grey band) (Cleveland *et al.*, 1992). When using less than 30 variables the accuracy is at a minimum, due to insufficient information for the classification algorithm to discriminate between species. The highest accuracy is achieved for 47 variables, after which the accuracy decreases with increasing number of variables. The lowest standard deviation however is achieved with 400 variables, although we still observe variation in the results.

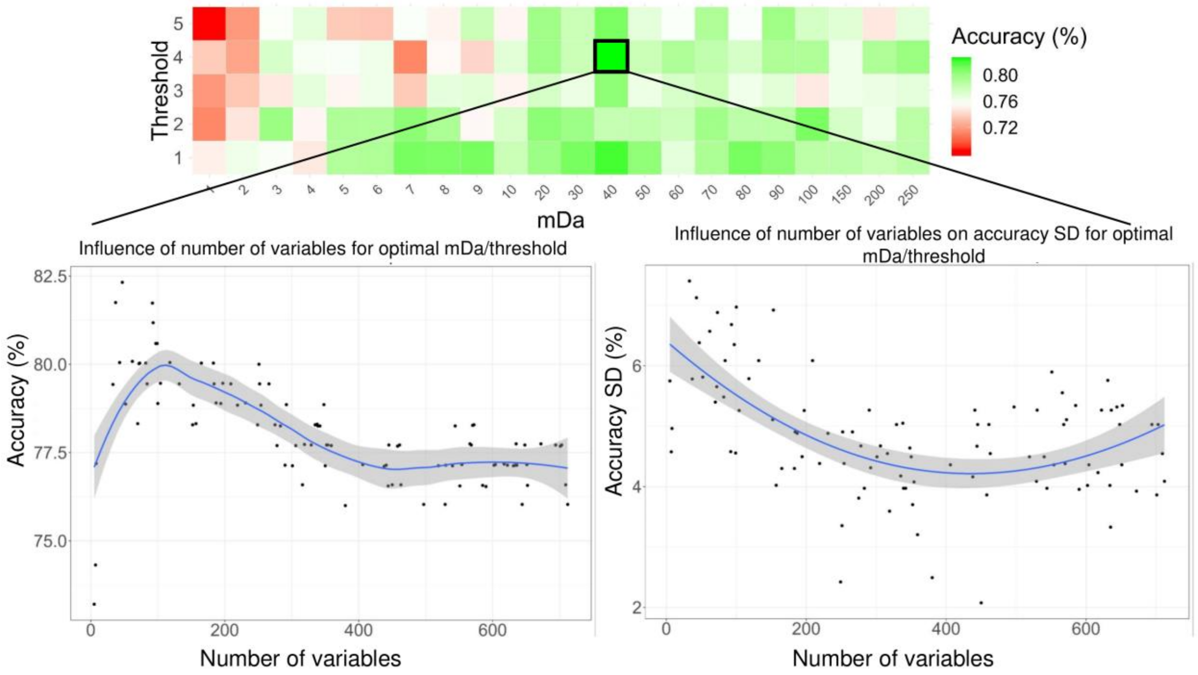
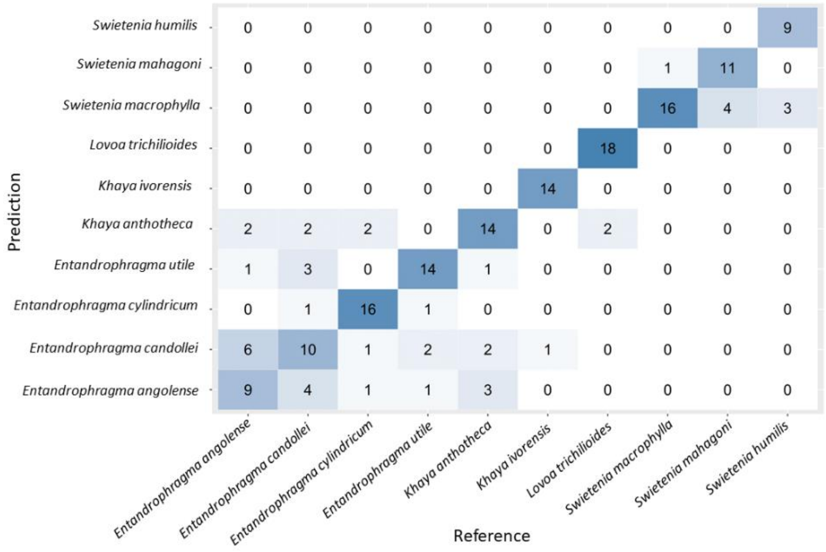


Figure 44: Above: Heatmap showing the random forest classification accuracy for each mDa binning size/threshold combination. Below: For the best mDa bin/threshold (40-4%) combination, both the effect of the number of variables on the accuracy (left) and the standard deviation (right) are shown for 100 random forest runs with a random choice of variable number (mtry). The blue line represents the LOESS fitted regression curve with 95% confidence intervals (grey band).

The confusion matrix after five-fold cross-validation (for more information see Deklerck et al. (2019a)) using the optimal pre-processing parameters and *mtry* settings can be seen in Figure 45. This allows us to evaluate the results of species identification and understand the correlation with the heatmap shown in Figure 43. We must note that this confusion matrix might slightly change when rerunning the cross-validation. However, the trends will remain the same, and the mis-identifications between species will remain.



Prediction	Entandrophragma angolense	Entandrophragma candollei	Entandrophragma cylindricum	Entandrophragma utile	Khaya anthotheca	Khaya ivorensis	Lovoa trichilioides	Swietenia macrophylla	Swietenia mahagoni	Swietenia humilis
Swietenia humilis	0	0	0	0	0	0	0	0	0	9
Swietenia mahagoni	0	0	0	0	0	0	0	1	11	0
Swietenia macrophylla	0	0	0	0	0	0	0	16	4	3
Lovoa trichilioides	0	0	0	0	0	0	18	0	0	0
Khaya ivorensis	0	0	0	0	0	14	0	0	0	0
Khaya anthotheca	2	2	2	0	14	0	2	0	0	0
Entandrophragma utile	1	3	0	14	1	0	0	0	0	0
Entandrophragma cylindricum	0	1	16	1	0	0	0	0	0	0
Entandrophragma candollei	6	10	1	2	2	1	0	0	0	0
Entandrophragma angolense	9	4	1	1	3	0	0	0	0	0

Reference

Figure 45: Out-of-sample confusion matrix (based on 5-folds) for the optimal mDa-threshold setting and random forest model. This is constructed by taking the sum of the 5 confusion matrices resulting from the 5-fold cross-validation.

Samples from *E. angolense* are most confused with *E. candollei* and *K. anthotheca*, and vice versa. This is in line with what can be noted in the heatmap, the three species have very similar chemical fingerprints. It has been shown before that *E. angolense* and *K. anthotheca* are difficult to adequately identify using texture analysis of anatomical transverse cross-sections (Rosa da Silva *et al.*, 2017), although there are minor anatomical differences. However, the classification success in the study by Rosa da Silva *et al*. (2017) is still higher, even with more species included, compared to using chemical fingerprints (74.8% to 57.1%). Although there is a group of ions for *E. angolense* (429 – 574 m/z) that are clearly unique, this is not consistent in all the samples. In the technique described by Rosa da Silva *et al*. (2017), *E. candollei* (97.3%) was very distinguishable from other *Entandrophragma* species. However, *E. angolense* and *E. utile* were harder to distinguish between each other, which is not a problem with chemical fingerprinting.

E. cylindricum is classified with high accuracy, as expected, since it has a unique chemical fingerprint showing three identifying ion groups (Figure 43). This is different from using convential light microscopy, where it is hard to distinguish between *E. cylindricum* and *E. utile*, especially when sampling is suboptimal.

There are some equivocal assignments between the different *Swietenia* – species, as their chemical fingerprint is similar. 25% of the *S. mahagoni* samples and a third of the *S. humilis* samples are being classified incorrectly as *S. macrophylla* (Figure 45). The difficulty of separating the *Swietenia* species was already indicated by Höltken *et al*. (2012) using DNA-analysis. Although the results are similar, as a technique, DART TOFMS shows fewer constraints for obtaining the wood samples; the only requirement is the need for heartwood. From the confusion matrix (Figure 45) it is clear that the classification of *Swietenia macrophylla* separately was more successful and similar to the results obtained by Bergo *et al*. (2016), although in their paper only four species were considered and species specific PLS-DA models based on NIRS were used. In addition, the random forest classification accuracy would greatly increase if only this *Swietenia* species was considered. The combination of wood images and convolutional networks achieved high classification success for both *Swietenia macrophylla* and *mahagoni* (100% and 91.4% resp.), yet *Swietenia humilis* was not included in that paper (Ravindran *et al*., 2018). Identification of species within the *Swietenia* genus using conventional light microscopy is less straightforward (Supporting Materials I2). It should be noted here that distinguishing between the genus *Swietenia* and *Khaya* using convential light microscopy is not straightforward, although this is not a problem with DART TOFMS spectra.

Both *K. ivorensis* and *L. trichilioides* perform well with only one or two sample(s) misidentified respectively. As shown in Figure 43, these species have a clear set of unique ions, which is consistent across all samples. In comparison, the method by Ravindran *et al*. (2018) to identify *K. ivorensis* performed poorly (76.1%), however different species from the Meliaceae family were included in that study. DART TOFMS could work complementary here, not only as a quick screening technique, but also to help identify difficult species. It should be noted here that the classification success for the discussed techniques highly depends on the parameters used and which samples or species that are included in the study.

Assessing the potential of DART TOFMS for geographical provenancing (Deklerck *et al.*, 2020)

As indicated above, an important question that needs to be answered in timber trade is where the timber comes from, or the geographical provenance of the timber. Although the previously mentioned techniques have shown promise (see above), another important aspect is the time and cost constraint. With containers waiting to be cleared in the harbour, a fast and accurate screening method is necessary to allow fast processing of shipments. This is where mass spectrometry might play a role. A first indication for the potential of mass spectrometry was the study by Pérez-Coello *et al*. (1997), where different origins of oak wood were discriminated based on volatile compounds. Recently, the speed and accuracy by DART TOFMS was shown for species identification (see Lancaster and Espinoza, 2012; McClure, Chavarria and Espinoza, 2015; Deklerck *et al.*, 2019a). In this technique, a wood sliver is introduced to an ambient ionization source where the small molecules held by the heartwood (or other plant tissue) are ablated from the surface. **The mass spectrum produced is in part due to the metabolome of the tree: the small molecule profile produced due to endogenous and exogenous factors impacting chemical expression of the genetics among other factors.** The use of DART TOFMS is gaining momentum for identifying tropical timbers given that it is minimally destructive for finished, high-value wood products, is a fast analysis and no sample preparation is needed, which is essential for time-restrained enforcement policies. Moreover, it is rather inexpensive compared to genetic or stable isotope analysis. However, its potential for individual tracking or even individual distinction is currently unknown. **Currently, studies concerning its potential for geographical provenancing are limited, however, since exogenous factors influence the chemical fingerprint, origin might be determined based on this variation.** Espinoza *et al*. (2014) distinguished wild from cultivated agarwood (*Aquilaria* spp.) based on single chemical fingerprints per specimen, with inferences to geographical region between China, Bornea, Vietnam and Thailand. Paredes-Villanueva *et al.* (2018) tried to classify multiple sampling sites within Bolivia for two Cedrela species based on DART TOFMS analysed heartwood samples but this proved to be difficult. Finch *et al.*(2017) differentiated coastal and Cascade populations of *Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii* in western Oregon based on three chemical fingerprints corresponding to separate tree rings along a pith – to – bark gradient. Time – series analysis based on mass spectra of rings can play a potential important role in assessing the site – specific conditions during the tree's life, providing as such the basis for geographical provenancing. This was already hinted at by Finch *et al.* (2017). Stem disks or radially oriented tree cores, which are easier to sample in the field, could be the optimal sampling strategy to start building towards fingerprints that allow a determination of the geographical provenance. As exogenous factors (for example: climate, site conditions,…) will influence the chemical structure within the growth rings we can use these rings as the basis for individual distinction and geographical provenancing. **We present here a proof-of-concept study to determine whether chemical fingerprints from pith-to-bark growth rings by DART TOFMS allow to distinct between (1)**

individuals from two species from the African tropical moist forest and (2) geographical provenances in West and Central Africa.

Heartwood slivers were collected from growth rings, referred to as samples along a pith – to – bark gradient from stem disks. Each stem disk is from a different individual, belonging to *Pericopsis elata* and *Terminalia superba* (Table 1.4 in Deklerck (2019)). Both species are valued timber species in the Congo Basin, and *Pericopsis elata* is even listed in the CITES Appendix II. For *Terminalia superba*, stem disks were collected in three study sites in the Democratic Republic of the Congo at the southern border of the Mayombe (two in the UNESCO Man and Biosphere Reserve of Luki, one near Tshela) and in in one study site in West Côte d'Ivoire. The stem disks of *Pericopsis elata* were sampled in Biaro (Democratic Republic of the Congo). For both species, stem disks were collected in previous studies and were archived in the Tervuren Wood Collection (RMCA) (De Ridder *et al*., 2014, 2013). The stem disks were cross-dated, which allowed taking wood slivers from individual rings (dated to the calendar year) as smallest sample unit. For the four *Pericopsis elata* disks (not for *Terminalia superba*), rings across multiple transects (sampling trajectories from pith to bark) were sampled. These transects were available from a previous study (De Ridder *et al.*, 2014) and they allowed to test potential intra-indiviual variability. All wood slivers were analysed with DART TOFMS and the text-files were exported to Microsoft Excel using a 5 mDa binning and 1% abundance threshold. Random forest in combination with cross-validation (see Deklerck (2019)) was used to determine whether it is possible to differentiate between individuals within a species (*Pericopsis elata* and *Terminalia superba*) and between countries for a species (*Terminalia superba*). In this report we will focus on the differentiation of *Terminalia superba* between the Democratic Republic of the Congo and Côte d'Ivoire. For more information on individual differentiation see Deklerck (2019).

Figure 46 shows the frequency (% of rings the ion is present in) of ions present in 50% or more of the tree rings for either CDI or DRC. Ions that could be identified in the KNApSack – database (search term *Combretaceae*) are beta-sitosterol (397.38 *m/z*), stigmasterol (395.364 *m/z*), trimethoxyphenanthrene (303.123 *m/z*), 2,7-Dihydroxy-3,4,6-trimethoxyphenanthrene (302.12 *m/z*), quinic acid (193.084 *m/z*, 194.085 *m/z*) and catechol (112.05 *m/z*). **Most notable ions are 177.054 *m/z* (present in 90.29 % of the rings for DRC, present in 28.13% of the rings for CDI), 191.072 *m/z* (69.78% – DRC, 11.17% – CDI) and 395.364 *m/z*, stigmasterol (3.60% - DRC, 52.13% - CDI). Figure 47 shows the resulting prediction accuracies of the random forest cross-validation for provenance allocation. Only 25 samples per country are needed to get a consistent 80% cross-validation prediction accuracy for both CDI and DRC.** For lower number of samples, the standard deviations are high. Sample identification for the DRC appears more difficult compared to CDI. The most important ions to discern between provenances, based on random forest classification with 25 random samples per country (10 repeats) and the Gini-index and MDA, are: 123.053, 134.07, 138.054, 186.075, 204.087, 251, 207.066, and 277.2 *m/z*. The random forest analysis on the country level with balanced training set and each *Terminalia superba* disk used as validation can be seen in Table 10. Overall, the tree rings of the different disks are successfully attributed to the correct country; however, the country allocation of Tw60802 (CDI) appears difficult with only 43% of the samples placed correctly.

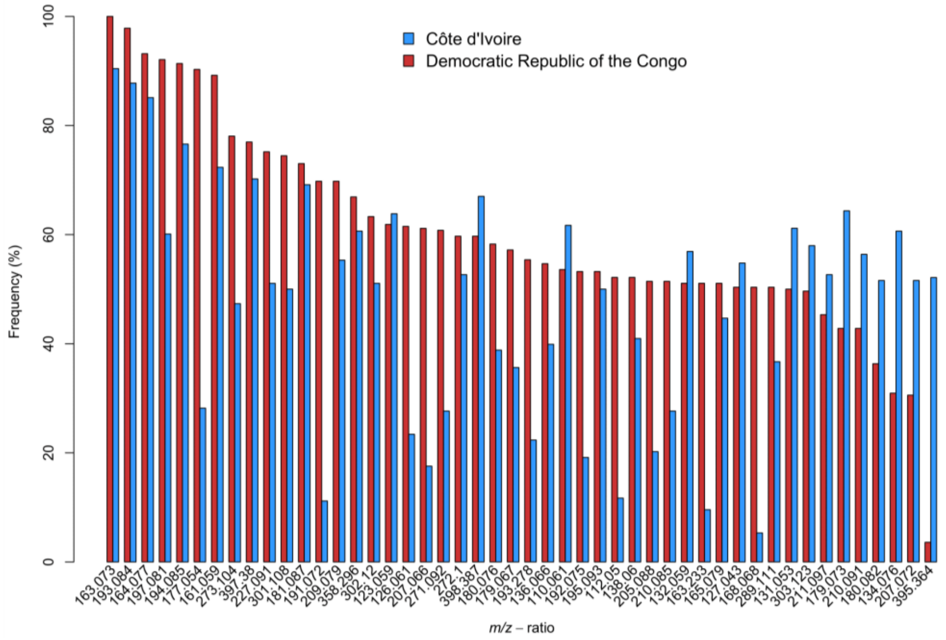


Figure 46: Barplot showing all ions that are present in 50% or more of the tree rings (Terminalia superba) for a certain provenance ranked from highest frequency (% of rings of the ion is present in) to lowest for the DRC. The corresponding frequency for the ion in the other country when it is not present in 50% of the treerings is shown as well. Y-axis indicated frequency (%) and the x-axis the m/z – ratio.

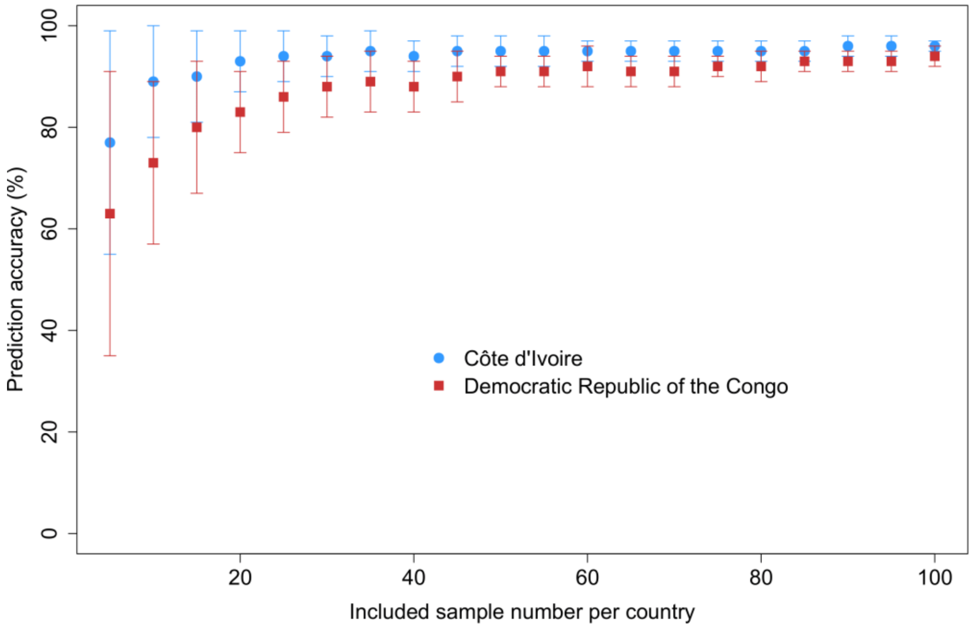


Figure 47: Prediction accuracy in function of the amount of samples for the provenancing of stem disks from Terminalia superba from Côte d'Ivoire and the Democratic Republic of the Congo through random forest analysis with 5 fold cross-validation. The black bars indicate the standard deviation per 100 runs.

Although stable isotopes analysis has shown its potential for agricultural products (see Kelly *et al*., 2005), the success using timber remains limited. Vlam *et al.* (2018) tried to trace back *Erythrophleum* spp. (tali) to concession in Cameroon and Republic of the Congo based on stable isotopes and DNA microsatellites. Concession assignment was successful with genetic markers, but not based on the isotopic signature, potentially due to low spatial variation in environmental conditions (Vlam *et al.*, 2018). Similar success with genetic methods was achieved by Jolivet and Degen (2012), who used DNA fingerprinting to determine whether cambium samples of *Entandrophragma cylindricum* (sapelli) originated from the forest concession in Cameroon. However, the degradation of the DNA in processed wood might be a problem (see also Höltken *et al.*, 2012).

Individual tracking will not always be possible (see Deklerck (2019)), and determining the provenance of timber might be necessary. **The effect of site-specific characteristics and local climate on the chemical fingerprint of an individual could be the basis for geographical provenancing, as individuals growing in a similar environment might have more similar chemotypes.** Species distribution plays an important role in geographical provenancing, it determines the potential exporting countries or regions. The distribution of *Terminalia superba* ranges from Sierra Leone to Angola (De Ridder *et al*., 2013; Groulez and Wood, 1985). Although there is currently no need to regulate the trade in *Terminalia superba*, it would still be interesting to collect tree cores in future fieldwork across the distribution pattern of the species, or a wide range of tropical species for that matter, to develop country or regional chemical fingerprints. As trees do not follow country borders, perhaps chemical fingerprints based on the local growing environment would make more sense. Paredes-Villanueve *et al.* (2018) stressed that site-specific characteristics and their influence on tree growth play a big role in geographical provenancing. Finch *et al*. (2017) mentioned that identifying climate responsive molecules should be possible by combining weather records with metabolite profiles. These molecules could then be the basis to determine the geographical provenance of timber based on differences in climate between regions. Although it should be noted here that other factors are likely to play a role as well, for example, soil, age, vitality of the tree and more. The two regions within the respective countries (DRC and CDI) where the stem disks were collected are both tropical but differ in average annual rainfall (see De Ridder *et al.*, 2013). The soils are pretty similar, with orthic Ferralsols and ferric Acrisols in the DRC study sites, and Ferralsols and Acrisols in the CDI study site.

Separating *Terminalia superba* between the CDI and DRC is possible, and the prediction accuracy increases with increasing sample amount. Differences in ion frequency between countries are shown in Figure 46. Some ions are a lot more frequent throughout rings for a certain country and could be a first indication. Important ions (*m/z*), based on the Gini-index and MDA do not show important frequency differences between countries (Figure 37), so relative abundance of the ion within a ring seems to be more important compared to frequency throughout rings. **Important to note is that when complete disks are removed from the dataset and used as validation, the results are ranging from 43.00 to 100 % (Table XII).**

Table XII: Country allocation per disk for Terminalia superba. The samples of the given disk are not included in the training set. The number is the number of samples per disk, the average prediction success (%success, % of rings placed in the correct country) and standard deviation (%std) across the 5 random forest runs are given.

Disk	Country	Number	%success	%std
Tw60796	CDI	50	94.50	4.56
Tw60799		50	72.00	3.58
Tw60802		20	43.00	2.45
Tw60804		29	96.55	3.78
Tw60815		39	70.26	4.17
Tw58841	DRC	25	100.00	0.00
Tw58842		20	86.00	5.83
Tw58843		27	92.59	4.06
Tw58844		33	98.79	2.42
Tw58845		20	79.00	8.60
Tw61004		36	97.78	1.11
Tw61005		35	100.00	0.00
Tw61006		50	89.20	1.60
Tw61009		32	93.13	1.25

Although most country allocations for a disk are correct, the rings of Tw60802 seemed difficult to attribute to the correct country, but the disk itself is clearly discerned from other disks (Figure 37). Possibly, the chemical fingerprint of this disk is more unique compared to other disks, even from CDI. Samples from the same individual are easily placed together, but not in a random sampling group across disks for country identification. Of course, trees do not follow political borders, and the country delineation is an artificial one. Not every tree within a country will follow the same exact chemical signature.

Metabolites of wood – added value of science in support of policy and development

Over 2000 DART TOFMS spectra (> 900 specimens, 55 species) were collected from Tervuren Wood Collection specimens, of which a part was added to the ForeST© database. This is the Forensic Spectra of Trees database, managed by the U.S. Fish and Wildlife Forensic Service. The database contains all spectra that are used to answer forensic timber identification questions with DART TOFMS. Due to the close collaboration between the Royal Museum for Central Africa and the U.S. Fish and Wildlife Forensic Laboratory, the RMCA can also use this database. Within HERBAXYLAREDD there has been significant progress on how to use DART TOFMS for timber identification and geographical provenancing. Especially when looking at the modelling aspect and classification accuracy optimization.

A close contact was also established with the Department of Environment (FOD-Belgium). Several partners of the HERBAXYLAREDD project joined environmental inspectors (responsible for CITES, EUTR and FLEGT) to the harbour of Antwerp for a sampling campaign. The object was to collect samples from random containers and try to identify the species based on DNA, wood anatomy and DART TOFMS. The results are currently being finalised. Several presentations have been given at the Department of Environment to explain DART TOFMS for timber identification. Finally, in December 2019, Victor Deklerck

(UGENT/RMCA – HERBAXYLAREDD) was invited by Frans Arijs (Coördinator Wetenschappelijk Comité CITES – België) to present DART TOFMS at the scientific working group of the European Union. Within the POLCARTIM project, a policy brief is being prepared at the Royal Museum for Central Africa that discusses the current capability of Belgium in timber identification and timber tracking.

Finally, Victor Deklerck became actively involved in the Global Timber Tracking Network (GTTN) (https://globaltimbertrackingnetwork.org/). GTTN groups scientists, policy makers and wood collections to enable a joint approach towards timber identification, on both the species and origin level. The Global Timber Tracking Network developed a sampling guide (Schmitz *et al*., 2019) that can be used as a basis for sampling expeditions, and that shows how to collect material for different identification or provenancing methods. Victor Deklerck helped writing this sampling guide, and a data-analysis guide is currently being created within GTTN.

4.3 Distribution mapping

4.3.1 Niche evolution within *Erytrophleum* (Gorel *et al*., 2019a)

In the Neotropics, relative high niche conservatism is generally assumed within genera, since genus resolution is considered to be suitable to identify floristic gradients (e.g. Higgins and Ruokolainen 2004). In contrasts, in Africa, a strong niche partitioning has been observed among congeneric species (see for instance Blach-Overgaard et al. 2010 for palms, Holstein & Renner 2011 for Coccinia). This strong niche differentiation among congeneric species was early demonstrated by White (1979) for *Diospyros* (Ebenaceae) and raised to the identification of major phytochories across Africa (White, 1979), and to the first comprehensive vegetation map for Africa (White, 1983). These phytochories (White, 1979) were confirmed numerically (Denys, 1980) and are still acknowledged in the most update biogeographical regionalization of Africa (Droissart *et al*. 2018, Linder *et al*. 2012). Examples of genera transcending biomes are quite common in Africa (Figure 48), while some tree genera have also been associated to specific biomes and floras (Linder, 2014).

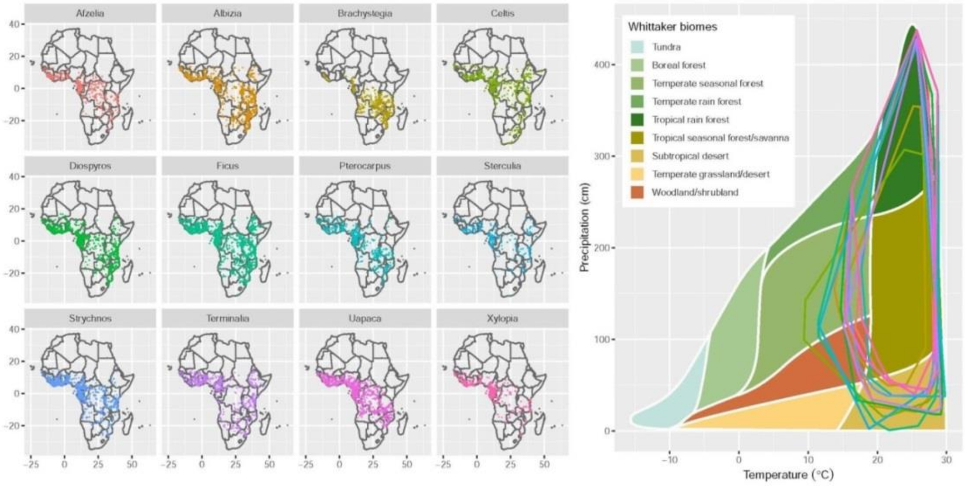


Figure 48: Examples of tree genera transcending the forest and savanna biomes in Africa, as shown by their individual distribution across Africa (left) and the environmental space they occupy in the Whittaker biome diagram (right, © Anais GOREL).

Among the set of genera transcending the forest and savanna biomes we selected the *Erythrophleum* genus (Fabaceae - Caesalpinioideae) within the HERBAXYLAREDD project as a case study (see Gorel, 2019), to deeply investigate trait and distribution, compiling field-based and herbarium-based evidence, in addition to the huge amount of information already available on the genetics (Duminil *et al*. 2010, 2013). Our study model, *Erythrophleum*, is a tropical tree genus widespread across Africa, and vastly investigated for genetics, that allowed to differentiate the two dense forest species, *E. ivorense*, characteristics of wet forests and *E. suaveolens*, characteristics of moist forests and extremely widespread across Africa, and gene pools among them. The two species are usually confounded in the field, and sold under the same commercial name, Tali (Gorel *et al*. 2019). The distribution of the savanna species *E. africanum* was also investigated, while *E. lasianthum*, absent from the Congo Basin, restricted to a small area in Mozambic, was not considered.

Bioclimatic constraints have been analysed on the distribution of three *Erythrophleum* species (*E. ivorense*, *E. suaveolens*, and *E. africanum*) and intra-specific gene pools of the two forest species (*E. ivorense* and *E. suaveolens*) in Africa, in order to identify the process of diversification (Gorel *et al.* 2019a). The isolation of populations inside forest refugia during past climate changes has widely been hypothesized as the major driver of tropical plant diversity, and investigated using genetic and phylogenetic approaches. Environmental conditions can also affect the inter- and intra-specific diversity by driving divergent selection leading to ecological speciation. Though needed to properly test hypotheses on diversification processes, the environmental space occupied by closely related species or gene pools is barely quantified. Species distribution models were used to determine the bioclimatic constraints on the distribution of closely related species and intra-specific gene pools of *Erythrophleum*. Specifically, the available phylogenetic data were combined with information on niche divergence to explore the role of ecology into diversification at the species and gene pool levels. **The results showed that ecological speciation through climate has played a key role in the evolution of the *Erythrophleum* species. The differential distribution and climatic niche of the species indicated adaptive divergence along rainfall gradients, that have probably been boosted by past climate fluctuations (Figure 49).**

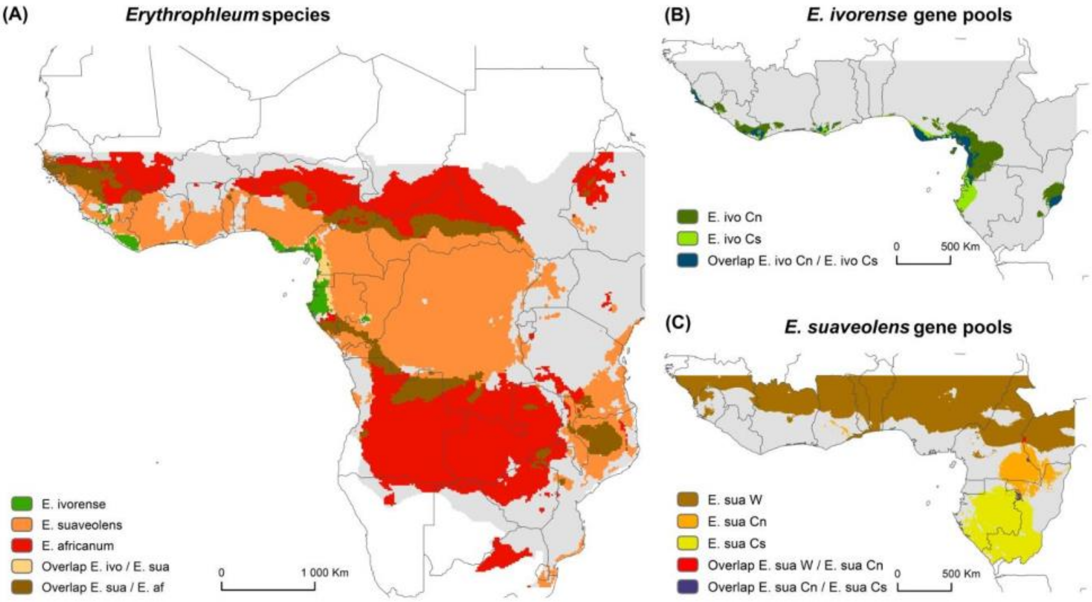


Figure 49: Potential distribution of Erythrophleum species (A), and of E. ivorense (B) and E. suaveolens gene pools (C, © Anais GOREL). Suitable areas were identified for the three species and five gene pools using a suitability threshold on the predictions of species distribution models. Overlap between pairs of species and pools are also given.

At the gene pool level, past climate changes during the Pleistocene have shaped genetic diversity, though within *E. suaveolens*, adaptive divergence also occurred. **The conclusion is that ecological speciation is a key mechanism of diversification for tropical African tree species, since such climatic niche partition exist among many other genera (Figure 48).** Modelling the environmental niche of closely-related taxa, and testing for niche differentiation, combined with divergence dates offered new insights on the process of diversification. A comparable approach could be used on many other genera for which niche partitioning is suspected among congeneric species, and for gene pools within largely distributed tree species as reviewed by Hardy *et al*. (2013).

4.3.2 The extent of forest and savanna distribution

The distribution of the forest and savanna biomes across Africa were studied and modeled, using floristic information. The manuscript, entitled “The extent of the forest and savanna biomes in Africa and implications for conservation and restoration” has been submitted to Nature (Aleman *et al*., submitted).

Tropical forests and savannas are important biomes that in some places represent alternative stable states (see also Deklerck *et al*., 2019c, *Rate of forest recovery after fire exclusion on anthropogenic savannas in the Democratic Republic of the Congo*). In the frame of the HERBAXYLAREDD we gathered already published woody species lists from specific savanna sites (Fayolle *et al*., 2019) that were combined to the species lists from specific forest sites already published (Fayolle *et al*. 2014). The environmental space shared by forests and savannas, i.e. the bistability area, was determined, such as the specific space used by forests and savannas, i.e. deterministic forests and savannas. **Two major environmental gradients were identified across Africa, with strong overlap between**

forests and savannas on the precipitation/aridity gradient, and strong partition of northern and southern savannas on the temperature/elevation gradient (Figure 50). Using floristic information for specific forest and savanna sites, we refined for Africa the distribution of the bistability area and of the deterministic forests and savannas, and our map showed slight/strong differences from earlier studies based on tree cover and climate, both remotely-sensed (Staver *et al*., 2011).

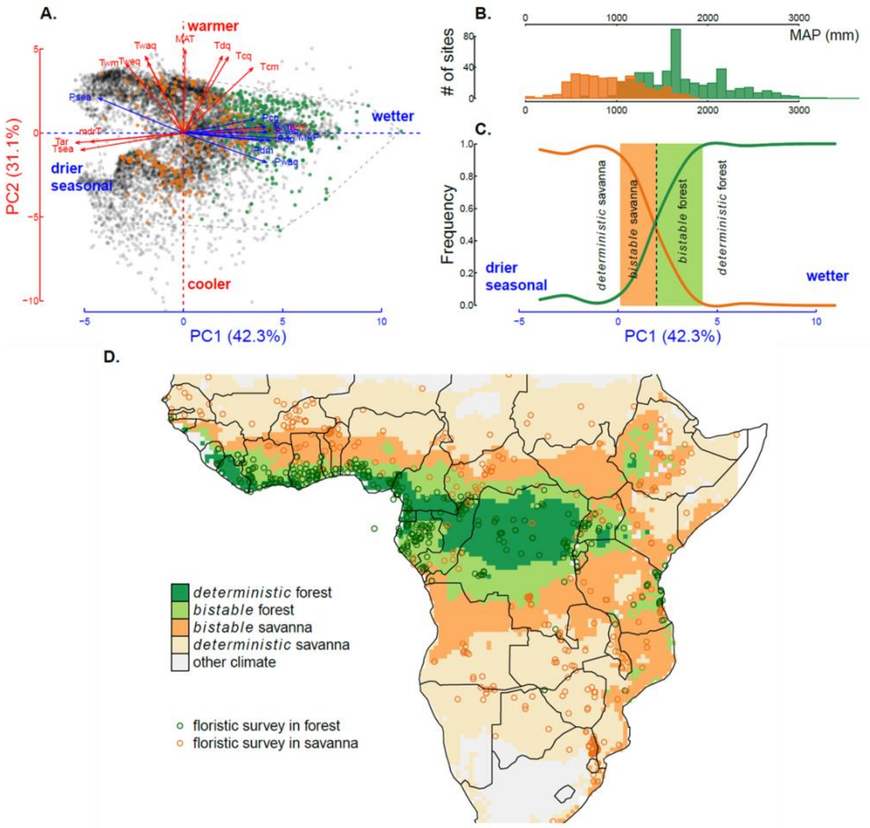


Figure 50: Climate envelopes of the forest and savanna biomes in Africa. (A) Climatic gradients were identified with a Principal Component Analysis of gridded climatic variables. One point corresponds to the centre of a 0.5° pixel, with pixels containing floristic surveys in forest (green) and savanna (orange) sites indicated. Red and blue arrows indicate the influence of temperature and precipitation variables, respectively. (B) Distribution of forest (green) and savanna (orange) sites along an axis of Mean Annual Precipitation (MAP, in mm). (C) Frequency distribution of forest (green) and savanna (orange) sites along a precipitation and seasonality gradient (PC1, A), with the climatic area where savanna and forest both occur plotted in light orange (where savanna is more common) and light green (where forest is more common). The dashed line corresponds to an equal probability of savanna and forest. (D) Map of deterministic and bistable forest, bistable and deterministic savanna, with location of floristic surveys in forest (green) and savanna (orange) sites. White pixels are outside the environmental range covered by the floristic surveys, as defined by a convex hull on the site scores on PC1 and PC2 (dashed line in A).

Generalist species, i.e. occurring in both forest and savannas, and specialist species of forests and savannas, were also identified using the species lists assembled. Specialist species and genera were retrieved for forest (Fabaceae - Caesalpinioideae) and savanna (Vachellia, Senegalia, Combretum), and interestingly, generalist species showed intermediate niche optimum on the precipitation gradient, but not necessarily larger niche breadth. The distribution of forest and savanna specialists, as well as generalist species, were expanded using the occurrence data derived from georeferenced collections (including that of Meise Botanic Garden, but also other collections). **While some tree genera were found to transcend the two biomes, the distribution of forest and savanna specialists, and of generalists, moreover confirmed the floristic specificity of the forest and savanna biomes, and demonstrated for the first time the mix composition of the bistability area, with only few generalist species (Figure 51).**

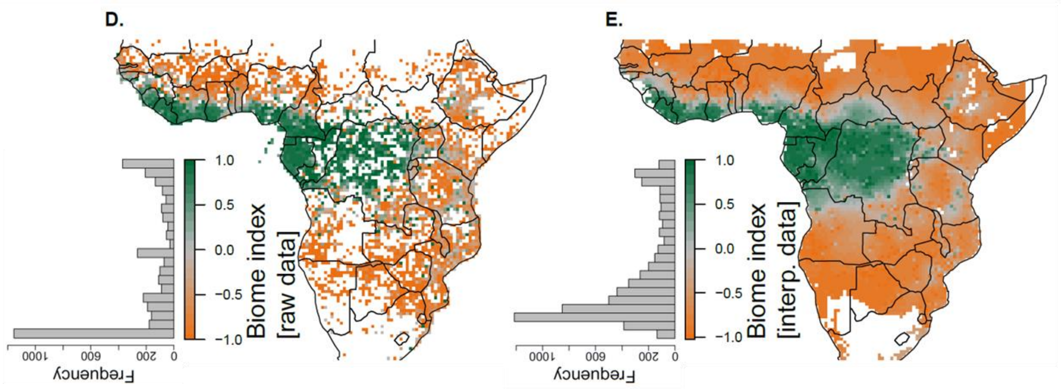


Figure 51: The biome index was computed at 0.5° resolution using (D) raw distribution data and (E) spatially interpolated distributions. White pixels in (D) correspond to a lack of data, and in (E) to areas outside the environmental range covered by the floristic surveys. For large areas with no data, such as eastern Angola, Sudan and South Sudan, the pure spatial interpolation might have led to oversimplification of the vegetation.

4.3.3 Distribution maps of *Scorodophloeus zenkeri, Prioria balsamifera and Staudtia kamurensis*

The distribution maps of *Pioria balsamifera* and *Staudtia kamurensis* can be seen in Figure 32 and Figure 29 respectively. The distribution map of *Scorodophloeus zenkeri* can be seen in Figure 52. These occurrence maps were created to visualize the distribution of genetic clusters within the respective species. Although these occurrence maps only include the samples for which the DNA was successfully amplified and analysed, they virtually cover the complete species' distribution range. Only the distribution map for *P. balsamifera* appears to be incomplete, since georeferenced records are lacking for some countries (e.g. Nigeria, Equatorial Guinea).

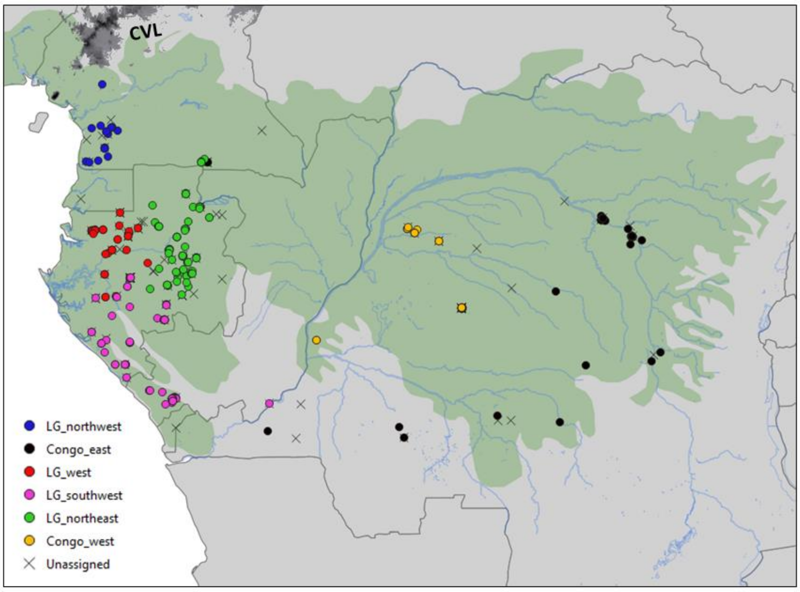


Figure 52: Distribution of genetic clusters in Scorodophloeus zenkeri as inferred with structure for the most likely scenario at K = 6. The green area depicts the natural distribution of rainforests in Central Africa. CVL Cameroon Volcanic Line

Distibution mapping – added value of science in support of policy and development

The species distribution provides spatial information for large scale biodiversity studies, which have a broader impact than purely for a scientific audience. This work is complementary with the RAINBIO project and allows guiding reforestation and plantation efforts, especially keeping in mind the potential distribution of a species.

4.4 Functional strategy

4.4.1 Trait evolution within the *Erythrophleum* genus

The variation in tree performance, tree hydraulics and wood anatomy associated with niche partitioning along the rainfall gradient, for the two forest species of *Eryhtrophleum* was also examined (Figure 53) (Gorel, 2019). Along rainfall gradients, shift in forest composition has been observed according to species drought-tolerance. However, the mechanisms to cope with drought are still poorly understood for tropical tree species, and hydraulics, wood traits and performance were used to examine niche partitioning among two congeneric species, *E. ivorense* and *E. suaveolens*, with known phylogeny and occupying contrasting environments in Africa (Gorel *et al*. 2019b). In the natural habitat, the vulnerability to cavitation, volumetric water content and capacitance and the underlying wood traits in branch and stem were quantified. Growth was also examined. A common garden was used to confirm that the observed differences are largely genotypic in origin rather than environmentally plastic.

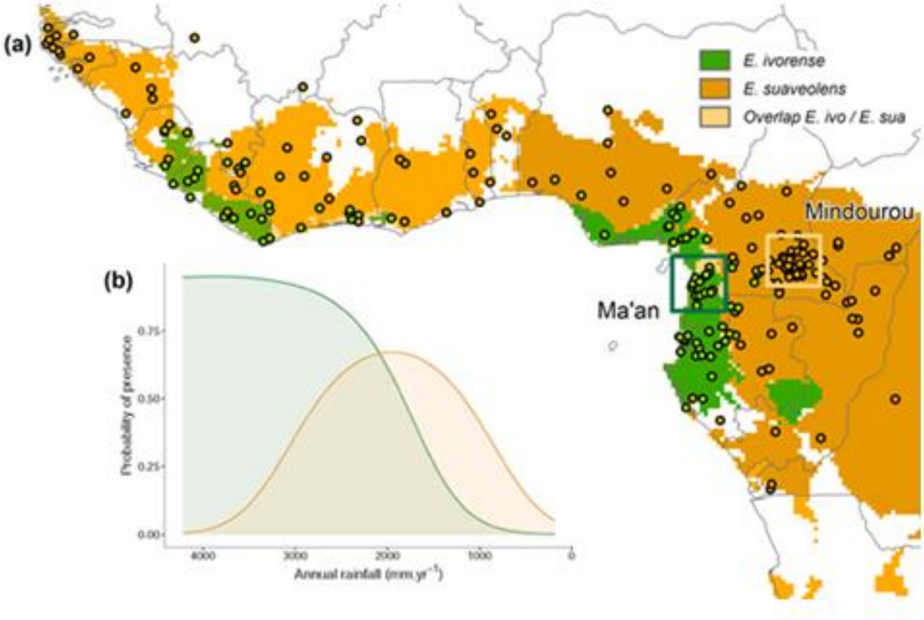


Figure 53: Species distribution and study sites. Potential distribution in Upper and Lower Guinea (a) and response curve (probability of presence) along the rainfall gradient (b) resulting from the distribution modeling of the two sister species Erythrophleum ivorense (green, wet forests) and E. suaveolens (orange, moist forest and savannas; occurrence data overlaid on the map). Note that the distribution of E. suaveolens is much larger across southern and eastern Africa, but genetic information is lacking (Duminil et al., 2010b). The location of the two study sites Ma'an and Mindourou in Cameroon, corresponding to the natural habitat of E. ivorense and E. suaveolens, respectively, are indicated on the map (colored squares). The common garden experiment was set up at the Mindourou site, i.e. in the natural habitat of E. suaveolens.

The wet forest species, *E. ivorense*, have wider vessels, lower vessel cell-wall reinforcement and wider intervessel pits than *E. suaveolens* (Figure 54). These traits allow a high hydraulic conductivity and the fast growth of *E. ivorense*, but confer high vulnerability to cavitation. These results confirm the trade-off between drought tolerance and growth rate as a key mechanism leading to niche partitioning along rainfall gradients. Lower water availability in drier environments excludes drought-sensitive species while in wetter environments drought-resistant species are expected to be outcompeted by drought-sensitive species with fast growth.

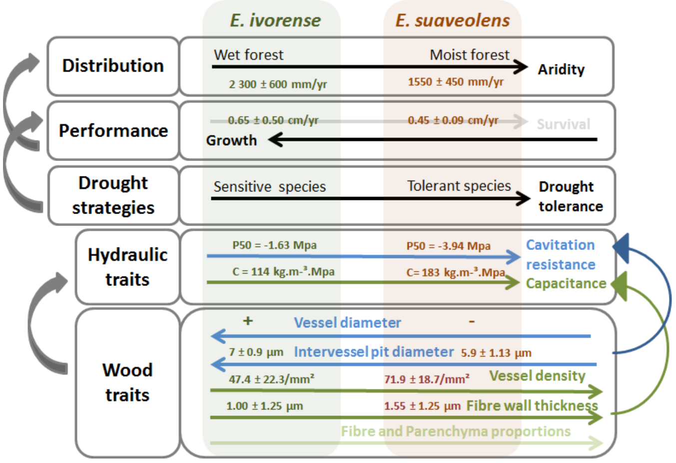


Figure 54: Multi-scale approach of the mechanisms behind niche partitioning along rainfall gradient between congeneric tropical tree species, integrating tree performance, tree hydraulics and wood anatomy, © Anais GOREL).

4.4.2 Functional strategy and growth

(1) Please see the Hubau *et al*. (2019) publication in ***nature plants*** for the full description of the results. They found that in spite of their much smaller size, in understory trees mean carbon age (74 years) is greater than in sub-canopy (54 years) and canopy (57 years) trees and similar to carbon age in emergent trees (66 years). The remarkable carbon longevity in the understory results from slow and aperiodic growth as an adaptation to limited resource availability. **The analysis by Hubau *et al*. (2019) reveals that while the understory represents a small share (11%) of the carbon stock, it contributes disproportionally to the forest carbon sink (20%). They conclude that accounting for the diversity of carbon age and carbon sequestration among different forest strate is critical for effective conservation management and for accurate modelling of carbon cycling.**

We also refer to the **Nature** publication by Hubau *et al.* (2020) for the full results and discussion. **The key result is that despite the past stability of the African carbon sink, the data by Hubau *et al*. (2020) suggest a post-2010 increase in carbon losses, delayed compared to Amazonia, indicating asynchronous carbon sink saturation on the two continents.** A statistical model including carbon dioxide, temperature, drought and forest dynamics accounts for the observed trends and indicates a long-term future decline in the African sink, whereas the Amazonian sink continues to weaken rapidly.

(2) The consistency of a functional scheme integrating leaf, stem and root traits, biomass allocation and stem anatomy was tested for 15 tropical tree species at the seedling stage (Mirabel, 2015). Trade-offs for resource use and the hydraulics of the plants shaped this functional scheme which was found to determine seedling growth (Mirabel *et al*. 2019). The functional strategy of 15 African tree species was assessed by (1) highlighting the structure of traits covariance and the underlying functional trade-offs, (2) inferring a whole-plant functional scheme and (3) testing the correlation of the functional scheme with plant performance for two early developmental stages (seedlings and saplings). A total of 10 seedlings for each of the 15 species studied were selected in a nursery in south-eastern Cameroon and 18 functional traits, including leaf, stem and root traits, biomass allocation and stem anatomy were measured. They assessed the height and diameter growth of the seedlings and the DBH growth and survival for the saplings of nearby plantations. Multivariate analyses highlighted the covariations among the functional traits of the leaf/stem/root, biomass allocation ratios and stem anatomy. **The major trait covariation axes were driven by two trade-offs, first between resource acquisition and conservation and second between hydraulic safety and efficiency (Figure 55).** The axes were integrated into a Bayesian network inferring a functional scheme at the wholeplant scale, which was found to predict the growth of the seedlings but not the performance of the saplings. The functional strategies of the seedlings were

determined by an integrated whole-plant scheme reflecting the trade-offs for resource use and plant hydraulics. The scheme predicted the growth of the seedlings through mechanistic pathways from the wood stem to all the plant traits, but it appeared to shift at the stage of the saplings.

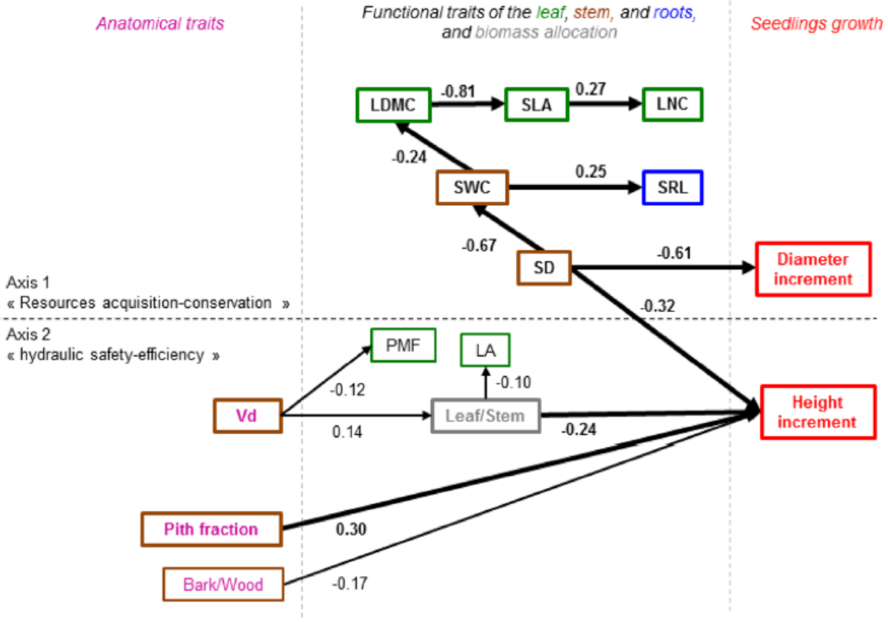


Figure 55: Directed acyclic graph (DAG) inferred from a Bayesian network analysis based on the functional traits determining more than 10% of the PCA axis and their relations to the seedlings' growth. The regression coefficients indicating the relationship between nodes were calculated from a mixed model with seedling species as grouping variable. The direction of the arrows was imposed from anatomical traits to functional traits, biomass allocation ratios and seedling's growth. Growth variables were defined as terminal nodes and no connections between traits defining the different axes were allowed.

(3) Ilondea *et al*. (under review) found that **precise increment measurements based on dated wood are advisable for small subsets of individuals, as is the case for the many less abundant species in a rainforest, and for functional species groups characterized by slow growth.** The dated wood approach made clear that many understory trees, with non-periodical rings, remain for a long time in a steady state. A dated wood approach offers also perspectives for studies of growth trajectories of individual trees which might be of importance for carbon assessments in degraded forests.

4.4.3 Reproductive traits and phenology

We also refer here to the work by Illondea *et al*. (2019 and under preparation) which can be found under 4.2.1 Leaf, seed and whole tree traits.

Phenology is a key biodiversity variable (Pereira *et al*. 2013) recognized by IPCC for monitoring response to climate changes. Strong evidence of shifts in reproductive phenology in response to global warming has been reported worldwide and for a variety of organisms (Walther *et al*. 2002) including plant species (Cleland *et al*. 2007). In this regard it is important to identify the climatic factors that control and impact reproductive phenology, and the huge amount of information stored in herbaria offers new opportunities for phenological studies (Lange *et al*. 2016), specifically in the tropics were phenological studies are scarce, much more recent in tropical than in temperate forests (Abernethy *et al*. 2018) and difficult and costly to implement in the field (Bush *et al*. 2018). Using collection date of fertile specimen is a complementary approach to tree crown observations in the field, that moreover offer large-scale analysis of reproductive phenology, as demonstrated by Zalamea *et al*. (2011) for the *Cecropia* genus across the Neotropics.

The temporal patterns in reproductive phenology for eight tropical African timber species and their spatial variation were studied (Figure 56) (Ouédraogo *et al*., 2018). Specifically, (1) the frequency and seasonality of reproduction at specific sites (n=7) in Central Africa was determined using field data, graphical analysis and circular statistics, and between-species and between-site differences were identified; (2) the latitudinal variation in the seasonality of reproduction at continental scale was investigated using herbarium data; and (3) the correlation between the spatial variation in the seasonality of reproduction and the seasonality of rainfall was tested. Annual or supra-annual flowering patterns were found, and most flowering events occurred during the dry season (21 out of the 25 populations studied). The analysis of herbarium specimens showed significant latitudinal variation in the timing of flowering and/or fruiting for five species, that was significantly related to the spatial variation in rainfall seasonality for three species (*Lophira alata*, *Milicia excelsa* and *Pterocarpus soyauxii*). **The results highlight the role of rainfall seasonality in the functioning of tropical African forests and the predicted change in rainfall seasonality due to global warming might thus severely affect these ecosystems.**

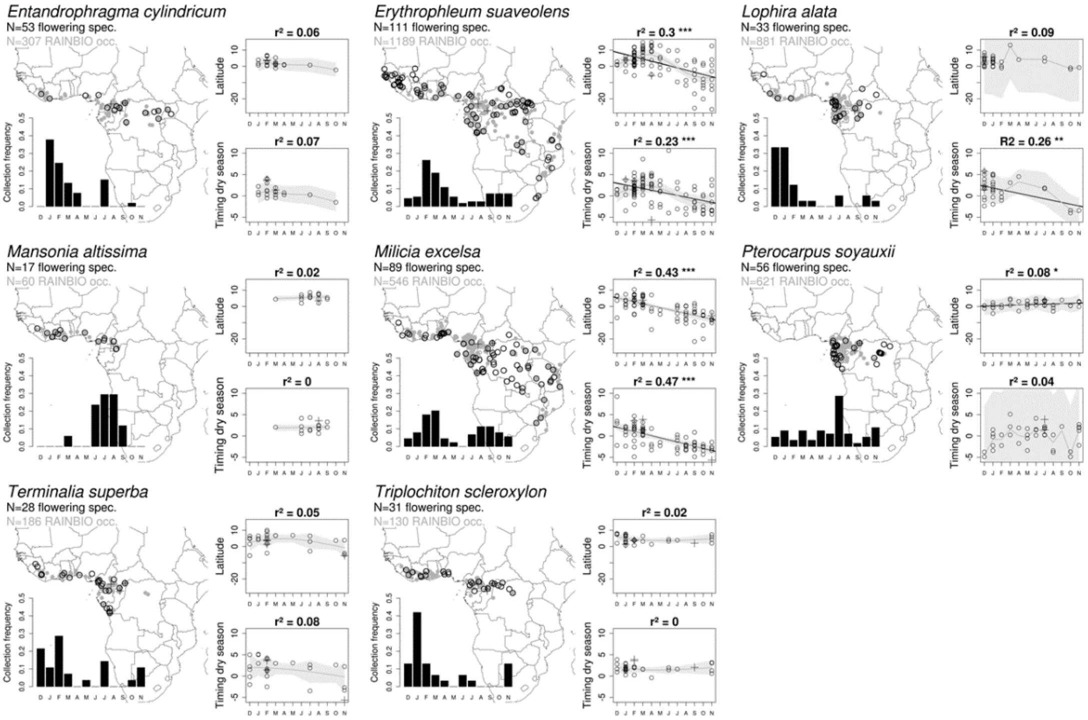


Figure 56: Location of flowering herbarium specimens (black circles, number N indicated in black) across Africa for the eight species (© Dakis OUEDRAOGO). Species spatial distribution i.e. location of RAINBIO occurrences (Dauby et al. 2016) is indicated with solid grey circles (number N indicated in grey), and crosses indicate field sites where the species has been surveyed. Insets show the

frequency of flowering herbarium collections by month (represented from December to November for clarity). Right panels show the relationships between the month of collection of flowering specimens and (top) latitude or (bottom) rainfall seasonality summarized by the first axis scores of a Principal Component Analysis on monthly rainfall, with positive scores indicating that the major dry season occurs in Dec-Jan-Feb, and negative scores indicating that the major dry season occurs in Jul-Aug. Trends are shown in grey (cubic smoothing spline fitted to the data), and significant correlations between latitude or timing of the dry season and month of collection are indicated with black lines and stars on the coefficient of determination r^2 (P-value < 0.05;, ** P-value < 0.01; *** P-value < 0.001). Crosses indicate the flowering month observed at field sites.*

Functional strategy – added value of science in support of policy and development

The presented data in this part allows guiding of seed collection for forest restoration and forest plantation. This based on native species instead of exotics such as Acacia, Eucalypt and so on.

4.5 Wood technology of lesser used species

A paradoxical relationship is the opposite relationship between two characteristics/properties then what we would expect from literature or known trends. As such, paradoxical species are species that have such a paradoxical relationship. For example, the general trend is that with increasing wood density, the amount of potential swelling increases as well. However, interesting timber species have the combination of medium to high wood density and low volumetric swelling (high dimensional stability). Several species could be identified with this interesting, valuable and paradoxical combination (*Afzelia* spp, *Pterocarpus* spp…) (see 4.2.4. Wood technological traits). **As we are now able to screen more species based on the developed techniques, we can now search for more paradoxical species. These species can play an important role in future timber use, mainly because they are currently overlooked as potential high-value species. This also means that we could shift from a single species focus towards a mix of species with similar characteristics for the same applications.** When we take it a step further, we could shift to a mix of specimens (and as such species) with similar characteristics.

Concerning the species for bio-energy we refer to the work presented by Esther Bustillo Vazquez and Laurent Raets (see 3.5.3 and online report).

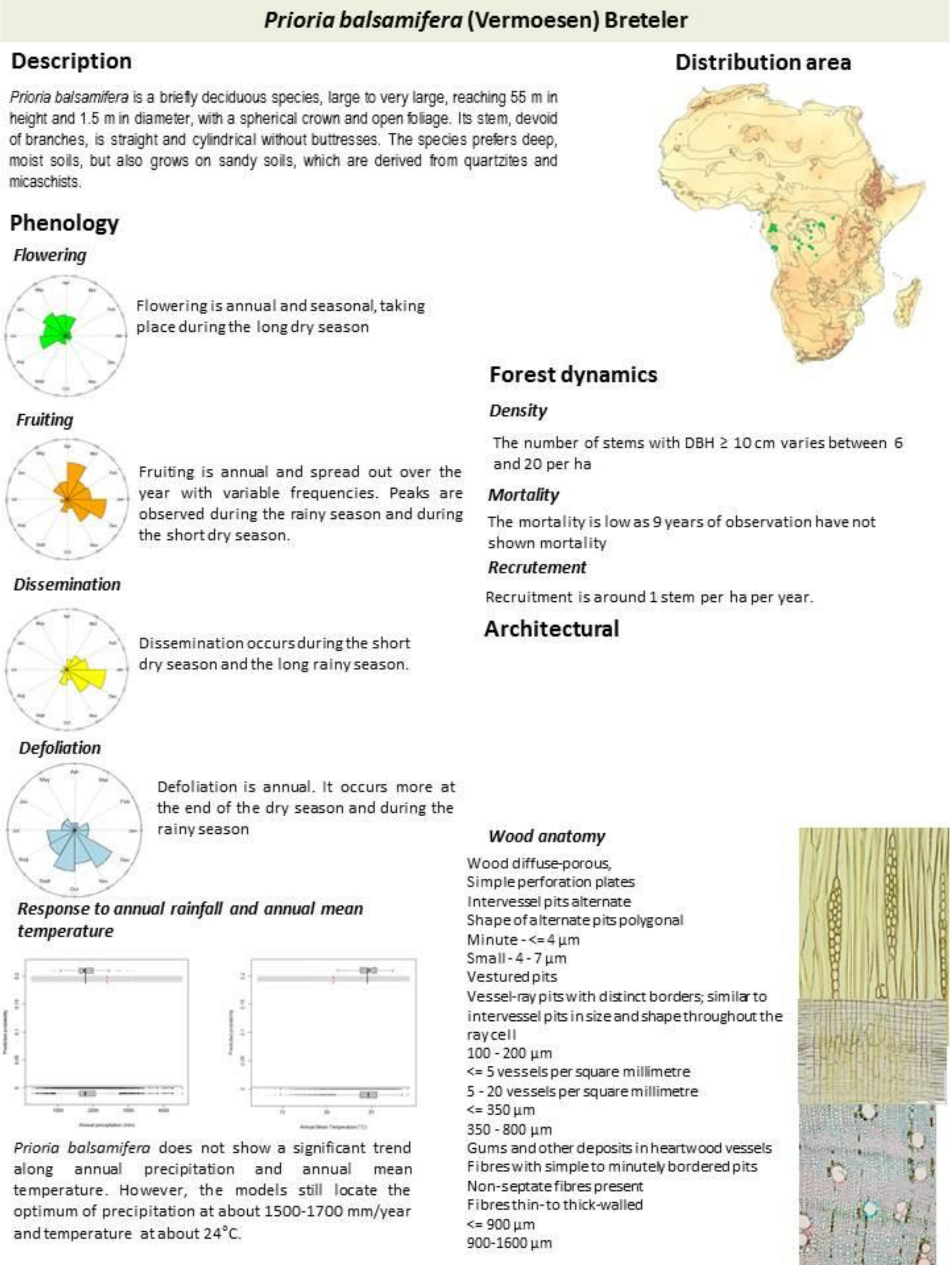
4.6 Identification keys/atlases

4.6.1 Visual keys

A visual key to identify the most important Congolese timber species based image material of their wood anatomical features has been updated and tested with trainees at the university of Kisangani and Antanarivo. For a full description, the specific pathway for identification and the actual software with the visual key, please visit: http://woodbiology.africamuseum.be/identificationkey. The Royal Museum for Central Africa (Hans Beeckman) also provided specimens for the “Atlas of Macroscopic Wood Identification” by Flavio Ruffinatto and Alan Crivellaro (2019). In the atlas there is a special focus towards timbers used in Europe and CITES-listed species. It offers anatomical descriptions and images but also information on natural durability and physico-mechanical properties.

4.6.2 Atlases with complete trait information

A draft example of the page for *Prioria* balsamifera in the atlas can be found in Figure 57. This is currently being prepared for all species.



Prioria balsamifera (Vermoesen) Breteler

Description

Prioria balsamifera is a briefly deciduous species, large to very large, reaching 55 m in height and 1.5 m in diameter, with a spherical crown and open foliage. Its stem, devoid of branches, is straight and cylindrical without buttresses. The species prefers deep, moist soils, but also grows on sandy soils, which are derived from quartzites and micaschists.

Phenology

Flowering

Flowering is annual and seasonal, taking place during the long dry season

Fruiting

Fruiting is annual and spread out over the year with variable frequencies. Peaks are observed during the rainy season and during the short dry season.

Dissemination

Dissemination occurs during the short dry season and the long rainy season.

Defoliation

Defoliation is annual. It occurs more at the end of the dry season and during the rainy season

Response to annual rainfall and annual mean temperature

Prioria balsamifera does not show a significant trend along annual precipitation and annual mean temperature. However, the models still locate the optimum of precipitation at about 1500-1700 mm/year and temperature at about 24°C.

Distribution area

Forest dynamics

Density

The number of stems with DBH ≥ 10 cm varies between 6 and 20 per ha

Mortality

The mortality is low as 9 years of observation have not shown mortality

Recrutement

Recruitment is around 1 stem per ha per year.

Architectural

Wood anatomy

Wood diffuse-porous,
Simple perforation plates
Intervessel pits alternate
Shape of alternate pits polygonal
Minute - <= 4 µm
Small - 4 - 7 µm
Vestured pits
Vessel-ray pits with distinct borders; similar to intervessel pits in size and shape throughout the ray cell
100 - 200 µm
<= 5 vessels per square millimetre
5 - 20 vessels per square millimetre
<= 350 µm
350 - 800 µm
Gums and other deposits in heartwood vessels
Fibres with simple to minutely bordered pits
Non-septate fibres present
Fibres thin- to thick-walled
<= 900 µm
900-1600 µm

Figure 57: Atlas page for Prioria balsamifera.

4.7 Future research

It is important to continue the collecting and archiving efforts as Africa is still undersampled in terms of plant and wood samples (Stropp *et al*., 2016). More efforts are needed in terms of logistical planning as leaf traits of tropical trees, and other more difficult ecophysiological measurements are hard to perform. Currently we heavily rely on statellite data (25*25km) for relating tree growth to climate, local meteorological stations would mean a big advancement in this area. As indicated in Hubau *et al*. (2020), Central African forest are less sensitive to recent climate changes compared to the Amazonia, and we need reliable climate data for further monitoring. Furthermore, trait-analyis could help explain why African species might be more adapted to drought conditions (see also Aguirre-Gutiérre *et al*., 2019). There is also the need for more long term sites which allow, for example, monitoring of forest dynamics and climate change (see for example the forest plot network indicated in Hubau *et al*., 2020). Attached to this is increased funding for research which is aimed at the long term and at the same time.

Future wood biological research should maximally focus on explaining detailed variability of wood anatomical features to understand the performance of individual trees within the ecosystem and eventually understand the vital rates of a forest community (regeneration, growth and mortality) which are responsive to climatic changes.

The methodologies developed to determine wood technological traits on xylarium specimens can now be used for a full screening of the xylarium. This allows discovering new interesting timber species and can help explaining the behaviour of several well know timber species. Future research should focus on developing a methodology to determine the Modulus of Elasticity of xylarium specimens. The xylarium also proved invaluable as reference collection for timber identification via DART-TOFMS as the sample requirement for this technique is small. However, little is known on the effect of potential pretreatment (heating…) of samples on the chemical fingerprint, this should be investigated further.

The genetic data generated in the frame of HERBAXYLAREDD is of great value for drawing-up management plans, conservation guidelines, and for timber tracking purposes. However, some (timber) species (e.g. *Prioria balsamifera*) are characterized by a weak genetic population structure, which often impedes accurate fine-scale origin determination when microsatellite markers are used. Therefore, future research will focus on high-throughput sequencing (HTS) methods, such as target capture sequencing, to assess whether those approaches can improve the spatial resolution for origin determination. Moreover, target capture data could be used to date population divergence and to assess the level of admixture in timber species. In addition, nuclear/chloroplast HTS data will be used to develop short family- or genus-specific barcodes that enable accurate species identification of unknown samples. The challenging part is that those barcodes need to contain enough discriminatory power at the species level, while being of short length, since DNA in (processed) wood samples is often fragmented and degraded.

5. DISSEMINATION AND VALORISATION

The partners within HERBAXYLAREDD took part in several international conferences and network meetings. Below is a list of the different conferences and presentations by the partners. The full list of publications is given in the next part. During the HERBAXYLAREDD-project, several meetings were organized between partners to optimise collaboration efforts and dissemination of the results. The results and recommendations given in this report, as well as future guidelines, have been disseminated by the HERBAXYLAREDD research group to the scientific community, policy makers (Belgian and international) and international organizations (World Wildlife Fund, World Resource Institute,…). Partners within the HERBAXYLAREDD project engaged in active discussions and meetings with the CITES department of the Department of Environment (FOD, Belgium). The partners also hosted the annual meeting of the Congo Basin Forest Partnership in the Royal Museum for Central Africa.

A project webstite was set-up (https://herbaxylaredd.africamuseum.be/) which allowed the different partners to give updates on their progress to a wider audience. The homepage consists of a full explanation of the project, complemented by an indicative figure of the different research subjects. The blog section gave interesting updates on both scientific progress and field missions. People interested in collaborating could contact the different partners on through the website and links were provided to both the Xylarium and Herbarium. A list was also given showing the publications made within HERBAXYLAREDD. Finally, partner projects were shown and download links are available for newly developed software.

Finally, a new proposal was submitted on Valorisation in the form of Policy briefs, where P1 (RMCA) can collaborate with GTTN, CITES and the FAO.

Victor Deklerck (Royal Museum for Central Africa, Ghent University) became part of the Global Timber Tracking Network (GTTN) and was present at several focus-group meetings with actors within GTTN (meetings: Forest Legality Meeting Washington D.C. 2017, progress GTTN meeting Wageningen 2018, database meeting Koli 2019). He wrote an article about the Society of Wood Science and Technology (SWST) and the Japan Wood Research Society (JWRS) conference in Nagoy (Japan) for the GTTN website. (https://globaltimbertrackingnetwork.org/2018/11/16/the-role-of-wood-science-and technology-in-a-sustainable-world/. He was also present at the 24th meeting of the CITES Plants Committee at the United Nations (2018, Geneva, Switzerland) and gave a radio interview on the German radio about the use of chemical fingerprints to identify timber. Victor Deklerck also gave a presentantion on timber identification at the SRG-workgroup meeting at the European Union in December 2019. Furthermore, press releases were organized about the Deklerck *et al*. (2019c), Hubau *et al*. (2019) and Hubau *et al*. (2020) papers. A sciencediscovery article was published on the Deklerck *et al*. (2019b) paper: https://sciencediscoveries.degruyter.com/sleeping-beauties-finding-timbers-future-searching-past/.

INTERNATIONAL CONFERENCES:

ATBC 2016 – Annual Meeting of the Association for Tropical Biology and Conservation (Montpellier, France, 19-23 June 2016)
Deklerck V., Van den Bulcke J., De Ridder M., Beeckman H., Van Acker J. (2016). Methodology to use Xylarium specimens to generate wood technological output.

Janssens S.B., Verstraete B., Hietz P., Ouédraogo D.-Y., Fayolle A., Delvaux C., Beeckman H., De Ridder M., Bogaerts A., De Smedt S. & P. Stoffelen (2016). Sleeping beauties are awaking: the story of the African herbarium of the Botanic Garden Meise, Belgium.

Vanden Abeele S., Hardy O, Janssens S.B. (2016). Population genetics of the tropical African tree *Staudtia kamerunensis* using newly developed microsatellite markers.

Verstraete B., Helsen K., Janssens S.B., Delvaux C., Beeckman H., Stoffelen P., Hietz P. (2016). Changes in functional leaf traits of Central African rainforest.

European Conference of Tropical Ecology (GTÖ) (Brussels, Belgium, 6-10th Feb. 2017)
Beeckman H. Public lecture within GTÖ on the importance of a xylarium in a context of tropical forest ecology and management.

Deklerck V., Gasson P., Finch K., Van den Bulcke J., Beeckman H., Van Acker J., Espinoza E.O. (2017). Species identification and separation based on chemotypes using DART-TOFMS.

Janssens S., Vanden Abeele S., Dauby G., Hawthorne W., Marschall C., Maurin O., Rejou-Mechain M., van der Bank M., Baya F., Beina D., Hardy O. (2017). How old is the African Flora? New insights in the evolution of African biomes based on large-scale dating and diversification analyses.

Vanden Abeele S., Hardy O., Donkpegan A., Migliore J., Janssens S. (2017). Phylogeographic study of the tropical African tree *Staudtia kamerunensis* using newly developed microsatellite markers.

Joint SYMPOSIUM Traits and function in the tropics, chairs: Vincent MERCKX, Sofia GOMES, Hans BEECKMAN, Maaike DE RIDDER, Adeline FAYOLLE

XXI AETFAT Congress (Nairobi, Kenya, 15-19 May 2017)
Vanden Abeele S., Hardy O., Donkpegan A., Migliore J., Janssens S. (2017). Population genetics of the tropical African tree *Staudtia kamerunensis* using newly developed microsatellite markers.

48th Annual Meeting of the International Research Group on Wood Protection (Ghent, Belgium, 4-8 June 2017)

Deklerck V., De Windt I., Defoirdt N.., Van den Bulcke J., Beeckman H., Espinoza O.E., Van Acker J. (2017). Assessing the natural durability for different tropical timber species using the mini-block test.

7th International Barcode of Life Conference (Krugerpark, South Africa, 20-24 November 2017)
Hardy O., Dauby G., Couvreur T.L.P., Droissart V., Rejou-Mechain M., Janssens S., Hawthorne W., Marshall C., Maurin O., van der Bank M., Thomas D., Kenfack D., Chuyong G., Beina D., Baya F. (2017). The phylogenetic structure of plant assemblages in tropical Africa: from local community to biogeographical scales.

10th World Dendro Conference (Thimpu, Bhutan, 02-22 June 2018)
Beeckman H. (2018). Tree growth in the rainforest understory.

18th Meeting of the CBFP parties (Tervuren, Belgium, 27-28 November 2018)
Beeckman H., De Mil T., Deklerck V., Hubau W., Mascarello M., Vanden Abeele S., Rousseau R., Van den Bulcke J., Janssens S. B. (2018). Identification of Congo Basin woods and enforcement of timber trade regulations.

Vanden Abeele, S., Hardy, O., Matvijev, K. & Janssens, S. B. (2018). Cryptic diversity in the African tree *Staudtia kamerunensis*.

6th Science week at University of Kisangani (UNIKIS) and Center for International Forestry Research (CIFOR) (Kisangani, DRC, 29 April 2019)
Beeckman H. (2019). Accurate measurements of tree increment.

50th Annual Meeting of the International Research Group on Wood Protection (Québec, Canada, 12-16 May 2019)
Deklerck V., De Ligne L., Van den Bulcke J., Espinoza O.E., Beeckman H., Van Acker J. (2019). Determining the natural durability on xylarium samples: mini-block test, wood powder and chemical profiling.

2018 Joint Convention: Society of Wood Science and Technology (SWST) and the Japan Wood Research Society (JWRS), Era of Sustainable World – Tradition and Innovation for Wood Science and Technology (Nagoya, Japan, 5-9 November 2019)
Deklerck V., Kondjo P., De Mil T., Beeckman H., Van Acker J., Van den Bulcke J. (2018). A new method for exploring and explaining dimensional stability of commercial and lesser-used wood species from the Congo Basin.

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6.1 Peer-reviewed publications

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Deklerck V., Finch K., Gasson P., Van den Bulcke J., Van Acker J., Beeckman H., Espinoza O.E. (2017). Comparison of species classification models of mass spectrometry data: Kernel Discriminant Analysis vs Random Forest; A case study of Afrormosia (Pericopsis elata (Harms) Meeuwen). Rapid Communications in Mass Spectrometry, 31: 1582-1588. https://onlinelibrary.wiley.com/doi/full/10.1002/rcm.7939

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6.4 Master thesis

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