Mangroves make a virtue of necessity

The vascular factor in the ecological success of *Avicennia* trees for survival in the precarious conditions of the mangrove forest

Elisabeth M. R. Robert

VUBPRESS

Mangroves make a virtue of necessity

Bomen zijn werkelijk.
Hun bladeren praten werkelijk
Met woorden veelzeggend en letterloos.

Hun toppen zingen.
Hun stammen zwijgen
hoorbaar.

Hun wortels houden
van de aarde.

Bij een boom
staande moet ik wel
ademen als een boom.

Naar een boom
ziende zie ik
hemel en aarde in elkanders
armen.

Want een boom,
een boom is een bruiloft.

HANS ANDREUS [1]

Print: Silhouet, Maldegem

2012 Uitgeverij VUBPRESS Brussels University Press
VUBPRESS is an imprint of ASP nv (Academic and Scientific Publishers nv)
Ravensteingalerij 28
B-1000 Brussels
Tel. +32 (0)2 289 26 50
Fax +32 (0)2 289 26 59
E-mail: info@vubpress.be
www.vubpress.be

ISBN 978 90 5718 121 4
NUR 922
Legal deposit D/2012/11.161/057

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The vascular factor in the ecological success of *Avicennia* trees for survival in the precarious conditions of the mangrove forest

Thesis

Submitted in fulfilment of the requirements for
the degree of Doctor of Philosophy in Sciences
of the Vrije Universiteit Brussel

by

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May 2012

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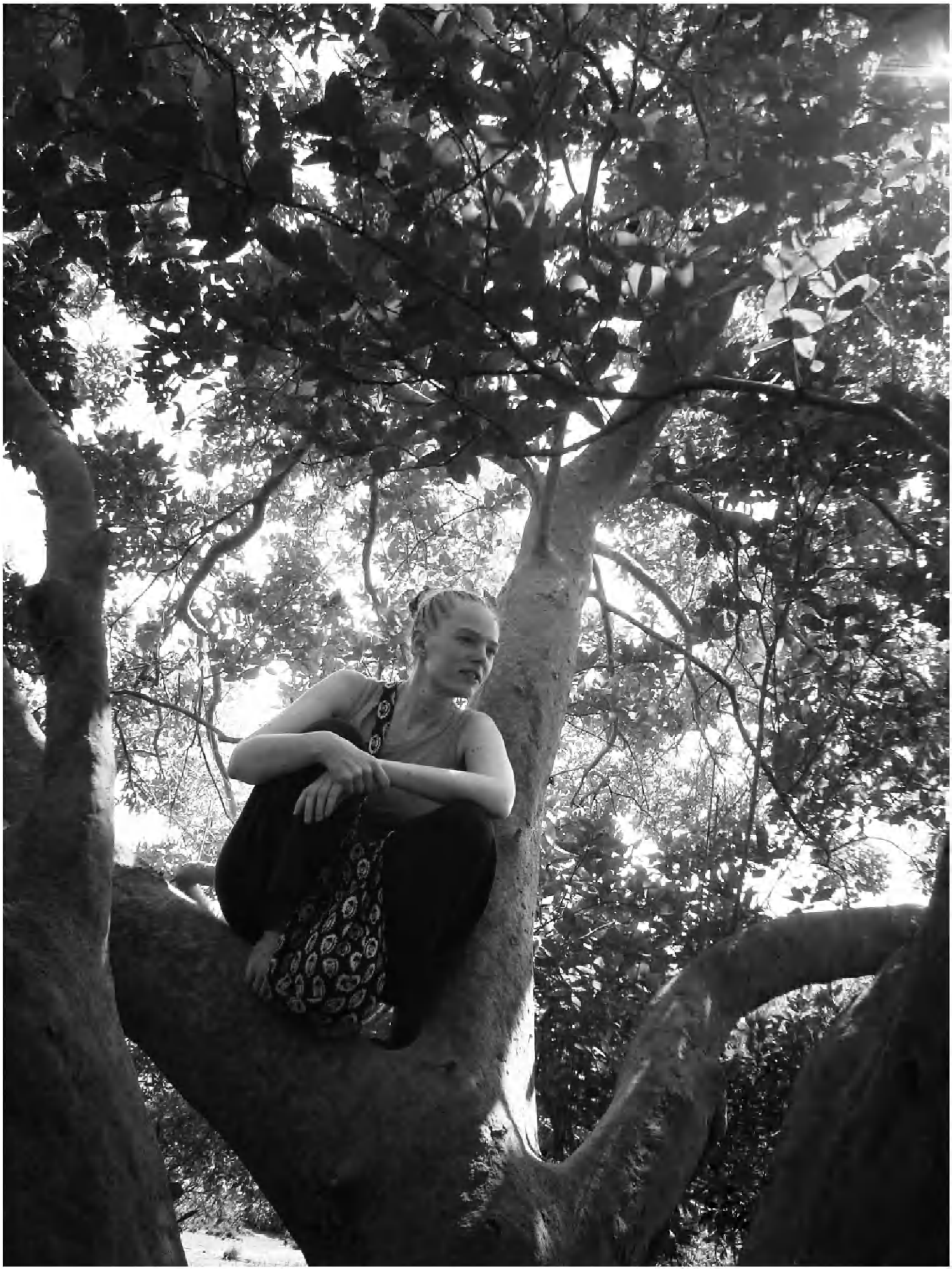
This research project was a collaboration between the Laboratory of Plant Biology and Nature Management (APNA) of the Vrije Universiteit Brussel (Brussels, Belgium) and the Laboratory of Wood Biology and Xylarium of the Royal Museum for Central Africa (Tervuren, Belgium). It was funded by the Agency for Innovation by Science and Technology (IWT, Flanders, Belgium). Research stays abroad were sponsored by the Research Foundation - Flanders (FWO, Flanders, Belgium), the Company of Biologists (COB) through the Society for Experimental Biology (SEB) and the European Cooperation in Science and Technology (COST) on the Terrestrial Biosphere in the Earth System (TERRABITES) via a Short Term Scientific Mission. Fieldwork expeditions were financially supported by the Research Foundation - Flanders (FWO, Flanders, Belgium), the Schure-Beijerinck-Popping Fonds (Koninklijke Nederlandse Akademie van Wetenschappen (KNAW), The Netherlands), the Flemish Interuniversity Council (VLIR, Flanders, Belgium), the King Leopold III Fund for Nature Exploration and Conservation (Belgium) and the 'Stichting ter bevordering van wetenschappelijk onderzoek in Afrika' (SBWOA, Belgium). The 'Universitair Ziekenhuis Brussel' (UZBrussel, Brussels, Belgium), SkyScan (Kontich, Belgium) and the Wageningen NMR Centre of Wageningen University (Wageningen, The Netherlands) enabled CT-scanning, micro-CT-scanning and MRI-scanning respectively. The Kenya Marine and Fisheries Research Institute (KMFRI, Mombasa, Kenya) and Burgers' Zoo (Arnhem, The Netherlands) contributed to the research.

Photos: Elisabeth Robert, Hilde Robert, Nele Schmitz



One should always be a little improbable.

OSCAR WILDE

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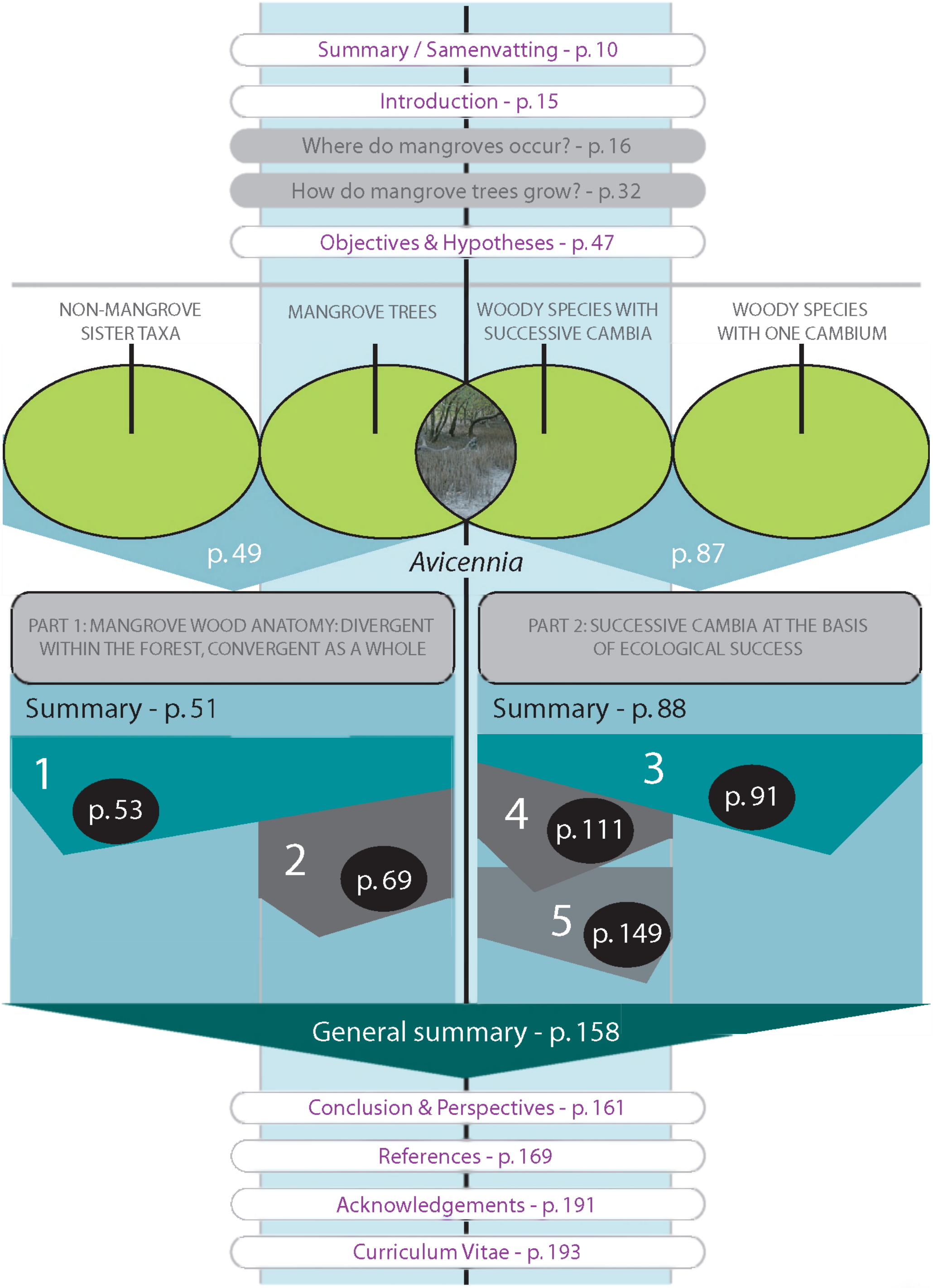

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Summary

Mangrove trees grow in an environment that is highly demanding for their water transport. Frequent tidal inundation may impede water uptake at the soil side, while in the prevailing tropical conditions, heat and wind are 'pulling' at the atmospheric side of the water column. On top, the mangrove environment is highly dynamic. Environmental conditions can quickly change in an unpredictable way. However, mangrove trees seem to make a virtue of necessity, thriving very well in these harsh environmental conditions. How can a water transport system of trees adapt to such environmental conditions? Is such adaptation part of the ecological success of the mangrove genus *Avicennia* in this environment as well as stretching its range into different climate zones? We address these two research questions through functional wood anatomy and ecophysiology, two research disciplines that search for anatomy-environment relationships both by physiological interpretation of observed internal structure and direct physiological experiments.

In **part one** of the study we investigate the requirements of the hydraulic system for survival in the mangrove environment and its adaptation to the gradient of environmental conditions within the intertidal zone. We compare the vessel characteristics of nine mangrove genera - representing all but one families with core mangrove species - with those of their respective non-mangrove sister taxa (Chapter 1). Furthermore, we assess the wood anatomy of *Avicennia marina* and *Rhizophora mucronata* - the two most widespread species of the only two pantropical mangrove genera - along the intertidal gradient in soil water salinity and inundation frequency of the mangrove forest in Gazi Bay (Kenya) (Chapter 2).

Our results show that survival in the mangrove forest requires a vascular system composed of numerous narrow water conducting vessels. This structure allows for bypassing air-filled vessel sections present to a greater extent in conditions of limiting water availability. With increasing soil water salinity and decreasing inundation frequency, the wood anatomy of mangrove trees has a more pronounced safety structure, *i.e.* numerous narrow vessels, and this at between-species and within-species level. *Avicennia* trees have the most pronounced hydraulic safety structure. This can partly explain the ecological success of the genus.

In **part two**, we focus on the study of the successive cambia in the genus *Avicennia*. Unlike in other mangrove trees, the radial growth of *Avicennia* trees occurs through several vascular cambia, characterizing the tree stem with internal phloem tissue. We investigate the possible ecological advantage this anatomical feature could offer *Avicennia* trees for survival in the mangrove environment, both by the presence of internal phloem (Chapter 3 and 5) and the special patchy nature of radial growth (Chapter 4). We make a three-dimensional reconstruction of the internal hydraulic structure of *Avicennia* trees along the intertidal gradient through (micro-)CT-scanning (Chapter 3), investigate the link between

species with internal phloem and drought-characterized habitats through a database analysis (Chapter 3), study the growth patterns of two *Avicennia* trees through a dendrometer analysis (Chapter 4) and assess the internal water content of three species with internal phloem, *Bougainvillea spectabilis*, *Avicennia marina* and *A.* cf. *officinalis*, a non-mangrove and two mangrove species respectively, through Magnetic Resonance Imaging (MRI) (Chapter 5).

The internal hydraulic structure of *Avicennia* is a complex network of xylem and phloem patches that can change extensively with small vertical distance. A large amount of internal phloem tissue is present in conditions that are highly demanding for the water transport, *i.e.* higher soil water salinity and less frequent inundation. Also, trees and shrubs with internal phloem mainly occur in habitats that are characterized by periodical or continuous physiological drought. This indicates the ecological significance of internal phloem tissue. Since internal phloem tissue shows high water content on MRI-scans, a high water storage capacity could allow trees with internal phloem to better overcome air-filling of vessel sections.

Avicennia trees show a high degree of patchiness in their anatomical structure and in the shrinking and swelling patterns of their stems. Different locations around the stem circumference can, but do not necessarily, react in a similar way to the prevailing environmental conditions. Correspondingly, radial stem increment can be patchy, but is not a systematic feature. In this way, *Avicennia* has a large adaptive toolbox of possibilities to interact with the environment and to optimally distribute available resources over the tree stem, dynamic both in space and in time, adding to the explanation for its ecological success within the mangrove forest.

We can conclude that the vascular factor is important in the survival of mangrove trees in their environment. It is the combination of a safe water conducting vessel system, an internal phloem tissue with high water content and a spatially and temporally dynamic growth system that contributes to the success of *Avicennia* in the mangrove environment, allowing for withstanding a gradient in soil water salinities and inundation frequencies.

This research adds to the general understanding of tree functioning since it has brought insight in important vascular factors for tree survival in harsh environmental conditions. Future research focusing on ecological wood anatomy in a phylogenetic context and on the link between anatomy and physiology could deepen these insights.

Samenvatting

Mangrovebomen groeien in een omgeving die veeleisend is voor hun watertransport. Frequente overstroming door zeewater bemoeilijkt wateropname uit de bodem, terwijl in de heersende tropische omstandigheden hitte en wind de vraag naar water verhogen aan de atmosferische kant van de waterkolom. Bovendien is het mangrovegebied zeer dynamisch. Omgevingscondities kunnen snel veranderen op een eerder onvoorspelbare manier. Toch lijken mangrovebomen van de nood een deugd te maken. Ze doen het namelijk goed in deze, voor bomen, moeilijke omgevingsomstandigheden. Hoe kan een watertransportsysteem van bomen zich aanpassen aan zulke omgevingsomstandigheden? Is deze aanpassing deel van het ecologisch succes van het mangrovegeslacht *Avicennia* in dit milieu en zorgt ze ervoor dat het geslacht kan voorkomen in verschillende klimaatzones? We benaderen deze twee onderzoeksvragen vanuit een functioneel houtanatomische en een ecofysiologische aanpak. In beide onderzoeksdisciplines wordt gezocht naar verbanden tussen anatomie en omgeving door fysiologische interpretatie van geobserveerde interne structuren en door directe fysiologische experimenten.

In **deel één** van de studie onderzoeken we wat de vereisten zijn voor een watertransportsysteem om overleven in het mangrovegebied toe te laten. We bestuderen ook hoe deze vereisten zich aanpassen aan de gradiënt van omgevingscondities binnen de intergetijdezone. We vergelijken de vatkenmerken van negen mangrovegeslachten - zij vertegenwoordigen op één na alle families met belangrijke mangrovesoorten - met die van hun respectievelijke zustertaxa (Hoofdstuk 1). Verder vergelijken we de houtanatomie van *Avicennia marina* en *Rhizophora mucronata* - de twee meest verspreide mangrovesoorten van de twee enige pantropische mangrovegeslachten - over de volledige intergetijdegradiënt in bodemwatersaliniteit en overstromingsfrequentie van het mangrovebos in Gazi Bay, Kenia (Hoofdstuk 2).

Overleven in het mangrovebos vereist een vasculair systeem dat is opgebouwd uit vele smalle vaten. Deze structuur laat toe de met lucht opgevulde delen van vaten - een veel voorkomend fenomeen bij beperkte beschikbaarheid van water - gemakkelijk te overbruggen. Met stijgende bodemwatersaliniteit en dalende overstromingsfrequentie heeft de houtanatomie van mangrovebomen een meer uitgesproken veilige structuur, *i.e.* vele kleine vaten, en dit op inter- en infraspecifiek niveau. *Avicennia*bomen hebben de meest uitgesproken veilige hydraulische structuur. Dit kan gedeeltelijk het ecologische succes van het geslacht verklaren.

In **deel twee** focussen we op de studie van de successieve cambia in the geslacht *Avicennia*. In tegenstelling tot andere mangrovebomen gebeurt de radiale groei in *Avicennia*bomen via meerdere vasculaire cambia. Hierdoor wordt de stam van *Avicennia* gekenmerkt door intern floëemweefsel. We onderzoeken welk potentieel ecologisch voordeel dit anatomisch kenmerk oplevert voor het overleven van *Avicennia*bomen in het mangrovegebied, en dit zowel door de aanwezigheid van intern floëem (Hoofdstuk 3 en 5) als door de bijzondere manier van gefragmenteerde diktegroei (Hoofdstuk 4). We maken een driedimensionale reconstructie van de interne hydraulische structuur van *Avicennia*bomen over de hele intergetijdegradiënt via (micro-)CT-scanning (Hoofdstuk 3), onderzoeken de link tussen soorten met intern floëem en habitats gekenmerkt door droogte via een database-analyse (Hoofdstuk 3), bestuderen de groeipatronen van twee *Avicennia*bomen aan de hand van een dendrometeranalyse (Hoofdstuk 4) en bepalen de waterinhoud van drie soorten met intern floëem, *Bougainvillea spectabilis, Avicennia marina* en *A.* cf. *officinalis*, een niet-mangrove en twee mangroven, via beeldvorming met magnetische resonantie (*Magnetic Resonance Imaging* - MRI) (Hoofdstuk 5).

De interne hydraulische structuur van *Avicennia* is een complex netwerk van xyleem- en floëemfragmenten dat sterk kan veranderen met minimale verticale afstand. Er is een grote hoeveelheid intern floëem in omstandigheden die veel van het watertransport vereisen, *i.e.* hogere zoutconcentratie en minder frequente overstroming. Bomen en struiken met intern floëemweefsel komen bovendien voornamelijk voor in habitats die gekenmerkt worden door periodieke of continue fysiologische droogte. Dit toont de ecologische betekenis van intern floëemweefsel aan. Omdat het intern floëemweefsel volgens de MRI-scans ook veel water bevat, zou een verhoogde wateropslagcapaciteit bomen met intern floëemweefsel kunnen toelaten hun met lucht gevulde vatdelen gemakkelijk weer op te vullen in tijden van droogte- of zoutstress.

*Avicennia*bomen hebben een hoge graad van fragmentatie in hun anatomische structuur en in de zwel- en krimppatronen van hun stam. Verschillende locaties rondom de stamomtrek kunnen verschillend of op dezelfde manier reageren op de heersende omgevingsomstandigheden. Ook de radiale groei kan al dan niet gefragmenteerd zijn. Op deze manier heeft *Avicennia* een waaier aan mogelijkheden om te interageren met haar omgeving en om de beschikbare hulpbronnen over de stam te verdelen op een dynamische manier in ruimte en tijd. Dit dynamische draagt bij tot het ecologisch succes van *Avicennia*bomen binnen het mangrovebos.

We kunnen besluiten dat de vasculaire factor belangrijk is in het overleven van mangrovebomen in hun omgeving. Het is de combinatie van een veilig houtvatensysteem, een met water gevuld intern floëemweefsel en een ruimtelijk en temporeel dynamisch groeisysteem dat bijdraagt tot het succes van *Avicennia* in het mangrovegebied en het geslacht doet overleven in een gradiënt aan zoutconcentraties van het bodemwater en overstromingsfrequenties.

Dit onderzoek draagt bij tot het algemeen begrijpen van het functioneren van bomen, aangezien het extra inzicht verschaft in de vasculaire factoren die belangrijk zijn voor bomen om te overleven in harde omgevingsomstandigheden. Verder onderzoek, toegespitst op ecologische houtanatomie in een evolutief kader en op het verband tussen anatomie en fysiologie, kan dit inzicht nog meer verdiepen.

The whole is more than the sum of its parts.

ARISTOTLE [3]

Introduction

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Mangrove trees occur in the intertidal zone between *ca.* 30°N and *ca.* 38°S. Of all mangrove genera, *Avicennia* has the largest latitudinal extent and is the most outspoken eurytopic genus growing along the entire intertidal ecotone.

2. What is so exceptional about the mangrove environment? - - - - - - - - - - - - - - - p. 18

Mangrove trees thrive in the peculiar environment of the intertidal zone, with high salinity and frequent flooding, which commonly constitute adverse and stressful conditions to plant life. This is exacerbated by the unpredictability and dynamic nature of the tropical intertidal environment.
> Robert & Schmitz *et al.* (2009) - Nature & Faune 24: 89-65 - - - - - - - - - - - - - - - - **p. 21**

3. What are the challenges for water transport in the intertidal zone? - - - - - - - - - p. 28

Water transport in the saline and drought-stressed conditions of the intertidal area is subjected to an increased risk of drought-induced cavitation, *i.e.* the formation of air bubbles in the vascular water column.

How do mangrove trees grow? - p. 32

1. Do mangrove trees show annual growth rings? - p. 32

Only in strongly seasonal conditions, mangrove trees form growth rings.
> Robert & Schmitz *et al.* (2011) - Trees - Structure and Function 25: 49-58 - - - - - - **p. 33**

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Avicennia trees are the only mangroves not having one cambium but successive cambia. These cambia generate a wood structured as a network of growth layers, allowing a basic growth rate all around the tree, with a more vigorous growth at one or several positions around the stem circumference.

Where do mangroves occur?

1. Where on Earth do mangrove trees grow?

Distribution area

Since mangrove trees are restricted to the intertidal zones in tropical environments, the geographical surface covered by mangroves is small. However, their north-south distribution, ranging from *ca.* 30°N to *ca.* 38°S, is large, as are the ranges that mangroves can cover along gradients of factors such as salt concentration of the soil water (from oligohaline to hyperhaline), substrate type (from muddy to sandy sediments) and inundation regime (from twice a day to twice a month or even micro-tidal systems). The two most widespread mangrove genera on global scale are *Avicennia* and *Rhizophora*, with *Avicennia* having a larger latitudinal extent than *Rhizophora* (Figure 1).

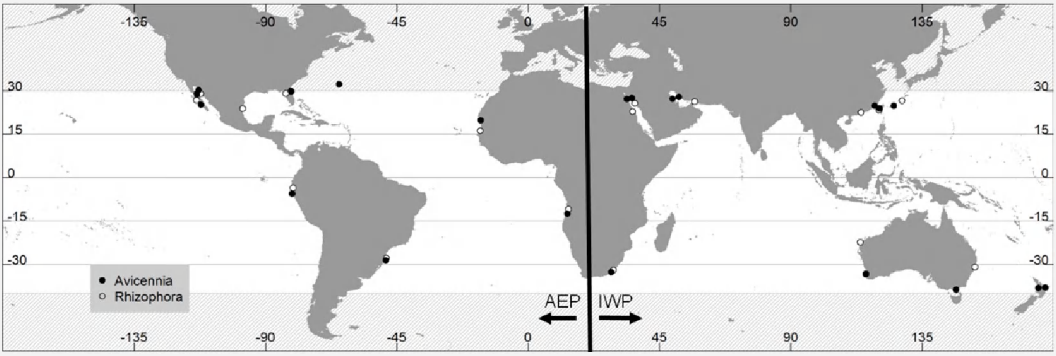


Figure 1 *- World map of the mangrove latitudinal limits for the genera Avicennia (full dots) and Rhizophora (open dots). AEP: Atlantic East Pacific Biogeographic Region; IWP: Indo-West Pacific Biogeographic Region.*

Range limits of *Avicennia* and *Rhizophora* are not defined by air or sea surface temperature on a global scale, *i.e.* no common isotherms defining mangrove range limits have been found. However, at more arid mangrove latitudinal limits, both *Avicennia* and *Rhizophora* have higher temperature requirements. In general, *Avicennia* can tolerate colder monthly mean temperatures than *Rhizophora*, possibly explaining the larger latitudinal extent of *Avicennia* spp. Along a same coastline, it is temperature that prevents *Rhizophora* from extending towards the colder *Avicennia* limit.

Trees - Structure and Function, accepted
Temperature variation among mangrove latitudinal range limits worldwide.
Katrien Quisthoudt, Nele Schmitz, Christophe F. Randin, Farid Dahdouh-Guebas, Elisabeth M.R. Robert and Nico Koedam
2012

Species distribution within the mangrove forest

Although a common species zonation pattern across mangrove forests worldwide is lacking, not all mangrove genera are able to grow along the entire intertidal ecotone (Tomlinson, 1994; Dahdouh-Guebas *et al.*, 2004; Spalding *et al.*, 2010). For example, *Rhizophora* is generally restricted to the seaward side of the mangrove forest where soil water salinity and inundation frequency are rather constant, while *Avicennia* can be found along the entire intertidal zone exposed to wide ranges of the same environmental factors (Figure 2). This indicates that, on a local scale, a difference exist between (i) more stenotopic mangrove genera, only capable of thriving in a narrow range of environmental conditions and (ii) more eurytopic mangrove genera, adapted to a wide range of environmental conditions in the mangrove forest.

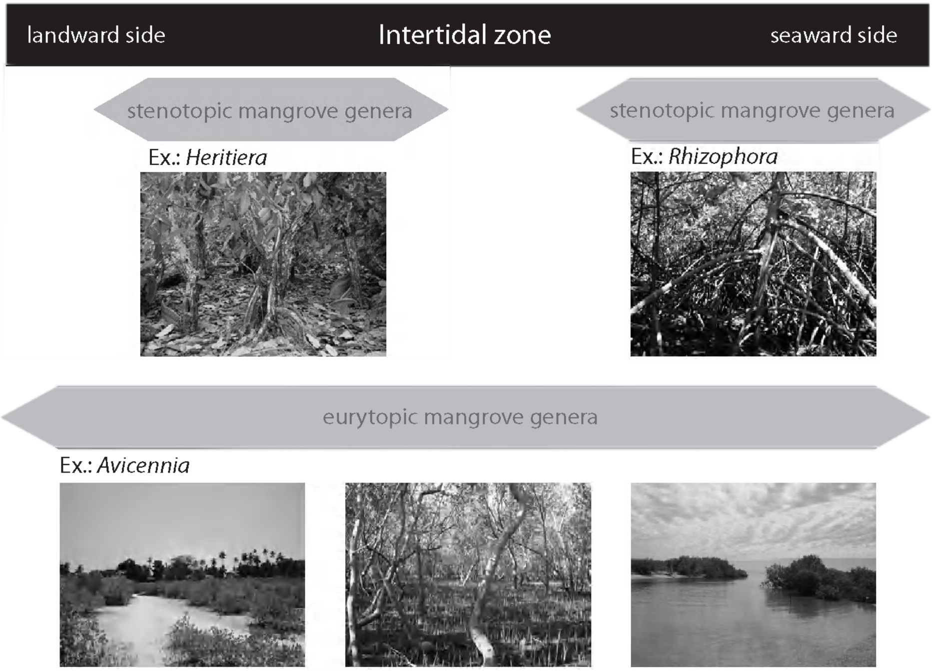


Figure 2 *- Distribution of mangrove genera along the intertidal ecotone. A distinction can be made between (i) stenotopic mangrove genera, occupying only a part of the intertidal zone and (ii) eurytopic mangrove genera, able to grow along the complete intertidal ecotone. As an example, the genera* Rhizophora, Heritiera *and* Avicennia *are depicted.*

Mangrove trees occur in the intertidal zone between *ca.* 30°N and *ca.* 38°S. Of all mangrove genera, *Avicennia* has the largest latitudinal extent and is the most outspoken eurytopic genus growing along the entire intertidal ecotone.

2. What is so exceptional about the mangrove environment?

The mangrove environment is harsh for trees because of the combination of salinity and flooding stress in a tropical environment (Figure 3). On top of this, the environment is extremely dynamic and unpredictable, with flooding levels and soil water salinities that can change within an hour due to inundation, rainfall and evaporation (Robert & Schmitz *et al.*, 2009 - p. 21). Possession of pneumatophores (Figure 4), processes such as salt exclusion, and in some species also salt secretion and viviparous development allow mangroves to thrive in this environment (Tomlinson, 1994).

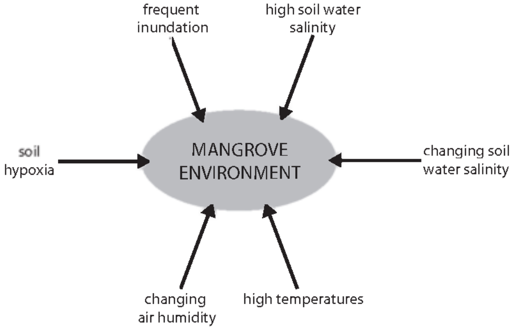


Figure 3 *- Most important environmental factors defining the mangrove environment.*

In order to survive in saline conditions, plants need to regulate cellular Na^+, Cl^- and K^+ concentrations (Flowers & Colmer, 2008). Mangrove trees deal with this salt stress by filtration of the soil water at root level and/or by secretion of salt crystals via salt glands on their leaves (Scholander *et al.*, 1962; Tomlinson, 1994; Sobrado *et al.*, 2004; Ye *et al.*, 2005). Despite these adaptations, growth, stature and productivity of mangrove trees decline with increasing salinity (Ball & Farquhar, 1984; Feller, 1995; McKee *et al.*, 2002; Lovelock *et al.*, 2004; Lovelock *et al.*, 2006; Naidoo, 2006; Naidoo *et al.*, 2011). This can be coupled to the morphological, physiological and ultrastructural changes in mangrove leaves under extreme salinity stress (Naidoo *et al.*, 2011). Examples are: reduced leaf production (Hoppe-Speer *et al.*, 2011) and smaller leaves with thicker cuticles (Naidoo *et al.*, 2011), decrease in stomatal conductance (Sobrado & Ball, 1999; Yan & Guizhu, 2007; Naidoo *et al.*, 2011) and swelling and desintegration of chloroplasts and mitochondria (Naidoo *et al.*, 2011).

For mangrove trees, salinity stress is combined with flooding. Flooded soils become oxygen deficient and accumulate chemical compounds and ions that negatively affect plant growth (Barrett-Lennard, 2003; Colmer & Flowers, 2009). Aerial roots of mangrove trees contain aerenchyma tissue, with air spaces that allow rapid diffusion of oxygen from lenticels to submerged or underground roots. Therefore, they allow mangrove trees to cope with the low oxygen conditions of their habitat (Scholander *et al.*, 1955; Purnobasuki & Suzuki, 2005). However, flooding tolerance differs between the different mangrove species, with

Avicennia considered the most tolerant (He *et al.*, 2007). Also, the duration of inundation influences the plant growth and performance (Ellison & Farnworth, 1997; Hoppe-Speer *et al.*, 2011), with infrequent and continuous inundation as the less favourable since they are associated with very high salt and very low oxygen concentrations respectively.

The dynamism of the mangrove environment demands a high flexibility from the organisms that live in this environment. Not only salt and flooding stress have to be tolerated, but also soil drought and increased salinity after periods of evaporation at locations of the mangrove forest that are not daily inundated. Rainfall and sunshine and their temporal patterns are extra variables that strongly influence water availability. Trees need to adopt a water transport system that can handle water qualities from fresh to highly saline, whether abundant or scarce. All aspects combined, the fact that organisms and especially long living and large trees can cope with this apparently hostile environment is most fascinating. It also offers the possibility to gain insight in the functioning of the water transport in trees and the limits imposed on this vital process.

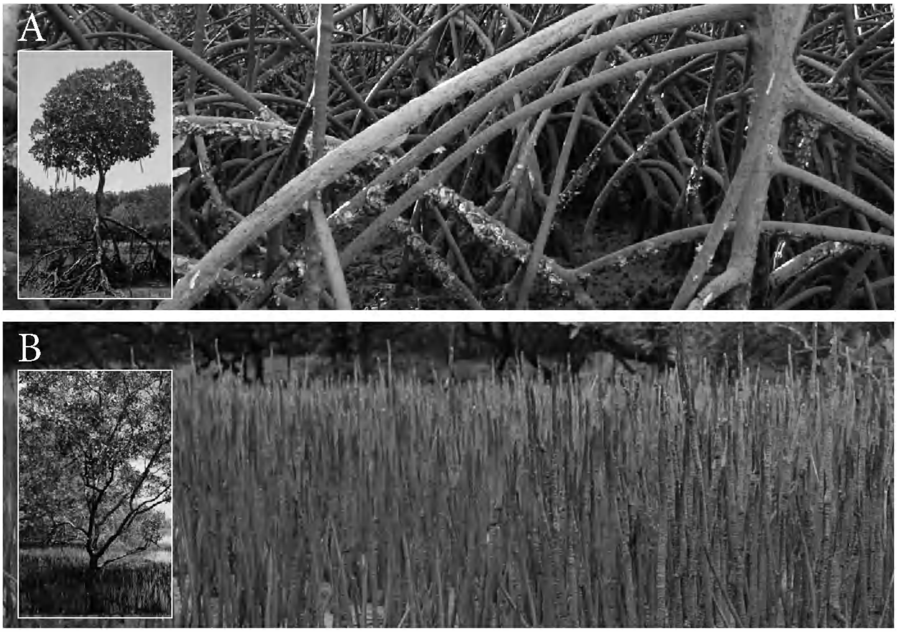


Figure 4 *- Pneumatophores of* Rhizophora mucronata *(A) and* Avicennia marina *(B) - insets: whole-tree pictures.*

Mangrove trees thrive in the peculiar environment of the intertidal zone, with high salinity and frequent flooding, which commonly constitute adverse and stressful conditions to plant life. This is exacerbated by the unpredictability and dynamic nature of the tropical intertidal environment.

Salinity fluctuations in mangrove forest of Gazi Bay, Kenya: lessons for future research

Published in: Nature & Faune 24: 89-95

Abstract

Studies on mangrove ecosystems that deal with the interaction of organisms and their environment very often draw conclusions based on only a restricted number of soil water salinity measurements. As inundation by salty water is the most typical characteristic of the mangrove environment, the authors addressed the temporal and spatial fluctuation of soil water salinity at seven locations in the mangrove forest of Gazi Bay, Kenya. As a pilot study, the research team measured soil water salinity twice a month, at neap tide and at spring tide, during one year. It can be concluded that the soil water salinity in mangrove forests can be highly variable in time as well as in space and depends on a complex interaction between inundation frequency, canopy closure, fresh water input and soil texture. Mangrove researchers should therefore pay attention to the differences in local site conditions inside the mangrove forest and conduct salinity measurements that cover the temporal and spatial fluctuations before drawing conclusions on the relationship to this environmental condition.

Introduction

As mangrove ecosystems are ecologically and economically of high importance but fast disappearing and degrading (Duke *et al.,* 2007), it is important to conduct scientific studies - fundamental as well as management based – to obtain the necessary knowledge to preserve, to protect and to restore mangrove forests. Ideally, such studies should not only deal with the organisms of the mangrove ecosystem – fauna as well as flora - but also with the interaction between these organisms and their environment. The most characteristic elements of the mangrove environment are a frequent inundation and saline water (Tomlinson, 1994), both not only strongly determining the life of the mangrove inhabitants (*e.g.* Fratini *et al.,* 2004; Schmitz, 2008; Robert *et al.,* 2009) but also, by selection, nature's diversity and assemblage composition.

Studies dealing with the relationship between organisms and their environment often generate conclusions from a restricted dataset – spatially and/or temporally – of environmental parameters. The purpose of this research is to study the variability of the soil water salinity on a spatial as well as on a temporal scale in the mangrove forest of Gazi Bay, Kenya, a well studied site as concerns mangrove research. The results of the study are intended to evaluate the fluctuation of one of the key parameters of the mangrove environment on an intra-annual scale and this for different study sites situated relatively close to each other.

Study area

Study sites

The study area is situated in Kenya, in the mangrove forest of Gazi Bay (39°30'E, 4°25'S), which covers about 600 ha (UNEP, 2001; Neukermans *et al.,* 2008) and is situated approximately 50 km south of Mombasa. The mangrove forest has a tidal amplitude of about 3.8 m with a maximum of 4.1 m (Kenya Ports Authority tide tables for Kilindini, Mombasa) and is characterized by a sloping topography (Matthijs *et al.,* 1999). Seven sites spread over the mangrove forest (Figure 1) were studied with as common characteristic the occurrence of the mangrove tree species *Avicennia marina*.

Climate description

The climate along the Kenyan coast is characterized by a bimodal distribution of the precipitation (Figure 1). A distinct dry season (January - February) is followed by a long (April - July) and a short (October - November) rainy season (Figure 1). During the wet season, the rivers Mkurumuji and Kidogoweni (Figure 1) provide an important freshwater source for the mangroves of Gazi Bay. The average temperature at the Kenyan coast ranges from 22 to 30°C, with a mean relative humidity of 65% to 81% (annual averages of minimal and

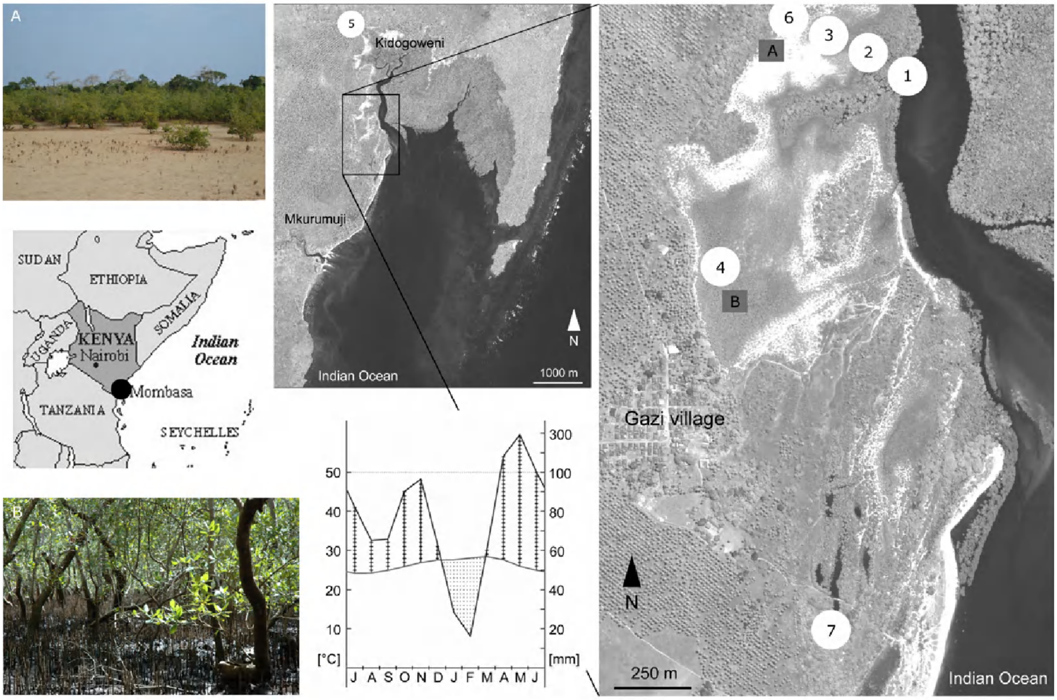


Figure 1 *- Description of the study area. Map of east Africa (left middle) showing the location of Gazi Bay on the Kenyan east coast (black dot) and two QuickBird satellite images of Gazi Bay (Kenya) (middle up and right), acquired in 2002 (Neukermans* et al.*, 2008) situating the seven study sites in the mangrove forest of Gazi Bay and the location the pictures A and B (left up and left down) have been taken. The two seasonal rivers that provide freshwater to the mangrove forest are indicated on the satellite image in the middle. Climate diagram (middle down) of Mombasa (adapted from Lieth* et al.*, 1999) representing the climate of the African east coast. Temperature (°C) is shown on the left and precipitation (mm) on the right vertical axe. Precipitation scale is reduced to 1/10 above the horizontal line. Pictures: Nele Schmitz and Elisabeth Robert.*

maximal values for Mombasa for the period 1972 - 2001, data from the Kenyan Meteorological Department, Mombasa, Kenya).

Material and Methods

Environmental data collection

For each of the seven sites (i) soil texture was determined by standard field characterization methods (GLOBE, 2005) and (ii) height above sea level and inundation frequency were calculated based on the Kilindini Harbour tide tables (Kenya, 39°39'E, 4°04'S). The local flooding level was measured with tracing paper impregnated with ecoline dye. For study site seven the exact height above datum and inundation frequency could not be determined. Here, the mangrove trees grow in a basin and are disconnected from the rest of the basin type forest by a raised road. Consequently, the inundation frequency differs in rainy and dry season since the water level reaches more frequently the height of the road during the former.

From April 2007 to February 2008, the salinity was measured at each site with a handheld refractometer (ATAGO, Tokyo, Japan / 0 – 100 ‰). Here for, soil water was sampled two times a month, once during neap tide and once during spring tide, at approximately 25 cm depth and at three positions scattered over each of the seven study sites. This was done with a punctured plastic tube connected to a vacuum pump or by digging a hole of the same depth when fine soil particles clogged the filtering tissue wrapped around the punctured plastic tube. Soil water salinity has only been measured at 25 cm depth so that the variation in soil water salinity with depth is not within the scope of this study.

Data analysis

To show the variation in soil water salinity, median, minimum and maximum values were calculated. However, at three of the seven sites no soil water could be extracted at certain dates (Figure 2, black stars). In these cases, the highest soil water salinity measured during fieldwork expeditions in the rainy season of 2005 and 2006 and the dry season of 2007 and 2009 was taken. For the basin forest the highest salinity of the water in the puddle was taken. Monthly rainfall data were averaged for the period 1966-2006 and are from the Kenyan Meteorological Department, Mombasa, Kenya.

Results

The seven study sites, although situated in one mangrove forest, differed seriously in environmental conditions (soil texture – height above sea level – inundation frequency) (Table 1). The annual average salinity as well as the pattern of variation of the soil water salinity over the year was different in different study sites (Figure 2).

Discussion

The study could not indicate one factor as being the main environmental characteristic determining soil water salinity. Instead, different environmental factors contribute to the annual average salinity as well as to the pattern of variation of the soil water salinity over the year. But although no clear-cut relationships between soil water salinity and environmental characteristics could be deducted from Figure 2, some trends were observed.

First, study sites that are daily inundated have a rather constant soil water salinity compared to study sites that are inundated only a few days a month (Figure 2, site 4-7 vs. 1-3).

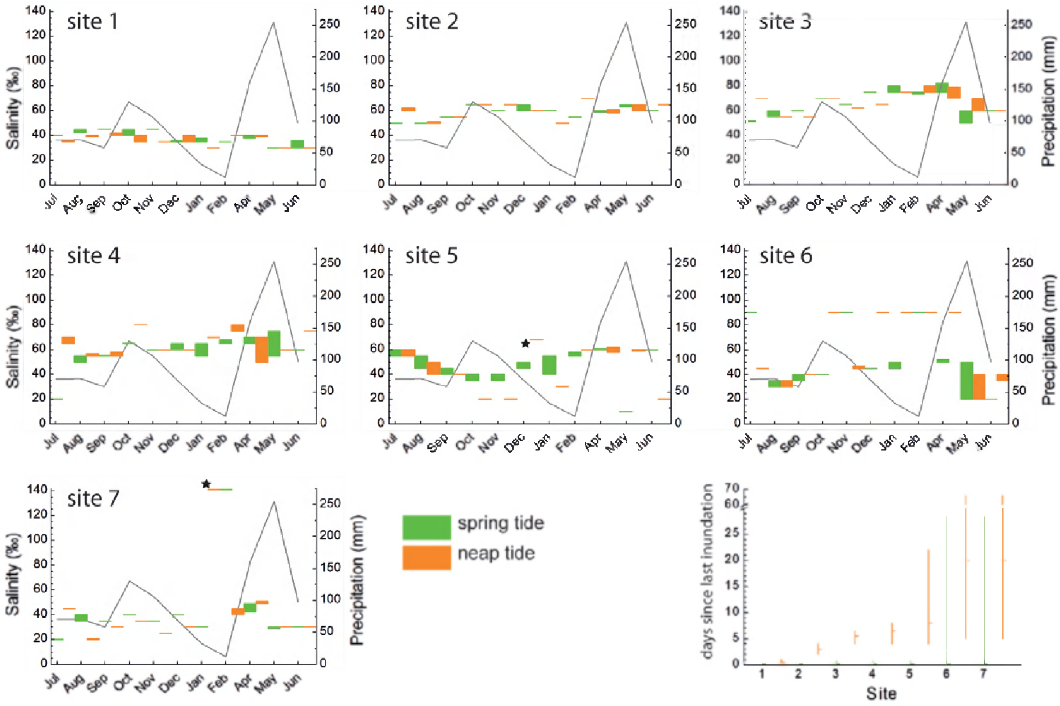
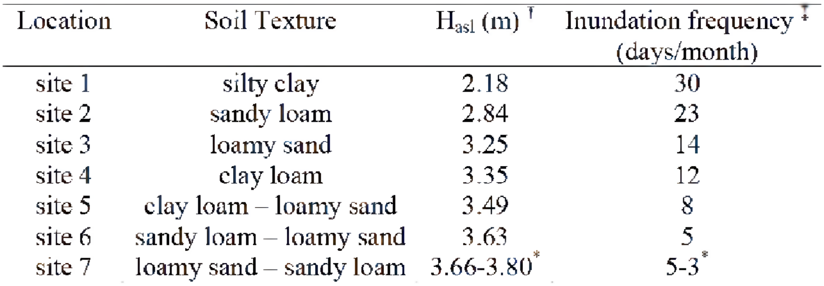


Figure 2 - *Spatial and temporal variation in soil water salinity in the mangrove forest of Gazi Bay, Kenya. Bars represent the salinity range of three measurements on the day when the high tide was highest during spring tide or lowest during neap tide. Black stars indicate moments when no soil water could be extracted and the highest soil water salinity measured in the period 2005-2009 was plotted (see also 'Material and methods – Data analysis'). The number of days since the last inundation on the day of measurement was plotted for each site as a monthly average (min-median-max).*

Non-frequent inundation implies longer dry periods during which the soil water evaporates leading to an increase in soil water salinity as alternating with periods of dilution by rainwater and salinity decrease. In contrast, frequent inundation permanently saturates the soil with water of seawater salt content. A similar effect was expected from the monthly tidal rhythm. At neap tide, study sites experience a drought period for one day up to more than two months (Figure 2). At spring tide, all sites are flooded at least once a day except for the most landward site and the site that is disconnected from the sea by a road (Figure 1 and 2, site 6-7) which experience drought periods of maximum one month. However, no link was found between tidal period and soil water salinity.

Second, the annual average salinity increased with decreasing inundation frequency in the seaward study sites (Figure 1 and 2, site 1-3). Further landward this trend broke down for the annual average but not for the maximum salinity, with study site 5 as an exception. In general, the disrupted trend with inundation frequency in site 4 to 7 could be related to changes in fresh water influence, canopy closure and soil texture. While site 3 is an open

Table 1 *- Environmental description of the seven study sites in the mangrove forest of Gazi Bay, Kenya.*



Location	Soil Texture	H_{asl} (m) †	Inundation frequency ‡ (days/month)
site 1	silty clay	2.18	30
site 2	sandy loam	2.84	23
site 3	loamy sand	3.25	14
site 4	clay loam	3.35	12
site 5	clay loam – loamy sand	3.49	8
site 6	sandy loam – loamy sand	3.63	5
site 7	loamy sand – sandy loam	3.66-3.80*	5-3*

† Height above sea level.
‡ Inundation frequency based on the Kilindini Harbour tide tables of 2009.
** See 'Material and methods – Environmental data collection' for more information.*

forest (Leaf Area Index of 0.23) with small trees (mean tree height: 2m) (Schmitz *et al.*, 2008b), the mangrove forest at study site 4 has comparably a closed canopy (Leaf Area Index of 1.18) (Schmitz *et al.*, 2008b) and a muddy soil (Table 1), both counteracting evaporation and thus also counteracting increase of soil water salinity. In study sites 5 and 6 fresh water input affects the soil water salinity. Site 5 is situated upstream the Kidogoweni river causing soil water salinity to match the precipitation curve. Site 6 is located at the border of the mangrove forest experiencing high amounts of freshwater runoff during the rainy season, leaching the sandy soil (Table 1). Due to the sandy soil in combination with an open canopy (Leaf Area Index of 0.62) (Schmitz *et al.*, 2008b), it is also study site 6 in which drought has the biggest impact. In study site 7 the main factor determining soil water salinity is the topography, which is like a basin. Seawater is thus standing and the salinity in the puddle gradually increased from the surface to the bottom. Stratification of the puddle water can thus explain why evaporation or fresh water input did not directly affect soil water salinity. Only in the middle of the dry season - around February - values up to 140 ‰ were measured in the last bit of water before the basin dried out completely.

Soil water salinity has a direct effect on the water relations in trees as high and fluctuating soil water salinity makes high demands on the water transport system by creating high risk of air blocking the water transporting canals (Cochard, 2006; Naidoo, 2006). This is especially true for mangrove trees inundated by saline water regularly to twice a day. Spatial differences in soil water salinity influence the species distribution of the mangrove forest due to differences in the ability to support high and fluctuating salinity between mangrove trees (Verheyden *et al.*, 2005; Schmitz, 2008; Robert *et al.*, 2009). Tree species distribution on its turn influences *e.g.* seedling dispersal (different root complexes act differently in propagule dispersal and establishment – Di Nitto *et al.*, 2008) and mangrove fauna (*e.g.* Smith, 1987), so that we can conclude that salinity is one of the major factors influencing and structuring life in the mangrove ecosystem.

Conclusion

The variability of the mangrove forest in terms of soil water salinity as observed in the mangrove forest of Gazi Bay (Kenya) should alert all those involved in mangrove studies. The factors contributing to this dynamism in time as well as in space, their interactions and the magnitude of the resulting fluctuations will vary between mangrove forests all over the world. Nevertheless, the message learnt from this study is of general importance. Soil water salinity cannot be predicted from inundation frequency alone and the additional influencing factors such as canopy closure, topography, fresh water input and soil texture can vary significantly within only a few hundred meters. The great number of micro-environments a mangrove forest can consist of should be taken into account to get better understanding of the functioning of the mangrove ecosystem and to be able to make high-impact conclusions that go beyond specific mangrove sites.

Recommendations

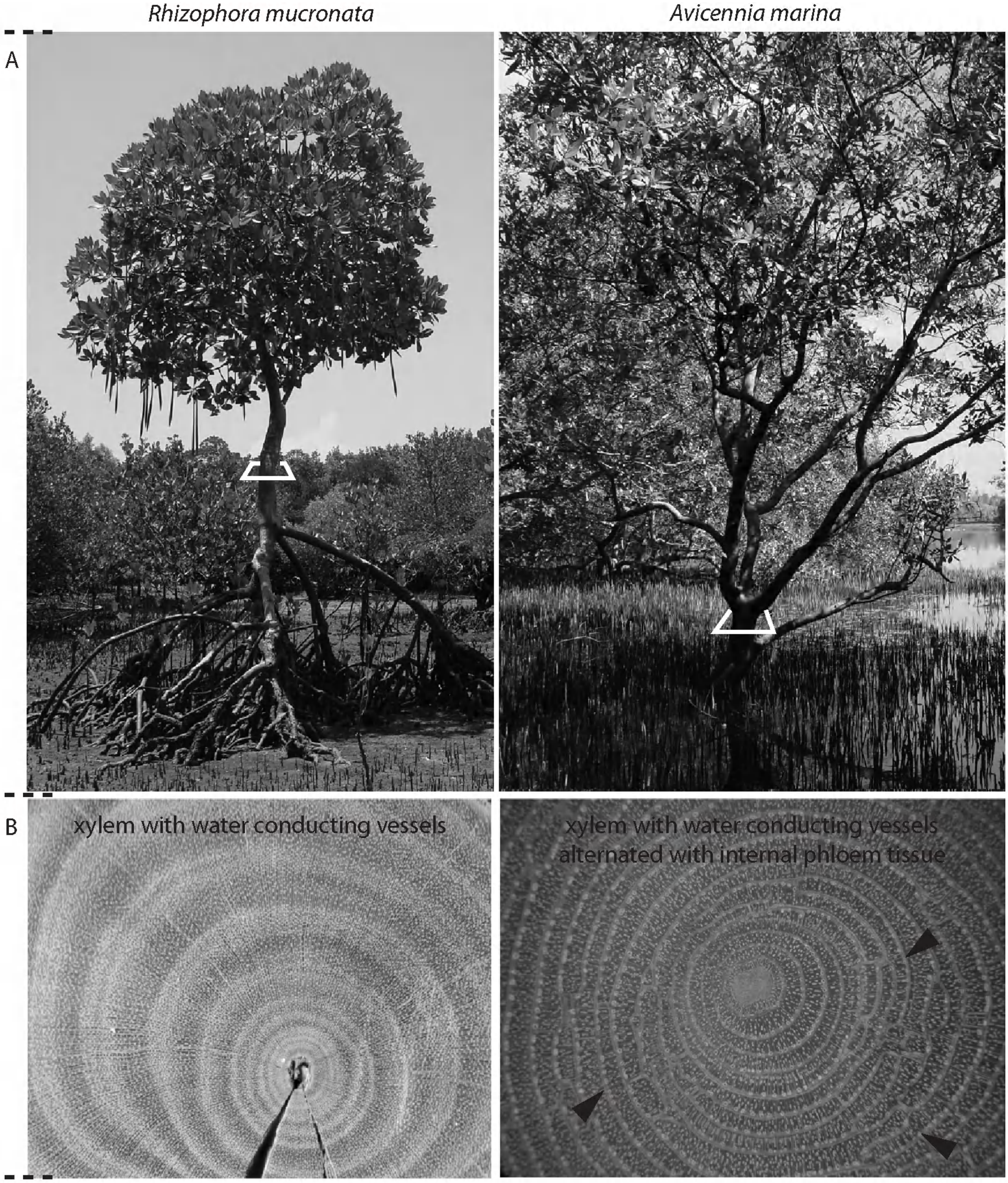
Soil water salinity, inundation frequency and soil texture can be determined with cheap and fast methods. The authors recommend that it should be standard practice for mangrove researchers to carefully compare different locations of a mangrove forest for these environmental parameters given their importance to obtaining a reliable reflection of the variation in soil water salinity. Moreover adequate number of measurements has to be carried out to cover the local variation not only in space but also in time. In the event that this is not possible, the limitations of the soil water sampling strategy should be reported and considered when drawing conclusions.

Acknowledgements

We thank James Gitundu Kairo of KMFRI for his assistance during fieldwork. The postdoctoral researcher (N.S.) is supported by the National Fund for Scientific Research (FWO, Belgium), but during this study she was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen) as is the PhD student (E.M.R.R.). This research was further sponsored by travel grants from the FWO, the Schure-Beijerinck-Popping Fonds (Koninklijke Nederlandse Akademie van Wetenschappen, Nederland) and the Flemish Interuniversity Council (VLIR).

3. What are the challenges for water transport in the intertidal zone?

In order to understand the biological response to the challenges for the water transport in trees under salinity stress and more or less frequent drought periods, one must understand the tree's internal anatomy of the water transporting tissues (Figure 5).



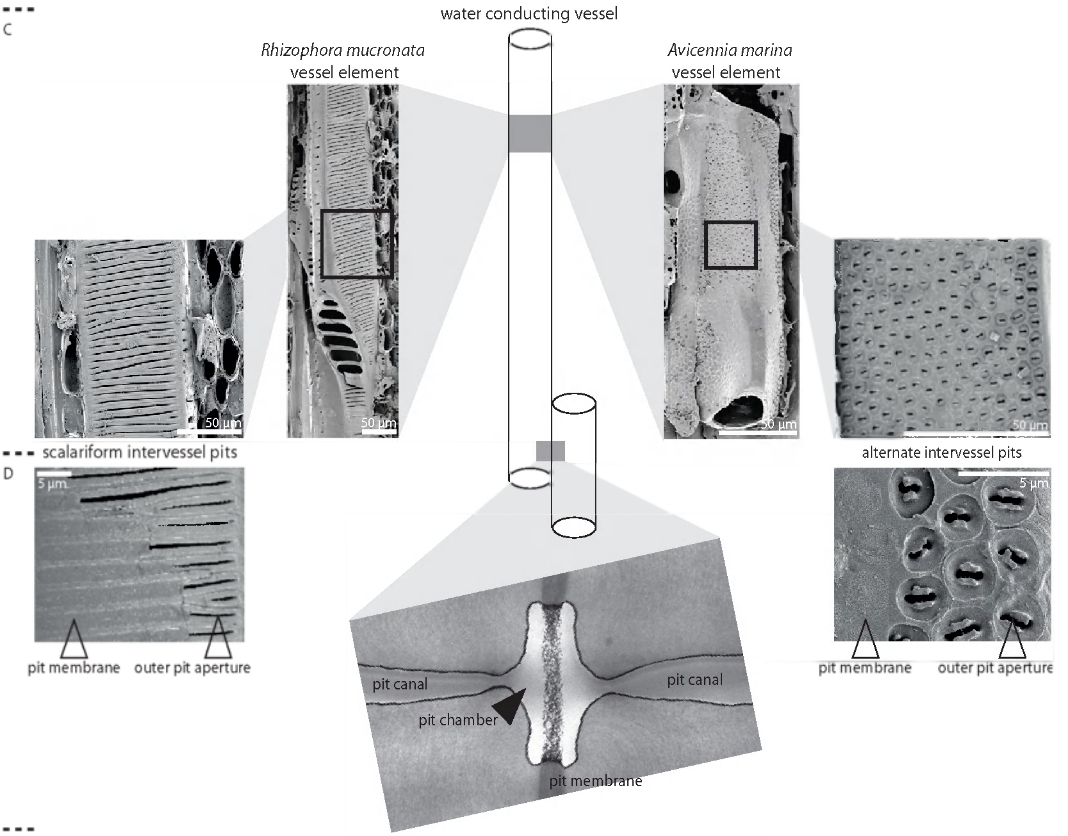


***Figure 5** - From tree to intervessel pits: visualization of the different structures of the hydraulic system of trees, starting from the macroscopic level (tree stem) up to the microscopic level (intervessel pit). Both* Rhizophora mucronata *(left) and* Avicennia marina *(right) trees have been taken as examples. (A) Whole tree images. The white rectangle is indicating the location of the transverse stem images shown in B. (B) Stem disc images showing the wood (xylem with water conducting vessels) and the absence (*Rhizophora*) or presence (*Avicennia - black arrow heads*) of internal phloem tissue. (C) Visualization of a water conducting vessel and scanning electron microscopy (SEM) images of the vessel elements, for both* Rhizophora *(left) and* Avicennia *(right). The outermost SEM images show the intervessel pits on the walls of the vessel elements. (D) SEM images showing the intervessel pits of both mangrove genera in more detail (outermost images) and TEM image of a* R. mucronata *pit indicating the different structures an intervessel pit is composed of (central image). SEM and TEM images are adapted from Schmitz* et al.*, 2007a.*

In 1894 Dixon and Joly explained water transport in plants by the cohesion-tension theory (CT-theory). First, a tension gradient pulls water passively through a plant, from roots to leaves. This tension gradient is created by, on the one hand, the adhesion of water to soil particles and, on the other hand, the evaporation of water from the leaf surface. Second, the cohesion forces between water molecules can keep the water column intact even with considerable tension. Although this theory is still the most widely accepted (Angeles *et al.*, 2004), it is also subject to controversy. Zimmermann *et al.* (2004) and Westhoff *et al.*

(2009), for example, studied the existence of continuous water columns from root to apex and discovered that water in trees is lifted in many steps, in a non-linear way, by independently operating short distance forces (capillary, osmotic and/or transpiration-bound tension gradients), by mobilisation of water in the parenchymatic tissues and the heartwood and by moisture uptake through lenticels. Furthermore, Laschimke *et al.* (2006) put forward that tension in the water conducting system is resulting from a wall adherent bubble system rather than from a gradient in negative pressure in the water column.

For more than a century water transport in plants has been regarded as a completely passive process. Recent findings have shown that plants can significantly control water flow and water distribution over the different stem tissues, for example during the refilling process of an air-filled vessel (Brodersen *et al.*, 2010; Secchi & Zwienicki, 2011), during radial transport from bark to xylem suggested to be mediated by boosted aquaporin abundance and/or activity (Steppe *et al.*, 2012) or by control of the swelling and shrinking of pectin gels at the level of the intervessel pits (van Ieperen, 2007; Jansen *et al.*, 2010). This makes plant water transport a combination of passive and active processes, with ongoing investigation on their respective significance.

Physiological drought, either caused by water shortage or salinity stress, can lead to cavitation events, *i.e.* the formation of air bubbles within the vascular water column, since it increases the tension on the water column. At critically negative sap pressures, air is being sucked into water-filled xylem conduits (air-seeding hypothesis - Zimmermann *et al.*, 1983; Tyree *et al.*, 1994; Christman *et al.*, 2009), after which the bubble can expand and entire vessel elements can become air-filled, *i.e.* embolised. Intervessel pits play an important role in drought-induced cavitation since, at tensions that exceed the threshold set by the pit characteristics, air bubbles can pass from already air-filled vessels, common in plants, to neighbouring water-filled vessels through small openings in the pit membranes (Hacke and Sperry, 2001). Although other mechanisms triggering xylem cavitation have been proposed, none besides air seeding can explain the experimental observations (Christman *et al.*, 2009). Besides drought, also frost can induce caviation (Hacke & Sperry, 2001), but this is of marginal importance since it only occurs at some of the mangrove latitudinal limits (Quisthoudt *et al.*, submitted).

Xylem embolisms are however not necessarily permanent since air-filled vessels trigger the active secretion of solutes into the air-filled vessel part after which water from the surrounding cells moves towards the air-filled vessel osmotically (Sauter *et al.*, 1996; Holbrook & Zwieniecki, 1999; Tyree *et al.*, 1999; Hacke & Sperry, 2003; Améglio *et al.*, 2004; Salleo *et al.*, 2006; Zwieniecki & Holbrook, 2009; Brodersen *et al.*, 2010). If embolized vessels receive enough coalescing water droplets to span the vessel width and to overcome the loss of water to neighbouring vessels, they can become functional again (Brodersen *et al.*, 2010). Remaining gas could be compressed until it dissolves into solution or escapes into hydrophobic microchannels in the vessel wall (Brodersen *et al.*, 2010). Sucrose leaking from the parenchyma and/or phloem tissue has been found to be the major solute having a role in the mechanism of embolism sensing (Secchi and Zwieniecki, 2011).

Although xylem cavitation negatively influences the water conductance of trees, several studies also indicate its potential positive role in regulating a tree's water status. Cavitated vessels are proposed to be locations of water storage (Stratton *et al.*, 2000; Melcher *et al.*, 2001) and cavitation of some parts of the water conducting system, such as distal ends and older xylem (Melcher *et al.*, 2003), can safeguard other, more essential sections (Hacke & Sperry, 2001; Choat *et al.*, 2005b; Domec *et al.*, 2006; Woodruff *et al.*, 2007).

In the mangrove habitat the risk for cavitation events is permanently high. While tropical sun and wind, typical for intertidal areas, increase the tension at one side of the water column, salt stress and/or water shortage raise(s) the tension gradient at the other end even more. Water transport in trees in a tropical intertidal environment is thus highly challenged. However, the mangrove vascular system is adapted to these conditions since mangrove trees form extensive forests with well-developed trees.

The adaptations of the vascular system of mangrove trees to their environment are addressed in Part 1 - Mangrove wood anatomy: divergent within the forest, convergent as a whole (p. 50).

Water transport in the saline and drought-stressed conditions of the intertidal area is subjected to an increased risk of drought-induced cavitation, *i.e.* the formation of air bubbles in the vascular water column.

How do mangrove trees grow?

1. Do mangrove trees show annual growth rings?

Trees are natural archives of the past climate since their wood anatomy stores information on the environmental conditions they live in (*e.g.* Hughes, 2002; Briffa *et al.,* 2004; Büntgen *et al.*, 2011). Tree ring analysis of annually formed growth rings thus allows diving in the environmental past of trees and forests. In the tropics lack of seasonality is often at the base of indistinct or non-annual rings, explaining why tropical dendrochronology has been less developed than dendrochronology on temperate tree species (Cherubini *et al.*, 2003). The possible wealth of information from annual tree rings made it however worthwile to check whether mangrove trees do show annual growth rings. The study of the mangrove species *Rhizophora mucronata* from Kenya revealed the presence of a periodical signal in wood anatomy variables such as vessel density (Verheyden *et al.*, 2005) and stable isotopes (Verheyden *et al.*, 2004a), introducing the perspective of mangrove dendrochronology. However, the study of several mangrove species and comparison between *Rhizophora* trees from different origins, showed that mangrove trees only show annual tree rings in strongly seasonal conditions (Robert & Schmitz *et al.*, 2011 - p. 33), making periodical signals in mangroves rather exceptional.

Only in strongly seasonal conditions, mangrove trees form growth rings.

Mangrove growth rings: fact or fiction?

Published in: Trees - Structure and Function 25: 49-58

Abstract

The analysis of tree rings in the tropics is less straightforward than in temperate areas with a demarcated unfavourable winter season. But especially in mangroves, the highly dynamic intertidal environment and the overriding ecological drivers therein have been a reason for questioning the existence of growth rings. This study aimed at casting light on growth rings in mangroves. In six mangrove species growing in Gazi Bay, Kenya (*Sonneratia alba*, *Heritiera littoralis*, *Ceriops tagal*, *Bruguiera gymnorrhiza*, *Xylocarpus granatum* and *Lumnitzera racemosa*), the occurrence of growth rings was examined. Growth rate of each species was determined based on a 1-year period using the cambial marking technique. The effect of climate was furthermore considered by comparing the results with a number of wood samples originating from contrasting climatic regions. We can conclude that for growth rings to appear in mangroves more than one condition has to be fulfilled, making general statements impossible and explaining the prevalent uncertainty. Climatic conditions that result in a range of soil water salinity experienced over the year are a prerequisite for the formation of growth rings. For species with an anatomy characterized by indistinct ring boundaries, this should be combined with a growth rate of at least 0.3 mm/year. The use of growth rings for age or growth rate determinations should thus be evaluated on a case by case basis.

Introduction

Because of the lush vegetation and the absence of clearly defined seasonality, prejudice against acceptance of formation of annual rings in tropical trees has existed for a long time (*e.g.* Jacoby, 1989; Walter and Breckle, 1999). From the beginning of the twentieth century, however, the existence of annual rings in tropical trees has been reported recurrently (Geiger, 1915; Coster, 1927; Coster, 1928; Berlage, 1931; Mariaux, 1967a; Mariaux, 1967b; Mariaux, 1969; Mariaux, 1981; Worbes & Junk, 1989; Worbes *et al.,* 1992; Worbes, 1995; Trouet *et al.,* 2001; Fichtler *et al.,* 2003; Worbes *et al.,* 2003; Fichtler *et al.,* 2004; Verheyden *et al.,* 2004b; Brienen & Zuidema, 2005; Roig *et al.,* 2005; Verheyden *et al.,* 2005; Schöngart *et al.,* 2006). The base of tree ring formation is the reaction of woody plants to seasonally fluctuating growth conditions: the alternation of specific wood anatomical features. This variance can result from a cambial dormancy, but also from a change in growth rate. In the tropics (1) the variation in precipitation between a rainy and a dry season (Worbes, 1997; Brienen & Zuidema, 2005; Verheyden *et al.,* 2005; Trouet *et al.,* 2006; Schmitz *et al.,* 2007b) and (2) annually occurring floods of great river systems (Worbes, 1985; Worbes, 1997; Schöngart *et al.,* 2004; Schöngart *et al.,* 2005) can affect annual tree growth. However, we should not deny that the absence of annual and/or distinct rings remains a common problem in dendro(chrono)logical and ecological studies of tropical trees (*e.g.* Worbes, 1990; Sass *et al.,* 1995; Dünisch *et al.,* 2002; Brienen & Zuidema, 2005; Hancock *et al.,* 2006).

Although studies on mangrove stem increment have been going on for almost a century (e.g. Baker, 1915; Amobi, 1974; Duke *et al.,* 1981; Shiokura, 1989; Aziz & Khan, 2001; Verheyden *et al.,* 2004b; Krauss *et al.,* 2006; Krauss *et al.,* 2007; Rajkaran & Adams, 2008; Schmitz *et al.* 2008b) it has never been clear whether annual growth rings are present in mangrove tree species. While some studies mentioned the absence of growth rings in several species (Marco, 1935; Van Vliet, 1976; Sun & Suzuki 2000), others did find rings although margins were not necessarily distinct (Amobi, 1974; Rao *et al.,* 1987; Srivastava & Suzuki 2001). More recent studies reported annual growth rings in more than one species (Menezes *et al.,* 2003; Verheyden *et al.,* 2004a; Verheyden *et al.,* 2004b; Yu *et al.,* 2004; Verheyden *et al.,* 2005; Yu *et al.,* 2007; Chowdhury *et al.,* 2008). Not water availability but soil water salinity (Robert & Schmitz *et al.,* 2009) was assigned as the major seasonal variable causing wood anatomical changes and hence growth rings. This discovery opens up new vistas to the analysis of mangrove growth rings for tree age and growth rate estimations. In view of the carbon sequestration programmes being set up in the framework of the Kyoto protocol, these approximations are essential to come to reliable predictions (Nabuurs & Mohren, 1995; Field, 1998; Vieira *et al.,* 2005; Manhas *et al.,* 2006; Mattsson *et al.,* 2009) and are currently not available. Insight into mangrove wood formation is also needed to optimize and to acquire a scientific basis for forest management and plantation activities.

In the present study we investigated the occurrence of growth rings in six mangrove species growing across the intertidal area of the Kenyan coast, including a limited number of *Rhizophora* congeners growing under different climate conditions. This allowed comparison of species within one climatic context as well as an intrageneric comparison over climate types. The aim of the study was to examine the growth rate of these mangrove species, as well as their wood anatomy for occurrence of putative growth rings in relation to climate and local site conditions (soil water salinity and inundation frequency). Making use of the cambial marking technique (Mariaux, 1967a; Mariaux, 1967b; Verheyden *et al.,* 2004a; Schmitz *et al.,* 2007b; Schmitz *et al.,* 2008b) growth rate of the different species was measured from May 2005 to June 2006, and the number of growth rings formed within this year was counted. We wished to understand the causation of ring formation, but first addressed the actual presence of tree rings.

Material and Methods

Study area

The research was conducted in the mangrove forest of Gazi Bay (42°50' S, 39°30' E) at the Kenyan coast (Figure 1). The forest has a total area of about 600 ha (UNEP, 2001; Neukermans *et al.,* 2008). Sampling was done in five sites (site 1–5, Figure 1) from the bay up into the creek and to the mouth of the Kidogoweni (Figure 1), a seasonal river functioning as an important source of freshwater for the forest during the rainy season (Kitheka, 1997). The climate along the Kenyan coast is characterized by a bimodal distribution of precipitation with a distinct dry season from December to March that is followed by a long and a short rainy season from April to July and from October to November, respectively (Figure 2c). Mean annual temperature ranges from 22 to 30°C, while mean relative humidity ranges from 65 to 81% (annual averages of minima and maxima from Mombasa for the period 1972–2001, data from the Kenyan Meterological Department, Mombasa, Kenya).

For each study site, soil water salinity and inundation class have been measured as described in Schmitz *et al.,* 2008b and Robert *et al.,* 2009. Soil water salinity measurements were made with a hand-held refractometer (ATAGO, Tokyo, Japan) at approximately 25 cm depth. Inundation classes are according to Tomlinson (1994) where class I, II, III and IV correspond to areas inundated by 100–76, 75–51, 50–26 and 25–5% of the high tides, respectively.

In addition to the sample set obtained by fieldwork, four stem discs of *Rhizophora* were selected from the Tervuren Xylarium to examine the presence of growth rings under different climatic and/or environmental conditions (Figure 2). One disc was taken from *Rhizophora apiculata* from Galle, Sri Lanka (Tw57236) that experiences an equatorial climate (Peel *et al.,* 2007), one from *R. mucronata* from Djibouti (Tw39263) with a warm desert climate (Peel *et al.,* 2007) and two discs from *R. mucronata* from site 1 (Tw56729) and site 3 (Tw55886) in Kenya (Figure 1, Table 1).

Cambial marking and wood analysis

Besides *R. mucronata* and *Avicennia marina* that have been analysed in previous studies by Verheyden *et al.* (2004b) and Schmitz *et al.* (2007b, 2008b), six other Kenyan mangrove species that occur widely in the forest of Gazi were investigated, *i.e.*, *Sonneratia alba*, *Heritiera littoralis*, *Ceriops tagal*, *Bruguiera gymnorrhiza*, *Xylocarpus granatum* and *Lumnitzera racemosa*. Two to six moderately sized trees were randomly sampled (with the post-experiment felling in mind) from two study sites for each species. For *C. tagal* and *L. racemosa* all studied trees exceptionally originated from site 4 (Figure 1, Table 1). The cambium of the mangrove trees was marked in May 2005 at 130 cm height using a hypodermic needle with a diameter of 1.2 mm. Four additional *C. tagal* trees (Tw57794-97), of which the cambium was marked in October 1999 by Anouk Verheyden, were included in the study. Before felling in June 2006, tree height was determined trigonometrically and stem circumference at 130 cm height was measured from which stem diameter was derived. Temperature as well as rainfall of the years studied are within the normal range for the region (Lieth *et al.*, 1999 and data from the Kenyan Meterological Department, Mombasa, Kenya).

All wood discs collected from the marked areas were air-dried and deposited in the wood collection of the Royal Museum of Central Africa in Tervuren, Belgium (Table 1). Sanded stem discs were analysed both macro- and microscopically in order to (1) describe the anatomy of the growth rings, (2) count the number of rings formed during the 1-year period between May 2005 and June 2006 and (3) measure the radial increment during this 1-year period using digital image analysis software (AnalySIS Pro version 3.2, Soft Imaging System GmbH, Münster, Germany). For the description of the ring boundary type (Table 2), the IAWA (International Association of Wood Anatomists) terminology (Wheeler *et al.*, 1989) was followed. The radial increment was measured at 2 mm distance left and right from the needle incision point to avoid the influence of wound tissue that is formed as a response to wounding (Iqbal, 1925). The change in wood anatomy near the wound indicated the position of the cambium at the time of the pinning.

Data analysis

Per species and study site, the mean of the radial increment measurements left and right of the cambial wound was calculated per stem disc and converted to an annual radial increment from the exact period between pinning and felling date. Afterwards, means were calculated per species and study site. A one-way ANOVA was conducted in Statistica 7.0 (StatSoft Inc., Tulsa, USA) to test differences
in annual radial increment between species.

Results

When comparing the wood anatomy and distinctness of growth rings in *Rhizophora* species

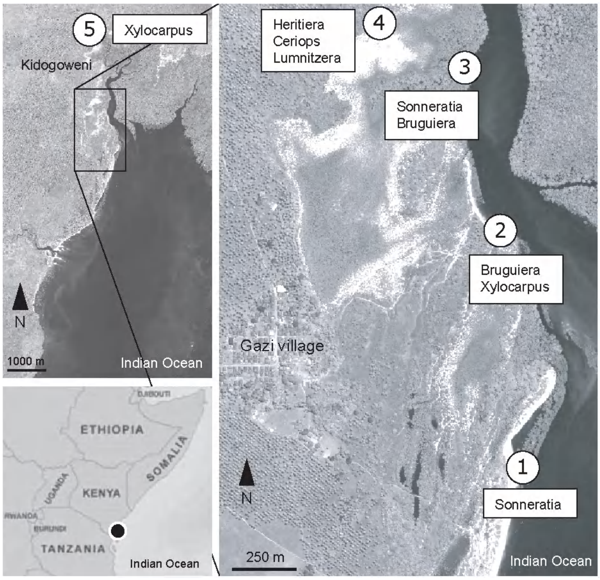


Figure 1 *- QuickBird satellite images of Gazi Bay acquired in 2002 (Neukermans* et al.*, 2008) and map of east Africa situating Gazi Bay on the Kenyan east coast (black dot). The five study sites together with the genera from which stem discs have been taken are indicated (site 1–5). The Kidogeweni, a seasonal river that provide freshwater to the mangrove forest, is indicated on the top left satellite image. Adapted from Robert* et al.*, 2009.*

growing under different climate types, clear differences were found. While *R. mucronata* from Djibouti showed quite distinct growth rings with striking changes in vessel density (Figure 2e), no obvious variation in wood anatomy was seen in *R. apiculata* from Galle (Sri Lanka) (Figure 2d) or in *R. mucronata* from site 3 in Gazi (Figure 1 and 2f). Although faint, the *R. mucronata* sample from site 1 in Gazi did show a fluctuation in vessel density (Figure 1 and 2g) that is at the base of the growth rings.

All six species macroscopically showed growth rings on stem discs. Ring boundaries, however, were not always distinct and sometimes they were even locally absent as in *S. alba* (Figure 3, Table 2). Indistinct ring boundaries in *C. tagal* and *B. gymnorrhiza* (Figure 3, Table 2) hindered ring counting, as did failed pinning of the cambium due to the thick bark in *H. littoralis*. Parenchyma bands, the most striking anatomical features that most likely indicate markers for growth-ring boundaries in mangrove species, were, however, observed in *H. littoralis* as well as in *C. tagal*, *L. racemosa* and *X. granatum* (Table 2). *L. racemosa* showed distinct growth rings, as did *X. granatum* under the two different flooding conditions (Figure 3, Table 2). *X. granatum* had formed two growth layers during the 1-year study period in four out of six trees; the other two trees had formed one and three rings. Only in *L. racemosa* annual ring formation could be suggested from the one ring that was formed during the 1-year experimental period (May 2005–June 2006).

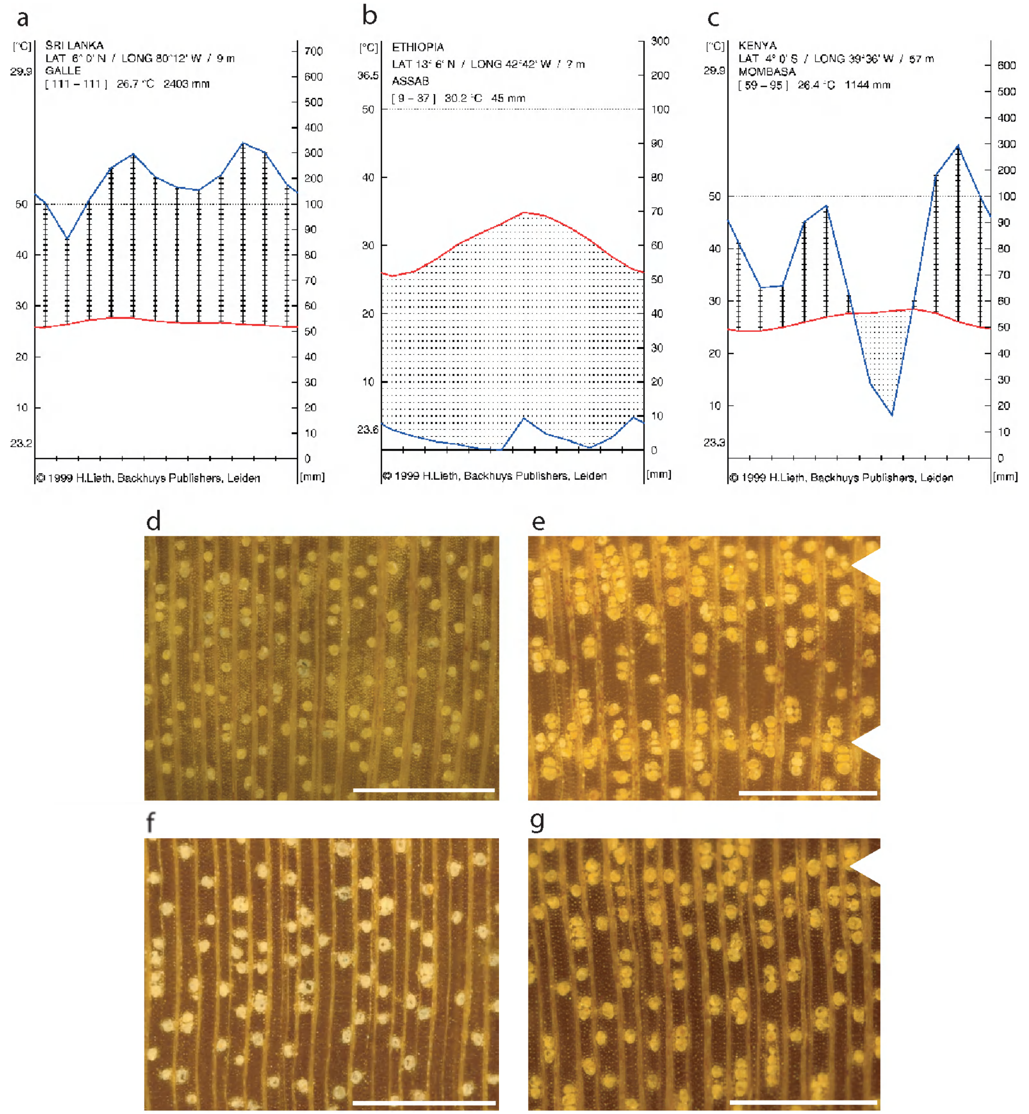


Figure 2 - Global distribution of mangroves implies growth under various climatic conditions (a–c) resulting in a wood anatomy showing growth rings more or less distinct (d–g). Also the internal dynamics of the mangrove environment play a major role as shown by the contrasting growth patterns at two Kenyan sites (f–g). Climate diagrams—July to June (Lieth et al.*, 1999) of (a) Galle, south west coast of Sri Lanka, (b) Assab, Ethiopia (nearest coastal station to Djibouti for which a climate diagram was available) and (c) Mombasa, south east coast of Kenya. Pictures showing wood anatomy are from sanded discs of (d)* Rhizophora apiculata*, Galle (Sri Lanka), (e)* Rhizophora mucronata*, Djibouti and (f–g)* R. mucronata *from Gazi Bay (Kenya), site 1 (g) and site 3 (f) (Figure 1). Arrowheads mark ring boundaries. Scale bars are 1 mm.*

Annual radial increments, based on the pinnings in May 2005 and felling in June 2006, differed significantly between species (F = 5.91, p\0.01, one-way ANOVA) with *S. alba* at study site 3 (Figure 1) recording the highest increment of the six species and *X. granatum* recording high growth rates, irrespective of the study site (Table 1). *C. tagal* (site 4) and *B. gymnorrhiza* trees growing at site 3 (Figure 1) had the lowest radial growth. Trees growing in study sites with low maximum soil water salinity values, coinciding with low salinity ranges experienced over the year and low inundation class, registered relatively high radial increments. Lower growth rates were found for trees growing under higher inundation class, associated with more extreme soil water salinity at least during one period of the year (Table 1). *S. alba* on the contrary showed a lower radial increment when maximum salinity and salinity range were lower (Table 1). This was most probably due to the insect infestation (*Salagena discata*, Lepidoptera) the *S. alba* trees were suffering from at site 1, but not at site 3 (Figure 1).

Discussion

In contrast to species with terminal parenchyma bands, clearly demarcating growth rings (Gourlay, 1995; Heinrich & Banks, 2006; Trouet *et al.,* 2006), *Rhizophora* exhibits no abrupt changes in wood anatomy that could indicate growth rings. Instead, the wood of *Rhizophora* shows gradual changes in vessel density that most likely reflect contemporary changes in soil water salinity caused by the variation in fresh water supply (Verheyden *et al.,* 2004b). However, climate did have an effect on the clarity of the growth rings in *Rhizophora* (Figure 2). While growth rings were extremely indistinct under the equatorial climate of south-western Sri Lanka, they were much more distinct in the Kenyan monsoonal climate (Verheyden *et al.,* 2004a; Verheyden *et al.,* 2004b; Verheyden *et al.,* 2005) and almost distinct in Rhizophora trees growing under the warm desert climate of Djibouti (Figure 2). The local environmental conditions, affected by a period of drought, are thus critical for growth ring formation in Rhizophora. Although Djibouti has an ever-dry climate, rainfall variations do occur on the field, causing fluctuations in soil water salinity and hence growth rings.

Consequently, the presence of a seasonal climate is no guarantee for growth rings in mangrove although for *Rhizophora* this does count. The mangrove trees of both Kenya and the Sunderbans in Bangladesh are subjected to a climate characterized by dry and rainy seasons, but only the latter shows growth rings (Figure 3a) (Chowdhury *et al.,* 2008). This is explained by the dissimilar effect of the climate on the locally experienced conditions. The *Sonneratia* trees in Gazi Bay, Kenya, are almost permanently flooded by seawater. The increased evaporation or input of fresh water during the dry and the rainy season, respectively, can therefor cause only minor changes in salinity (inundation class I, Table 1). These do not seem to be enough to create distinct growth-ring boundaries by a gradually changing vessel density (Figure 3a, Table 2). The Bangladeshi mangroves, in contrast, experience a much bigger drop in salinity during the rainy season due to the network of streams and rivers criss-crossing the forest (Hoque *et al.,* 2006; Wahid *et al.,* 2007). The moderating

Table 1 *- Annual radial increment of the six mangrove species studied in a 1-year period between May 2005 and June 2006 together with the characteristics of study sites (soil water salinity and inundation class) and trees (tree height and stem diameter at 1.3 m)*

Species	Specimen[a]	Study site[b]	Soil water salinity: min–max (range) (‰)	Inund. class	Tree height (m)	Stem diameter at 1.3 m (cm)	Annual radial increment (mm/years)
Sonneratia alba	Tw58983,85,86	3	21–46 (25)	I	6.0 ± 1	4.7 ± 0.7	2.6 ± 2
	Tw58981,82,84	1	25–36 (11)	I	6.0 ± 2	6.0 ± 2	1.8 ± 0.5
Heritiera littoralis	Tw58978-80	4	10–90 (80)	IV	4.5 ± 0.6	6.0 ± 2	–
	Tw58976,77	5	5–68 (63)	III	5.0 ± 4	5.0 ± 3	–
Ceriops tagal	Tw58998,99	4	10–90 (80)	IV	2.6 ± 0.4	4.0 ± 1	0.28 ± 0.09
Bruguiera gymnorrhiza	Tw58971,74,75	3	21–46 (25)	I	3.4 ± 0.5	3.0 ± 1	0.2 ± 0.1
	Tw58970,72,73	2	25–36 (11)	I	3.8 ± 0.7	4.4 ± 0.6	0.9 ± 0.5
Xylocarpus granatum	Tw58965-68	2	25–36 (11)	I	3.9 ± 0.6	5.0 ± 0.8	2.5 ± 1
	Tw58963,64	5	0–11 (11)	III	7.0 ± 1	4.1 ± 0.7	2.3 ± 1
Lumnitzera racemosa	Tw58995-97	4	10–90 (80)	IV	3.0 ± 1	4.0 ± 1	0.81 ± 0.08

Soil water for salinity measurements was taken at about 25 cm depth. Inundation classes are according to Tomlison (1994). Values for tree height, stem diameter and radial increment are means ± standard deviation
min *minimum,* max *maximum,* Inund. class *inundation class, “–” in* H. littoralis, *the thick bark hindered the wounding of the cambium so that annual radial increment could not be measured*

a Accession number of the Tervuren wood (Tw) collection
b See Figure 1

Table 2 *- Wood anatomical description of the growth rings of seven Kenyan mangrove species indicating the variables that change in gradual or abrupt way leading to indistinct or distinct ring boundaries, respectively (Figure 3) as defined by Wheeler* et al. *(1989)*

Species (family)	Variable	Type of boundary
Sonneratia alba (Lythraceae)	Vessel density and/or fibre wall thickness (discontinuously)	Indistinct-absent
Heritiera littoralis (Malvaceae)	Axial parenchyma band of 2–4 cells	Distinct
Ceriops tagal (Rhizophoraceae)	Vessel density|axial parenchyma band of 2–3 cells (only occasionally)	Indistinct
Bruguiera gymnorrhiza (Rhizophoraceae)	Vessel density	Indistinct
Xylocarpus granatum (Meliaceae)	Axial parenchyma band of 1–5 cells	Distinct
Lumnitzera racemosa (Combretaceae)	Vessel density|axial parenchyma band of 1–3 cells|fibre cell wall thickness|vessel diameter (minor difference)	Distinct
Rhizophora mucronata (Rhizophoraceae)	Vessel density	Indistinct*

Nomenclature is according to the Angiosperm Phylogeny Group (Stevens, 2001 onwards)
** Data from Verheyden* et al. *(2004b)*

effect of permanent flooding can also be observed within the mangrove forest of Gazi Bay when different sites are compared. The *R. mucronata* trees growing close to site 1 (Figure 1) are protected from frequent flooding by a dune in comparison with the trees of site 3 (Figure 1). This results in a larger salinity fluctuation between seasons (Table 1) causing a bigger difference in vessel density and resulting in more distinct growth rings (Figure 2f and g). Next to exogenous factors, the genetically determined wood anatomy affects the distinct-

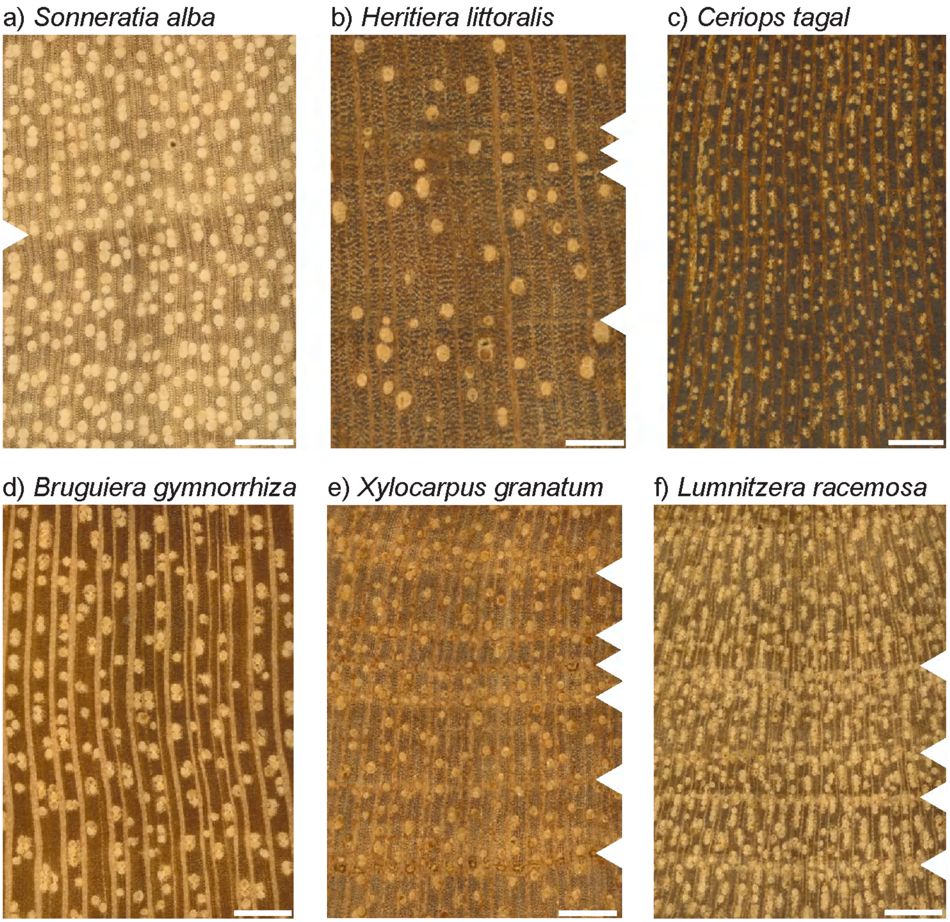


Figure 3 *- Wood anatomy of six Kenyan mangrove species showing distinct ring boundaries for* Heritiera littoralis, Xylocarpus granatum *and* Lumnitzera racemosa *and indistinct or no ring boundaries for* Sonneratia alba, Ceriops tagal *and* Bruguiera gymnorrhiza. *Arrowheads mark distinct (right) and indistinct (left) ring boundaries. Scale bars are 500 μm.*

ness and expression of growth-ring boundaries (Worbes, 1999). Species in which gradual changes in wood anatomy, such as vessel density, occur as indicator for seasonal changes need a minimum growth rate to guarantee that these changes in vessel characteristics can be detected. For *R. mucronata* in Gazi the minimum growth rate to be able to detect growth-ring boundaries was 0.5 mm/year (Verheyden *et al.*, 2004a; Verheyden *et al.*, 2004b). Only when the cambium is active enough to form a considerable amount of wood with a different anatomy depending on prevailing wet or dry conditions a growthring boundary will be visible. When cambial activity is generally low as in *C. tagal* and *B. gymnorrhiza* (Table 1), no variation in wood anatomy can be detected as small zones of high and low vessel density merge resulting in obscured growth rings (Figure 3c and d, Table 2). In both *L.*

racemosa and *X. granatum*, showing distinct growth rings, parenchyma bands mark the ring boundaries (Figure 3e and f, Table 2). Therefore, the formation of distinct rings was not hindered by the rather low growth rate of *L. racemosa* (Table 1). The presence of growth rings in *X. granatum*, growing in sites with only small variation in salinity values (salinity range 11‰, Table 1), indicated that this was sufficient to cause the formation of a parenchyma band. The less pronounced second dry season was probably even the source of the second ring boundary formed during the 1-year study period in all but one tree. The cause for missing second ring could be a slightly different microenvironment, as the sandy soil in site 2, where the tree was growing, is susceptible for small variations in fresh water supply. The observation of three rings in another tree illustrates that parenchyma bands can also be caused by other factors [*e.g.* defoliation creating false rings (Heinrich & Banks, 2006)].

A tenfold range in annual radial increment has been observed in the six mangrove species studied (Table 1). Compared with the radial growth rate of *B. gymnorrhiza* (1.2–3 cm/year) and *S. alba* (1.5–5 cm/year) in Micronesia (Krauss *et al.,* 2007), the annual growth of our trees from Kenya is very low, which emphasizes the diversity between mangrove forests. The high rainfall in the Micronesian study sites compared with the rainfall in the areas of the present study is most probably the cause of the faster growth, although nutrient effects cannot be excluded (Lin & Sternberg, 1992; Feller *et al.,* 2003; Lovelock *et al.,* 2004; Krauss *et al.,* 2007; Krauss *et al.,* 2008). Since mean radial increment was calculated from only six trees in the maximum, coming from two different sites, the result might not be relevant for the species. Therefore, site- and tree-specific effects have to be taken into account when comparing these data with other growth rhythm information.

We can conclude that due to the global distribution of mangroves across climatic regions (Duke, 1995; Duke *et al.,* 2002) and their local distribution across the intertidal zone, the presence of growth rings is dependent on climatic conditions that result in a variation of soil water salinity over the year (Figure 4, climate seasonality and periodic extreme environmental conditions). In addition, species with ring boundaries characterized by parenchyma bands are more likely to form distinct rings (Figure 4, Table 2). In species with a gradual change in vessel density demarcating tree rings, slow growth leads to merging of rings (Figure 4). Radial increment varied but was limited by a large soil water salinity fluctuation over the year, which is related to a high annual maximum salinity and a low inundation frequency. For tree rings to be formed, a growth rate above 0.3 mm/year and a strong seasonal signal in fresh water input and soil water salinity, not obscured by tidal inundation, should occur (Figure 4, dampening local conditions). For the three studied species showing distinct growth rings, more research is needed to clarify the annual nature of the observed rings. The study period should include more than 1 year and trees of different sizes should be included in order to be able to assess ontogenetic effects on anatomy and distinctness of growth rings (Brienen & Zuidema, 2005; Heinrich & Banks, 2005; Esper *et al.,* 2008). For now, we advice that tree rings in mangroves should be handled on a

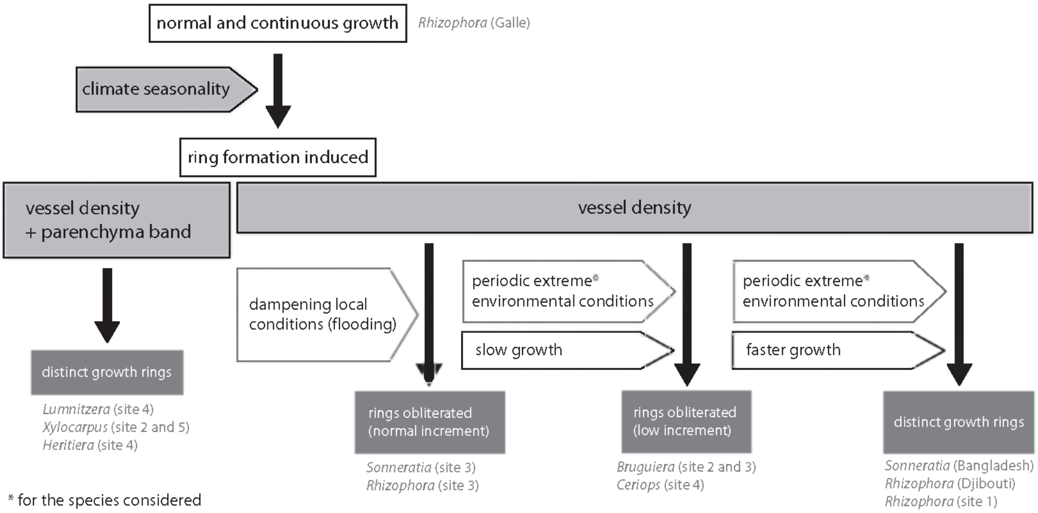


Figure 4 *- Overview of the observed ring clarity for all mangrove species studied in function of the climate they grow in or the location in the mangrove forest (between brackets) as mentioned in Figure 1 and 2. The climatic factors (grey arrows), the environmental factors (white arrows) and the species-specific factors (grey boxes with black text) at the base of the presence of absence of distinct growth rings are indicated.* Sonneratia *trees from study site 1 (Figure 1) have not been integrated in the figure as they are suffering from an insect infestation.* Sonneratia *trees from Chowdhury* et al. *(2008), however, were.*

case-by-case basis and should only be used for age or growth rate estimations after at least a preliminary investigation of the annual nature of the wood anatomically definable zones that can be seen in the wood.

Acknowledgements

We thank James Gitundu Kairo of the Kenya Marine and Fisheries Research Institute (KMFRI) for the logistic help during field expeditions, Hamisi Ali Kirauni for the help during the field work and Anouk Verheyden for the well collected samples deposited in the Tervuren xylarium.We are grateful to two anonymous reviewers for their critical comments on an earlier version of the manuscript. N.S. is supported by the National Fund for Scientific Research (FWO, Belgium) but during set up of this study she was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen) as is E.M.R.R. J.A.O. is supported by the Flemish Interuniversity Council (VLIR). Additional support came from the AFRODENDRO project of the Royal Museum for Central Africa (RMCA), Tervuren. This research was further sponsored by travel grants from the FWO, the Schure-Beijerinck-Popping Fonds (Koninklijke Nederlandse Akademie van Wetenschappen, Nederland) and the Flemish Interuniversity Council (VLIR).

2. What is uncommon about the secondary growth in *Avicennia*?

The secondary growth in the mangrove genus *Avicennia* takes place through successive cambia, *i.e.* not one but several vascular cambia are at the base of radial stem thickening. As a result, the stems of *Avicennia* trees show a succession of xylem tissue and internal phloem bands (Figure 6). The most striking difference between stems of trees with and without successive cambia is the presence respectively absence of internal phloem tissue. Since phloem mainly consists of living cell types, in contrast to xylem or wood tissue that predominantly consist of cells with a secondary cell wall that are dead at maturity, its presence inside the stem changes the water household of the tree.

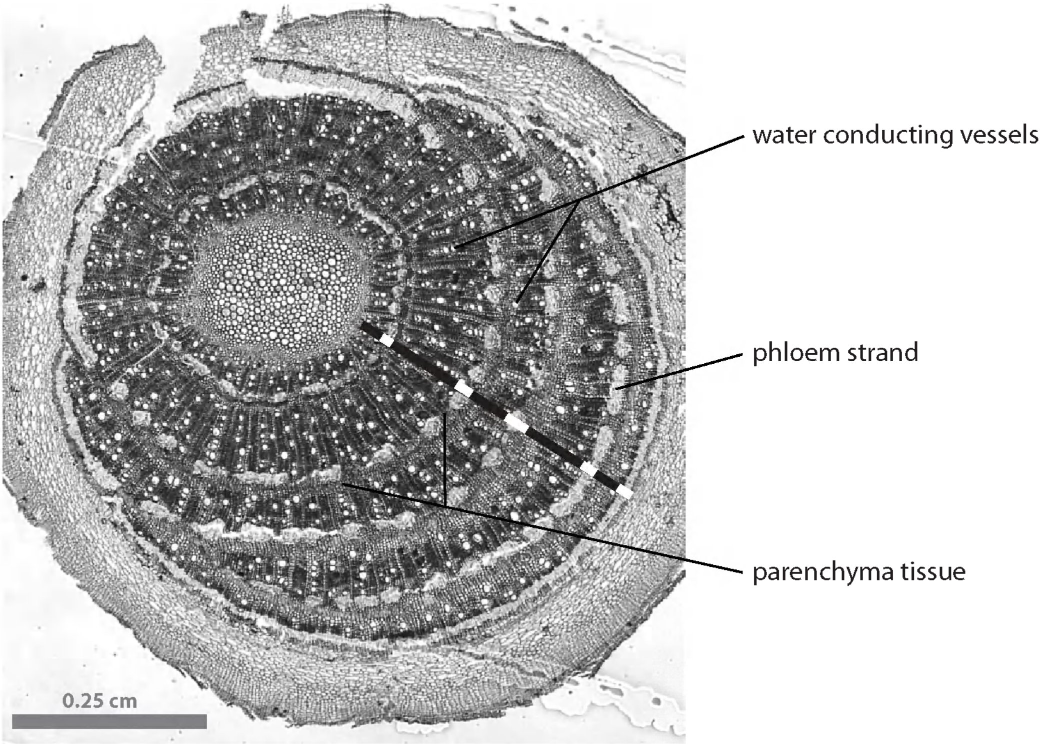


Figure 6 *- Microsection of an* Avicennia marina *branch, showing the characteristic alternating xylem tissue (black) and phloem tissue (white) bands. Within the xylem bands, water-conducting vessels can be observed, as well as a layer of parenchyma cells. The phloem bands consist of phloem strands with phloem parenchyma in between. The branch was sectionned using a sliding microtome and double stained with safranin-fast green after which a picture was taken via a camera connected to a microscope (Olympus BX60).*

In *Avicennia* the growth layers, composed of xylem and phloem tissue, are mostly non-concentric, forming a growth layer network. Investigation on the formation of these growth layers revealed their non-annual nature, with only a part of a growth layer up to four growth layers formed during one year. Moreover, growth layers could be formed simultaneously, indicating that more than one cambium could be active at the same time. Average growth layer width decreased with increasing soil water salinity values of the study site and decreasing tree height. Annual radial increment was however found to be independent from the studied environmental factors (soil water salinity, nutrient concentrations of the soil water (NO_3^-, NH_4^+ and P), soil texture and inundation frequency). This suggests that cambium differentiation is mainly controlled endogenously, but influenced by current environmental conditions such as soil water salinity when longer time spans are considered.

A patchy growth mechanism is therefore proposed for *Avicennia*, by which trees have a basic growth rate all around the stem circumference with the locations of active growth changing with time. This mechanism would offer *Avicennia* trees the possibility to interact with the environment in a particular way, *i.e.* at every moment in time, at least at one side of the tree stem, a wood segment is adapted to the environmental conditions imposed onto the tree at that moment.

This hypothesis will further be tested and discussed in Part 2 - Successive cambia at the basis of ecological success (p. 88).

Dendrochronologia 25(2): 87-96
Successive cambia development in *Avicennia marina* is not climatically driven in the seasonal climate at Gazi Bay, Kenya.
Nele Schmitz, Anouk Verheyden, James G. Kairo, Hans Beeckman and Nico Koedam
2007

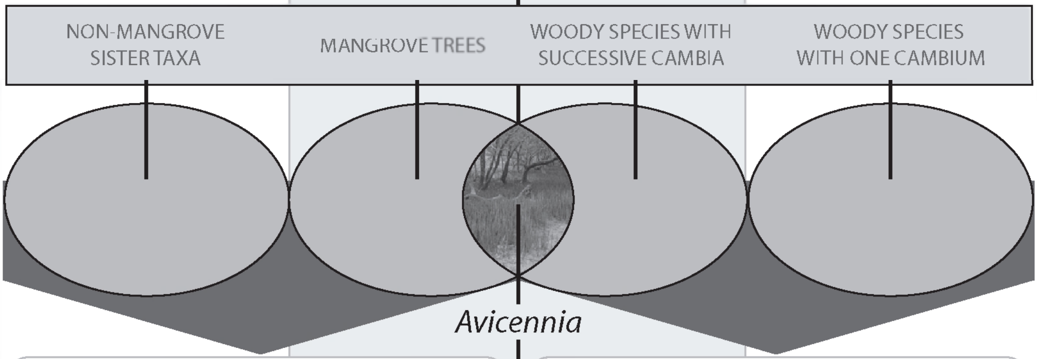
Annals of Botany 101: 49-58
A patchy growth via successive and simultaneous cambia: key success of the most widespread mangrove species *Avicennia marina*?
Nele Schmitz, Elisabeth M.R. Robert, Anouk Verheyden, James G. Kairo, Hans Beeckman and Nico Koedam
2008

***Avicennia* trees are the only mangroves not having one but successive cambia. These cambia structure the wood in a network of growth layers, allowing a basic growth rate all around the tree, with a more vigorous growth at one or several positions around the stem circumference.**

Objective and Hypotheses

Objective

Mangrove trees are the only trees able to cope with the highly dynamic and unpredictable conditions in the tropical intertidal environment. Within mangrove trees, Avicennia *species hold a special position since they are highly eurytopic, showing the broadest local and global distribution among mangrove genera. What makes* Avicennia *spp. trees so ecologically successful in comparison to other mangrove species? In this study we aim to clarify the relation between the mangrove environment and the hydraulic architecture of various species of mangrove trees, with special attention for* Avicennia *trees. By focusing on the development and functioning of the water transport system of trees growing in extreme conditions, we gain deeper knowledge of the biology of trees in general.*



Hypothesis 1

The ecological success of trees is dependent on how well the water transport system is adapted to prevent cavitation or minimize its effects.

We hypothesize that the water transport system of mangrove tree species, although they are phylogenetically diverse, is characterized by the same or equivalent properties important in preventing and overcoming embolized conduits. Furthermore, we expect differences in the hydraulic system being correlated to local environmental conditions, at inter- and infraspecific level. The risk of drought-induced cavitation is one of the major driving forces determining the wood hydraulic characteristics.

>> PART 1: Mangrove wood anatomy: divergent within the forest, convergent as a whole

Hypothesis 2

Wood development via successive cambia leads to stems with internal phloem tissue, important in refilling of embolized vessels (Nardini *et al.*, 2011).

We test the hypothesis that species with successive cambia are especially suitable for survival in environments that are characterized by limited access to water. We foresee an ecological advantage for *Avicennia* trees in the extremely saline and highly dynamic conditions of the mangrove forest, based on the water storage capacity of the internal phloem tissue as well as on the peculiar patchy growth system through a network of successive cambia.

>> PART 2: Successive cambia at the basis of ecological success

The most beautiful and profound emotion we can experience
is the sensation of the mystical. It is the power of all true science.

ALBERT EINSTEIN [4]

Part 1

Mangrove wood anatomy: divergent within the forest, convergent as a whole

Chapter 1 - Spare tubes for survival. Convergent evolution in the water transport system of mangroves.
> Robert *et al.* (2012) - Submitted - p. 53

Chapter 2 - A safe hydraulic architecture as wood anatomical explanation for the difference in distribution of the mangroves *Avicennia* and *Rhizophora*.
> Robert *et al.* (2009) - Functional Ecology 23: 649-657 - - - - - - - - - p. 69

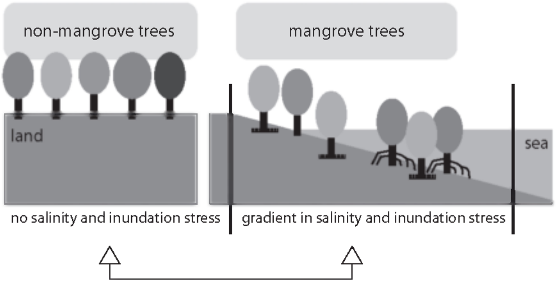
Summary

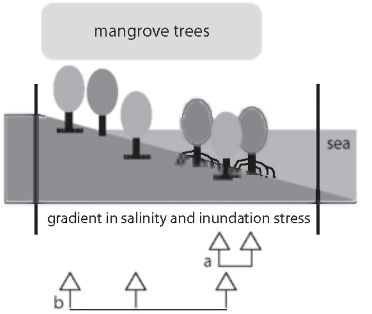
How is the vascular system of mangrove trees adapted to the mangrove environment?

Chapter 1 | Chapter 2

APPROACH 1 | APPROACH 2

Ecological setting





Research questions

What are the requirements for the vascular system of trees to survive in the conditions of the mangrove habitat?

How is the vascular system of mangrove trees adapted to the gradient in salinity and inundation stress within the mangrove forest?

Methodology

Comparison of the wood anatomy of 9 mangrove genera with that of their respective non-mangrove sister genera.
- 1) literature review
  - > defining the sister genera of mangroves
- 2) sample selection from wood collections
  - > 145 samples, 14 mangrove and 38 non-mangrove species
- 3) wood anatomical measurements & analysis

Comparison of the characteristics of the vascular system between
- a) the 7 different mangrove species growing in the mangrove forest of Gazi Bay (Kenya)
- b) populations of the same species along the ecological gradient within the mangrove forest
  - > with a focus on *Avicennia* versus *Rhizophora*

Results & Discussion

> Vessel density is higher in mangrove trees compared to their respective non-mangrove sister taxa.

> Vessel size is narrower and less variable in mangrove trees compared to their respective non-mangrove sister taxa.

Convergence in the mangrove vascular system: numerous small vessels allow bypassing air-filled vessel sections for sustained water flow in conditions of limiting water availability.

> With higher soil water salinity and less frequent inundation, mangrove trees show a higher vessel density and more narrow vessels, *i.e.* there is a clear ecological signature at between-species and within-species level

> The wood anatomy of *Avicennia* is safer than that of *Rhizophora*, with higher vessel density and vessel grouping and smaller vessels and vessel elements

A wood anatomy that is more directed to cavitation protection allows mangrove trees to grow at locations in the mangrove forest that are harsher.

Conclusion

To survive in the mangrove environment, trees need a vascular system that is specialized in protection against air in the water column. This is obtained through numerous small water conducting vessels. The more trees and species have this signature, the more they are able to stand high soil water salinity and low inundation frequency. The genus *Avicennia* has the most pronounced hydraulic safety signature corresponding to its ecological success within the mangrove forest.

Spare tubes for survival. Convergent evolution in the water transport system of mangroves

Abstract

Mangrove species are considered to have evolved in ecologically very distinct clades in approximately 20 families (Stevens, 2001; Ricklefs *et al.*, 2006; Spalding *et al.* 2010). Mangrove trees are distributed solely but widely along tropical coasts. Transition to the mangrove biotope may have happened through dry saline back-mangrove systems rather than through non-saline swamps, since the early Tertiary (Ricklefs *et al.*, 2006). In any case it was accompanied by specialized adaptations to the saline intertidal environment. Lifting water against gravitation is a challenge for any tree, but in mangrove species this is exacerbated by the low water potential of seawater, causing low water availability to plants. The shift to the mangrove habitus puts a high demand on the performance of the trees' water transport system. We found that the vascular systems in 14 mangrove species, representing 8 of the 11 core mangrove genera and all but one families with core mangrove species (Spalding *et al.* 2010), stand apart by having numerous narrow water-conducting vessels, unlike their respective closest non-mangrove tree relatives. This pattern holds despite the diverse eudicot origins of mangrove lineages. Such a hydraulic architecture facilitates bypassing air-filled vessel sections, allowing sustained water flow when trees face conditions of limiting water availability. Convergence in functional features of the water transport system in mangrove lineages indicates the importance of water-related environmental drivers in mangrove anatomy and evolution and adds to convergence observed in other characteristic mangrove traits, like vivipary or salt secretion (Shi *et al.*, 2005). With the reported increase of salinized land area on Earth (Flowers et al., 2010) and expected climate changes affecting world biomes, insight in the evolutionary biological response to physiological drought, here of long-lived trees, adds a dimension to our understanding of the possible changes that life-support ecosystems and their keystone species may undergo.

Among the halophytes, mangrove trees hold a special ecological position since they are the only trees growing in the marine intertidal environment (Spalding *et al.*, 2010). To thrive in the peculiar conditions of salinity and flooding stress of the tropical intertidal areas, exacerbated by the unpredictability and dynamic nature of this environment (Robert & Schmitz *et al.*, 2009), mangrove tree species are characterized by various combinations of functional traits. Such traits are the possession of pneumatophores, processes like salt secretion and salt exclusion and prevailing viviparous development (Tomlinson, 1994). Since wood anatomy and the conductive function of vascular tissues are key to a tree's water transport efficiency (Westoby & Wright, 2006), these traits may be equally critical for the survival of mangrove trees in their water-limited environment (Sobrado, 2007).

Mangrove trees belong to different families, well distributed over the eudicot phylogeny (Figure 2A) (Stevens, 2001; Bremer *et al.*, 2009). Because salt tolerance has been suggested to have evolved multiple times (Flowers *et al.*, 2010) and salt secretion and vivipary clearly have multiple evolutionary origins in mangroves (Shi *et al.*, 2005), we raised the question of the extent to which the hydraulic anatomy of mangrove species bears ancestral features shared with their non-mangrove relatives. There is a high diversity in wood anatomy features between species (Wheeler *et al.*, 2007), including mangrove species (Panshin, 1932; Robert *et al.*, 2011). A hydraulic architecture common to all mangroves would bring exciting new insight in the hydraulic requirements for trees to be able to thrive in saline conditions. This then needs to be seen from the perspective of phylogenetic signals evident in wood anatomy characteristics such as vessel diameter (Baas *et al.*, 2003) and the many alternative strategies for responding to a given environmental stress factor (Chave *et al.*, 2009; Zanne & Falster, 2010). In the spectacular evolutionary colonization of a wide range of environments by vascular plants and particularly angiosperms, understanding the part that mangrove species played is a challenge that can shed light on plant-water relations and adaptation to extreme environments (Flowers *et al.*, 2010).

When comparing the different mangrove species with their respective non-mangrove relatives, the phylogenetic background proved to have less bearing on the hydraulic structure of mangrove trees than the demands of the characteristic mangrove environment (Figure 1). In general, mangrove trees can rely on a system of numerous narrow conduits to transport water while their closest non-mangrove relatives all have a system of fewer but wider conduits. Clearly, a system with numerous narrow conduits suits all mangroves, enabling them to deal with the combination of salt and flooding stress, regardless of their diverse wood anatomy ancestral "blueprints" (Figure 2) (Baas *et al.*, 2000; Baas *et al.*, 2003).

Within the range of mangrove species, vessels are narrower and more numerous in trees which grew under low inundation frequency and high soil water salinity (Figure 3) (Yañez-Espinosa *et al.*, 2001; Schmitz *et al.*, 2006; Sobrado, 2007; Robert *et al.*, 2009). Also, within individual mangrove trees, wood formed during a dry season has narrower and more nu-

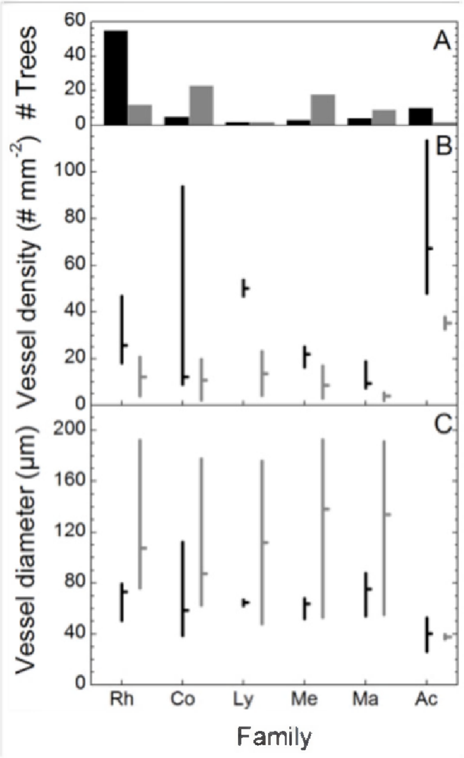


Figure 1 - Mangrove taxa evolved a common wood anatomical strategy to guarantee their water supply, different from that of closely related non-mangrove tree species. *(A) Total number of individual trees studied from the different mangrove (black) and related non-mangrove (grey) genera within the six studied families containing core mangrove genera (see also Table S3). (B) The density of water transporting conduits was significantly higher in mangrove trees from all families and was more variable (see also Table S4). (C) Conduit size was significantly smaller in mangrove trees than in their non-mangrove relatives and varied less (see also Table S4). B,C, Values are 5% percentile, median and 95% percentile. Rh: Rhizophoraceae, Co: Combretaceae, Ly: Lythraceae, Me: Meliaceae, Ma: Malvaceae, Ac: Acanthaceae.*

merous vessels than wood formed during a rainy season, when the constraints on water supply are temporarily lifted (Schmitz *et al.*, 2006). These trends clearly point to an environmental factor in the mangrove biotope that hinders the formation of wide vessels in smaller numbers. Vessel width depends on the differentiation rate (Aloni, 2004; Anfodillo *et al.*, 2012) and on turgor pressure (Arend *et al.*, 2007), which is needed for the cell to enlarge from a small cambium cell to a wider conduit during wood formation. Lack of water leads to small cambial derivates and consequently to narrow vessels (Woodward, 2004; Arend *et al.*, 2007). A fast differentiation with early secondary wall formation and lignification, possibly resulting from environmentally influenced auxin concentrations (Schrader *et al.*, 2004; Popko *et al.*, 2010), hinders further cell expansion (Aloni, 2004) and leads to narrow conduits.

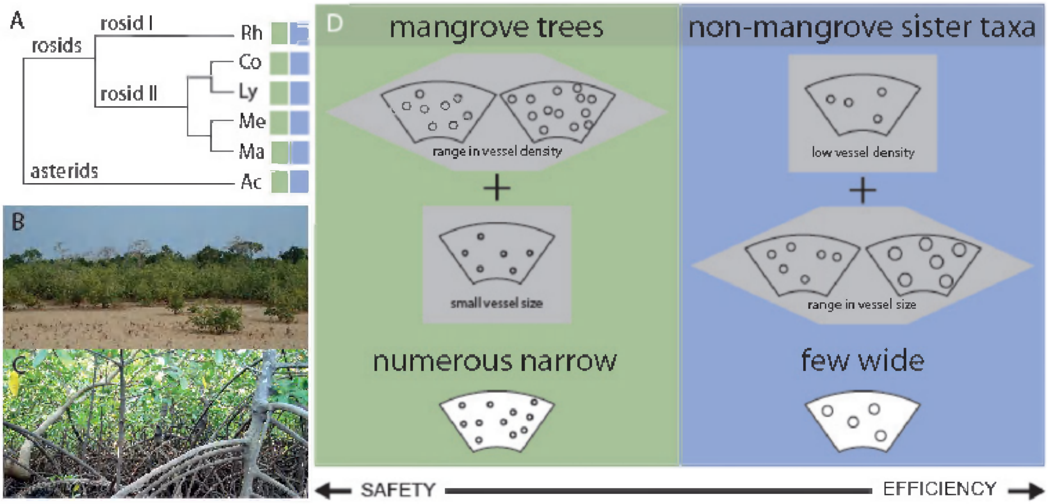
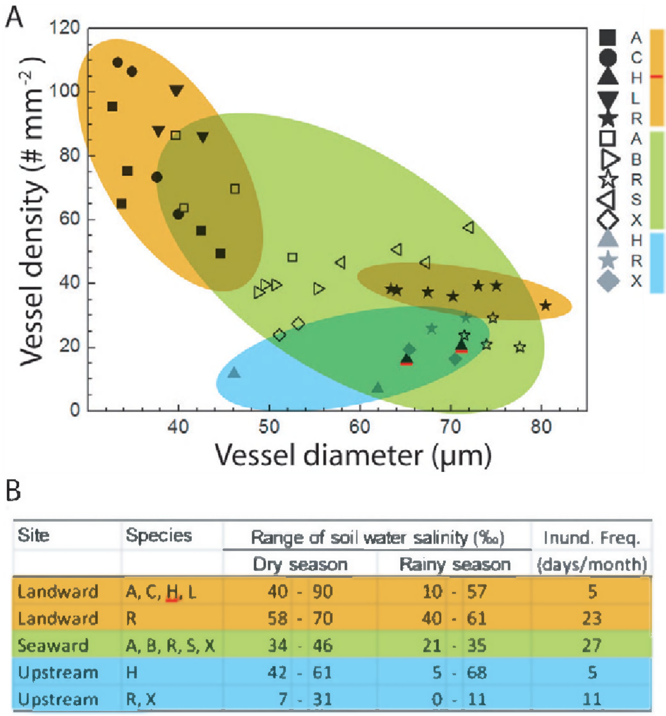


Figure 2 - Mangrove wood anatomy is convergent in vessel size and vessel density in comparison to non-mangrove relatives. *(A) Mangrove genera belong to different families well distributed over the eudicot phylogeny* (Stevens, 2001). *(B)* Avicennia marina *trees (Acanthaceae) at the landward and (C)* Rhizophora mucronata *trees (Rhizophoraceae) at the seaward side of the mangrove forest in Gazi Bay, Kenya. (D) In comparison to their respective non-mangrove relatives (blue), mangrove trees (green) show a range of high vessel densities combined with narrow vessels. Numerous narrow vessels offer bypass possibilities for air-filled vessel sections (safety) while wide vessels have better volume flow rate performance (efficiency). Rh: Rhizophoraceae, Co: Combretaceae, Ly: Lythraceae, Me: Meliaceae, Ma: Malvaceae, Ac: Acanthaceae.*

While in mangrove trees vessel width appears to be less variable than vessel density, the opposite is true for non-mangrove trees (Figure 1). The pattern found within the mangrove forest ecotone (Figure 3) is recurrent and is mirrored in the comparison between mangroves and their non-mangrove relatives, that is, (i) harsher conditions within the mangrove biotope require numerous narrow vessels and (ii) a trade-off exists between a safe and an efficient water transport system (Figure 2). In the non-saline conditions outside the intertidal forest, wider vessels allow sufficient water supply, while a higher vessel density is not a requirement since the loss of conducting area due to cavitation and subsequent air-filling of the vessels is less of a risk.

Although mangrove species and their respective close relatives are composed of the same hydraulic cell types, they do not share their quantitative vessel characteristics, much as they do not share the same ecology. Ecological and niche conservatism, which since Darwin has often been claimed to be a general principle (Losos, 2008), evidently does not apply to the evolutionary adaptation to the intertidal mangrove environment. Convergent evolution and a persistent phylogenetic signal as its antithesis are both evolutionary options for any functional trait at any phylogenetic level, requiring case by case scrutiny (Losos, 2008; Losos, 2011). Our study reveals a remarkable convergence in mangrove wood anatomy that gives strong circumstantial evidence for what is critical for trees to survive in the mangrove



Site	Species	Range of soil water salinity (‰)		Inund. Freq.
		Dry season	Rainy season	(days/month)
Landward	A, C, H, L	40 - 90	10 - 57	5
Landward	R	58 - 70	40 - 61	23
Seaward	A, B, R, S, X	34 - 46	21 - 35	27
Upstream	H	42 - 61	5 - 68	5
Upstream	R, X	7 - 31	0 - 11	11

Figure 3 - The structure of the water transport system of mangrove trees differs between species as well as between sites within one mangrove biotope. *(A) Mangrove trees growing more landward (closed black symbols) in the mangrove forest of Gazi Bay, Kenya, have more but narrower conduits than mangrove trees growing near the sea (open symbols) or upstream of the river Kidogoweni (grey symbols). For* Rhizophora mucronata *(R), the same trend can be observed at the intraspecific level despite the fairly high inundation rate of the most landward site where this species can be observed.* Heritiera littoralis *(H) at the landward side of the forest has fewer and wider vessels than would be expected based on the position in the forest, possibly explained by its higher amount of axial parenchyma helping in vessel embolism repair (Salleo* et al.*, 2009; Nardini* et al.*, 2011). (B) Differences in soil water salinity and inundation frequency at the different sites in the mangrove forest of Gazi Bay, Kenya. Each site is characterized by a different set of species. A:* Avicennia marina*, B:* Bruguiera gymnorrhiza*, C:* Ceriops tagal*, L:* Lumnitzera racemosa*, X:* Xylocarpus granatum.

environment: a water transport system composed of numerous narrow vessels. This convergence can be observed despite the characteristic differences in wood anatomy between the families studied (Baas *et al.*, 2000; Baas *et al.*, 2003), indicating the origination of a similar wood anatomy from different backgrounds.

Based on fossil records and phylogenetic studies, it has been suggested that colonization of the mangrove biotope at the shores of the Tethys Sea took place during the Tertiary, without substantial extinction of mangrove lineages ever since (Ellison *et al.*, 1999; Ricklefs *et al.*, 2006). But how were trees capable of colonizing the saline intertidal environment? Adaptations such as those needed by halophytes (Hacke *et al.*, 2006; Flowers & Colmer, 2008) are also required *a fortiori* by mangrove trees. The divergence in the structure of the water transport system between mangrove trees and their non-mangrove relatives corresponds to a divergence in β niche parameters (*sensu* Silvertown *et al.*, 2006), suggesting habitat divergence as the first stage of speciation with subsequent divergence in α niche parameters for further speciation and radiation (Ricklefs *et al.*, 2006). The mangrove environment is thus a filter allowing different combinations of functional traits as survival strategies, amongst others the requirement for strict minima such as a water transport system composed of numerous narrow water conducting vessels. This is in accordance with the stress dominance hypothesis stating that in stressful environments stabilizing selection limits variation (Swenson & Enquist, 2007). It may explain why mangroves, although well distributed amongst eudicots, are found in a limited range of families and constitute species-poor assemblages.

Although they are a treasure trove, xylarium samples do not allow wood anatomy, leaf area, or stomatal pore area to be functionally assessed by hydraulic conductivity in a tree. Understanding these relationships should, however, be our next goal as the function of vessels is to provide the leaves with water. Studies of fossil mangrove and non-mangrove wood of closely related clades could give insight in the ancestral wood anatomy, offering circumstantial evidence on the specific requirements for mangrove wood anatomy as a function of the environment and on how these peculiar trees may have evolved.

Experimental procedures

Sample selection

Based on global distribution and importance in the mangrove ecosystem, nine mangrove genera out of six families (Bremer *et al.*, 2003; Mabberley, 2008; Bremer *et al.*, 2009) were selected for the study: *Rhizophora* L., *Ceriops* Arn., *Bruguiera* Sav. (Rhizophoraceae), *Laguncularia* Gaertn. F., *Lumnitzera* Willd. (Combretaceae), *Sonneratia* L.F. (Lythraceae), *Xylocarpus* Koenig. (Meliaceae), *Heritiera* Dryand. (Malvaceae) and *Avicennia* L. (Acanthaceae). This selection covers eight of the eleven core mangrove genera and all families containing core mangrove species except one, the Tetrameristaceae with the rare and restricted mangrove genus *Pelliciera* (Duke, 1992; Spalding *et al.*, 2010). Although not a core mangrove genus, *Heritiera* has been added to the selection since it is composed of mangrove and non-mangrove species. Published phylogenetic studies on these genera and families were consulted to identify the non-mangrove sister genera of each mangrove genus (Table S1 and S2). Species and genera have been considered non-mangrove if not mentioned in the list of mangrove species set by Duke 1992 and Spalding 2010.

Although studies on mangroves and their sister taxa are not numerous, we could define the sister genera of all but one mangrove genus with sufficient support. For *Heritiera* and *Sterculia* L. the sister relationship is only weakly supported (Table S1) (Alverson *et al.*, 1999; Wilkie *et al.*, 2006). In spite of this, we decided to consider them as such, since all available studies regarding the phylogeny of *Heritiera* and *Sterculia* found them to be sister taxa (Alverson *et al.*, 1999; Wilkie *et al.*, 2006).

The genera most closely related to mangroves are genera of woody plants, mostly trees and shrubs. *Sonneratia* is an exception, as it is most closely related to *Trapa* L., a genus of annual aquatic plants (Table S2). The genera most closely related to *Sonneratia* and comprising woody plants are *Lagerstroemia* L. and *Duabanga* Buch.-Ham. (Huang & Shi, 2002; Graham *et al.*, 2005).

Wood samples of each mangrove genus and most woody non-mangrove sister genera were selected from the Tervuren wood collection (Royal Museum for Central Africa – Tervuren – Belgium) according to several criteria. Firstly, the available thin sections were screened for their suitability for quantitative measurements. Sections taken from branches were not selected and, if possible, sections covering at least 100 measurable vessels were selected over other samples. Secondly, the Köppen-Geiger climate classification index (Peel *et al.*, 2007) was used to preferentially select samples originating from the tropical climate zone (code A). This choice reduces effects of climate in the higher latitudes with low mean temperatures and annually varying day length conditions. Samples with unclear climatic origin were only taken into the analysis if their wood anatomy was not significantly different from the remainder of the samples of the same family according to variability plots. If available, at least two samples per species were measured. Since not all genera were well represented in the Tervuren wood collection, six samples of the National Herbarium of the Netherlands (Leiden University – Leiden – The Netherlands) were added. This generated a sample collection of 145 samples distributed over 14 mangrove species out of 9 genera and 38 non-mangrove sister species out of 21 genera (Table S3). Species identification of the samples is according to the collections they are retrieved from. Family names are according to the Angiosperm Phylogeny Group (Bremer *et al.*, 2003; Bremer *et al.*, 2009). *Pseudocalyx saccatus* (Tw46553), synonym to *Thunbergia chrysochlamys* and *T. deflexiflora* and *Entandrophragma cylindricum* (Tw26441, 52), synomym to *Swietenia angolensis* according to the Tervuren Xylarium Wood Database have been added to the sample selection (Table S3) in order to increase the suitable Acanthaceae and Meliaceae samples respectively. The genera *Bucida* and *Terminalia* as well as *Combretum* and *Quisqualis* are considered synonyms by Mabberley 2008 (Table S2).

Measurements and statistical analysis

For each sample, we determined vessel density (number of vessels per mm^2) and vessel diameter, calculated as the equivalent diameter, D_e (Lewis, 1992), enabling interspecific comparison of vessels with diverse cross-sectional shapes. From the short (a) and long (b) vessel axes, D_e is calculated as:

$$D_e = \sqrt{\frac{2a^2b^2}{a^2 + b^2}}$$

All measurements were done on transverse sections, for at least 125 vessels per sample using an Olympus BX60 microscope and the image analysis software AnalySIS Pro version 3.2 (Soft Imaging System GmbH, Münster, Germany). The resulting dataset was analysed with the software STATISTICA version 7.0 (StatSoft Inc.; Tulsa, Texas, USA). As data were not normally distributed, Mann-Whitney U tests were used to test for differences between mangrove and non-mangrove trees per family (Table S4) as this test is the most powerful nonparametric alternative to the t-test for independent samples. Trees were used as replicates giving a total n of 79 and 66 for mangrove and non-mangrove trees, respectively. We used a significance level of 0.05 (two-tailed).

Mangrove study

For the wood anatomical analysis within mangrove trees species, only the wood samples from Gazi Bay (Kenya) were considered. Here all samples were taken at 130 cm tree height and microsections were made from wood of the outer part of the stem and of the medium to largest radius for asymmetric stem discs. The different species grow at different sites in the mangrove forest of Gazi Bay, which were grouped according to soil water salinity and inundation frequency in seaward sites, landward sites and sites upstream of the Kidogoweni river. For each site, inundation frequency was calculated by determining the height above sea level and making use of tide tables (Kenya Ports Authority tide tables for Kilindini, Mombasa, 2009). Soil water salinity was measured at about 23 cm depth with a hand-held refractometer (ATAGO, Tokyo, Japan) at 2 to 7 different days per season between May 2005 and February 2009.

Acknowledgements

We thank Wim Tavernier for all help concerning the xylarium of Tervuren, Frederic Lens for sending us wood samples from the National Herbarium of the Netherlands, Hilde Robert for helping us with the selection of samples from the database by building Table S3, Kim Roelants and Sunita Janssenswillen for their advice on the phylogenetic background, and the Kenya Marine and Fisheries Research Institute (KMFRI, Mombasa, Kenya) for help and support with sample and data collection in the field. We are very grateful to Kim Roelants for his comments on an earlier version of this manuscript. This work was financially supported by the Royal Museum for Central Africa (RMCA, Tervuren, Belgium), the Agency for Innovation by Science and Technology (IWT, Flanders, Belgium), the Research Foundation - Flanders (FWO, Flanders, Belgium), the Schure-Beijerinck-Popping Fund of the Royal Netherlands Academy of Arts and Sciences (KNAW, The Netherlands), and the Flemish Interuniversity Council (VLIR, Flanders, Belgium).

Supplemental information

Supplementary Table 1 - Phylogenetic studies used to identify mangrove sister taxa. *Overview of the literature research showing the phylogenetic studies used to identify the sister taxa of the selected mangrove genera. For each study we summarize the number of taxa, the methods, the DNA sequences and the support methods that were used in the respective studies. We furthermore give (i) the total number of genera within a family according to the Angiosperm Phylogeny Website (Stevens, 2001) (Taxa column – in bold) and (ii) support values that indicate the strength of the phylogenetic relationship between the selected mangrove genera and their sister groups (Support column – in bold).*

Family	Taxa	Method	Genes/DNA	Support	Reference
Rhizophoraceae	30 species (13 mangrove species); 13/**16** genera	ML	1) *mat*K (= cpDNA) 2) *rbc*L (= cpDNA)	bootstrap **90%**	Zhong *et al.*, 2002
Rhizophoraceae	17 samples, 13 species (9 mangrove species); 6/**16** genera	MP	1) *mat*K (= cpDNA) 2) ITS regions (= nrDNA)	bootstrap **100%**	Shi *et al.*, 2002
Rhizophoraceae	28 species (13 mangrove species); 13/**16** genera	MP	1) *rbc*L 2) trnL-trnF spacer 3) *atpB-rbcL* spacer = cpDNA 4) ITS regions (= nrDNA)	bootstrap **(94-)100%**	Schwarzbach & Ricklefs, 2000
Rhizophoraceae	12 species (4 mangrove species); 12/**16** genera	MP	*rbc*L (= cpDNA)	bootstrap **98-100%**	Setoguchi *et al.*, 1999
Combretaceae	101 (sub)species (3 mangrove species); 14/**14** genera	- MP - Bayesian analysis	1) *rbcL* 2) *psaA-ycf3* spacer 3) *trnH-psbA* spacer = cpDNA 4) ITS regions = nrDNA	- bootstrap **77(-100)%** - Bayesian PPs **1.0**	Maurin *et al.*, 2010
Combretaceae	16 species (3 mangrove species); 8/**14** genera	- MP - ML	1) *rbcL* 2) *psaA-ycf3* spacer (= cpDNA) 3) ITS regions (= nrDNA)	- bootstrap **100%** - quartet puzzling **99%**	Tan *et al.*, 2002

ML = Maximum Likelihood; MP = Maximum Parsimony

Family	Taxa	Method	Genes/DNA	Support	Reference
Lythraceae	137 samples, 53 species (4-5 mangrove species); 13/31 genera	- MP - ML - Bayesian analysis	1) *rbc*L 2) trnL-trnF spacer 3) *psaA-ycf3* spacer (= cpDNA) 4) ITS regions (= nrDNA)	- bootstrap (62)96-100% - Bayesian PPs 1.0	Graham *at al.*, 2005
Lythraceae	21 species (3 mangrove species); 16/31 genera	- MP - ML	1) *rbcL* 2) *psaA-ycf3* spacer (= cpDNA) 3) ITS regions (= nrDNA)	- bootstrap 89% - quartet puzzling 96%	Huang & Shi, 2002
Meliaceae	44 species (1 mangrove species); 44/50 genera	- MP - Bayesian analysis	*rbc*L (= cpDNA)	- bootstrap 56% - Bayesian PPs 1.0	Muellner *et al.*, 2006
Malvaceae	24 species (1 mangrove species); 13/243 genera	MP	*ndh*F (= cpDNA)		Wilkie *et al.*, 2006
Malvaceae	70 species (2 mangrove species); 70/243 genera	MP	*ndh*F (= cpDNA)		Alverson *et al.*, 1999
Acanthaceae	85 species (4 mangrove species); 56/229 genera	- MP - Bayesian analysis	1) *trn*T-L 2) *trn*S-G 3) *trn*L-F (= cpDNA) 4) ITS regions (= nrDNA)	- bootstrap 92% - Bayesian PPs 1.0	McDade *et al.*, 2008
Acanthaceae	40 species (3 mangrove species); 12/229 genera	- MP - Bayesian analysis	1) *rps16* intron 2) *rpl16* intron 3) *trnT-trnL* intergenic spacer (= cpDNA regions)	- bootstrap 68% - Bayesian PPs 0.98	Borg *et al.*, 2008

ML = Maximum Likelihood; MP = Maximum Parsimony

Supplementary Table 2 - Mangrove sister genera. *Selected mangrove genera together with their respective most related non-mangrove relatives based on the available phylogenetic studies mentioned in Table S1. This table indicates whether or not the sister genus of each mangrove genus also is a mangrove genus ("Mangrove?") and whether the closest non-mangrove relatives are genera of woody species ("Woody?"). Genera have been considered non-mangrove if not mentioned in the list of mangrove species set by Duke 1992 and Spalding 2010.*

Mangrove	**Family**	**Closest relatives**	**Closest non-mangrove relatives**	
		Mangrove?	Woody?	Genera
Rhizophora	Rhizophoraceae	1	1	*Carallia, Crossostylis, Gynotroches, Pellacalyx*
Ceriops	Rhizophoraceae	1	1	
Bruguiera	Rhizophoraceae	1	1	
Laguncularia	Combretaceae	1	1	*Anogeissus, Buchenavia, Bucida*, Calopyxis, Calycopteris, Combretum**, Guiera, Meiostemon, Pteleopsis, Quisqualis**, Terminalia*, Thiloa*
Lumnitzera	Combretaceae	1	1	
Sonneratia	Lythraceae	0	0	*Trapa*
Xylocarpus	Meliaceae	0	1	*Capuronianthus, Carapa, Lovoa, Pseudocedrela, Swietenia*
Heritiera	Malvaceae	0	1	*Sterculia, Heritiera* (non-mangrove)
Avicennia	Acanthaceae	0	1	*Mendoncia, Thunbergia*

*0 = no; 1 = yes; * and ** = synonyms according to Mabberley, 2008*

Supplementary Table 3 - Sample list. *Sample collection indicating the measured samples per species, per genus and per family. For each sample it is indicated from which collection it is retrieved, whether it is a mangrove tree or not and from which biogeographical zone, place and climatic zone (Köppen index) (Peel* et al.*, 2007) it originates.*

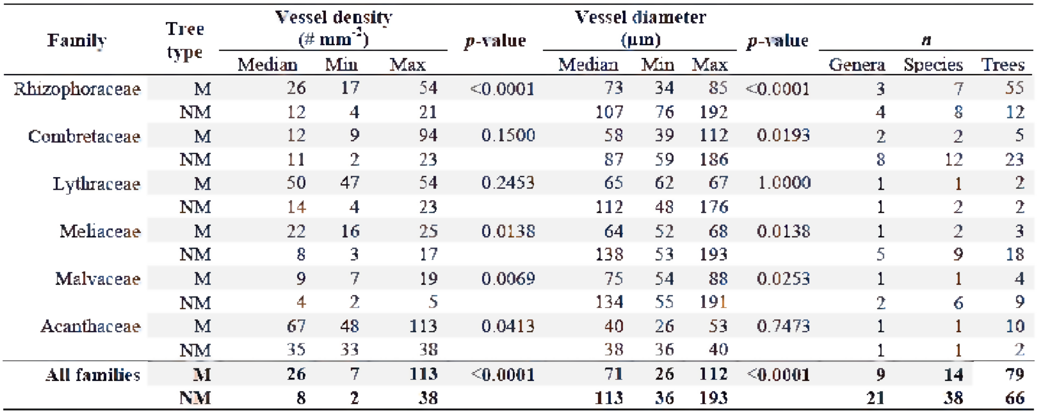
Family	Genus	Species	Collection number	M/NM	Region	Country (Location)	Köppen index
Rhizophoraceae	*Carallia*	*brachiata*	Tw28029	NM	IWP	Indonesia (Papua)	Af/Am/Aw
		eugenoidea	Tw47981	NM	IWP	Malaysia	Af
		lucida	Tw25255	NM	IWP	Bangladesh (Chittagong)	Am/Aw
			Tw28030	NM	IWP	Malaysia	Af
	Crossostylis	*grandiflora*	Tw28036	NM	IWP	New Caledonia	Af/Am/Aw
		multiflora	Tw28037	NM	IWP	New Caledonia	Af/Am/Aw
		richii	Tw47985	NM	IWP	Fiji	Af
	Gynotroches	*axillaris*	Uw21144	NM	IWP	Malaysia	Af
			v. Royen3690	NM	IWP	New Guinea	Af/Am/Aw
			Og B450	NM	IWP	Brunei (Belait, Habi)	Af
	Pellacalyx	*saccardianus*	Tw28038	NM	IWP	Malaysia	Af
			Tw47988	NM	IWP	Indonesia (Sumatra)	Af/Am
	Bruguiera	*eriopetala*	Tw28025	M	IWP	Malaysia (Sabah)	Af
		gymnorrhiza	Tw58970-1	M	IWP	Kenya (Gazi Bay)	Aw
		sexangula	Tw10883	M	-	-	-
			Tw28028	M	IWP	Indonesia (Papua)	Af/Am/Aw
	Ceriops	*candolleana*	Tw21614	M	IWP	Malaysia (Sabah)	Af
			Tw47982	M	IWP	Tanzania	Aw
		tagal	Tw58999	M	IWP	Kenya (Gazi Bay)	Aw
	Rhizophora	*apiculata*	Tw57219,31,34-6	M	IWP	Sri Lanka (Galle)	Af
		mucronata	Tw55872-3,76,80-1,83-94,Tw55905-8,58,75, 78, 90-1, Tw56705,14, 21-30,33-4,36	M	IWP	Kenya (Gazi Bay)	Aw
			Tw57256	M	IWP	Sri Lanka (Rekawa)	Aw
	Anogeissus	*leiocarpus*	Tw30077	NM	AEP	Guinea (Kouroussa)	Aw
	Buchenavia	*capitata*	Tw18558	NM	AEP	Puerto Rico	Af/Am/Aw
			Tw22417	NM	AEP	Brazil	Af/Am/Aw/BWh/BSh/Cwa/Cwb/Cfa/Cfb

Family	Genus	Species	Collection number	M/NM	Region	Country (Location)	Köppen index
Combretaceae		*grandis*	Tw20071	NM	AEP	Peru (Loreto)	Af/Am
			Tw25164	NM	AEP	Peru (Huanuco)	Af
	Bucida	*buceras*	Tw20744	NM	AEP	Cuba	Aw
			Tw25167	NM	AEP	Guatemala (El Peten)	Af/Am/Aw
	Combretum	*glutinosum*	Tw30660	NM	AEP	Congo (Kouilou)	Aw
			Tw41418	NM	AEP	Senegal (Casamance)	Aw
		molle	Tw23497	NM	AEP	Rwanda (Forêt de Bugesera)	Aw
			Tw26054	NM	AEP	Rwanda	Aw
	Guiera	*senegalensis*	Tw41450	NM	AEP	Senegal (Oriental)	Aw/BSh
			Tw46952	NM	AEP	Burkina Faso	Aw/BWh/BSh
	Pteleopsis	*diptera*	Tw28134	NM	AEP	Angola (Malanje)	Aw
			Tw32281	NM	AEP	Congo (Bas-Congo)	Aw
		kerstigii	Tw44437	NM	AEP	Togo (Région centrale)	Aw
			Tw49465	NM	AEP	Mali (Sikasso)	Aw
	Quisqualis	*latialata*	Tw32885,Tw35809	NM	AEP	Congo (Yangambi)	Af
	Terminalia	*macroptera*	Tw41319,Tw41423	NM	AEP	Senegal (Casamance)	Aw
		mollis	Tw24303	NM	AEP	Burundi	Aw
			Tw26061	NM	AEP	Rwanda	Aw
	Laguncularia	*racemosa*	Tw25391	M	AEP	Suriname	Af/Am
			Uw1378	M	AEP	Suriname (Wia Wia)	Af
			Uw1504	M	AEP	Suriname (Coronie)	Af/Am
	Lumnitzera	*racemosa*	Tw58995,97	M	IWP	Kenya (Gazi Bay)	Aw
L	*Lagerstroemia*	*excelsa*	Tw48011	NM	-	-	-
		piriformis	Tw48009	NM	IWP	Indonesia (Papua)	Af/Am/Aw
	Sonneratia	*alba*	Tw58981,83	M	IWP	Kenya (Gazi Bay)	Aw
Meliaceae	*Carapa*	*grandiflora*	Tw742	NM	AEP	Rwanda (Rubengera)	Aw
			Tw7506	NM	AEP	Rwanda-Burundi	Aw
		guianensis	Tw13882	NM	AEP	Brazil	Af/Am/Aw/BWh/BSh/Cwa/Cwb/Cfa/Cfb
			Tw25537	NM	AEP	Suriname	Af/Am/Aw
		procera	Tw26906,Tw27949	NM	AEP	Congo (Katanga)	Aw/Cwa/Cwb
	Entandrophragma	*cylindricum*	Tw26441	NM	AEP	Ivory Coast (Sud-Est)	Am/Aw
			Tw26452	NM	AEP	Cameroun (Nkam)	Am
		utile	Tw44043	NM	AEP	Gabon	Am/Aw

Family	Genus	Species	Collection number	M/NM	Region	Country (Location)	Köppen index
	Lovoa	*trichilioides*	Tw46876	NM	AEP	Congo (Kivu)	Aw
			Tw449	NM	AEP	Congo (Kikwit)	Aw
			Tw25775	NM	AEP	Gabon (Estuaire)	Am/Aw
			Tw26487	NM	AEP	Ghana (Central)	Aw
	Pseudocedrela	*kotschyi*	Tw22774	NM	AEP	Nigeria	Am/Aw/BWh/BSh
	Swietenia	*krukovii*	Tw34262	NM	AEP	Brazil (Acre)	Af/Am/Aw
		mahagoni	Tw43882	NM	AEP	Brazil (Amazonas)	Af/Am/Aw
			Tw11177	NM	-	-	-
			Tw20754	NM	AEP	Cuba	Aw
	Xylocarpus	*granatum*	Tw58963,68	M	IWP	Kenya (Gazi Bay)	Aw
		moluccensis	Tw14127	M	IWP	Kenya	Aw
Malvaceae	*Sterculia*	*appendiculata*	Tw39072	NM	IWP	Tanzania	Aw
			Tw39195	NM	IWP	Kenya	Af/Am/Aw/BWh/BSh/ Csb/Cwb/Cfb
	Heritiera	*foetida*	Tw43687	NM	IWP	Malaysia	Af
		quinqueloba	Tw761	NM	AEP	Congo (Katanga)	Aw/Cwa/Cwb
		densiflora	Tw22816	NM	AEP	Gabon	Am/Aw
			Tw25716	NM	AEP	Gabon (Estuaire)	Am/Aw
		ornithocephala	Tw45053	NM	IWP	Fiji	Af
		utilis	Tw26716	NM	AEP	Liberia (Bassa)	Af/Am
			Tw50666	NM	-	-	-
		littoralis	Tw47562	M	IWP	Philippines (Zamboanga del Norte)	Af/Am/Aw
			Tw47563	M	IWP	Philippines	Af/Am/Aw/Cfb
			Tw58977,80	M	IWP	Kenya (Gazi Bay)	Aw
A	*Mendoncia*	*vs. aspera hoffmannseggiana*	Uw22800	NM	AEP	Suriname (Avanavero)	Af/Am
			Uw23160	NM	AEP	Suriname (Lawa river)	Af
	Avicennia	*marina*	Tw58900-4,10-13,28	M	IWP	Kenya (Gazi Bay)	Aw

A = Acanthaceae; L = Lythraceae; Tw =Tervuren wood collection (Royal Museum of Cenrtral Africa, Tervuren , Belgium); Uw-, v. Royen3690 and Og B450 = Utrecht collection (National Herbarium of the Netherlands, Leiden, The Netherlands); M = Mangrove; N = Non-mangrove; AEP = Atlantic East Pacific group associated; IWP = Indo West Pacific group associated; "-"= No information available; Af = Tropical, Rainforest; Am = Tropical, Monsoon; Aw = Tropical, Savannah; BWh = Arid, Hot desert; BSh = Arid, Hot steppe; BSk = Arid, Cold steppe; Csb = Temperate, Dry summer, Warm summer; Cwa = Temperate, Dry winter, Hot summer; Cwb = Temperate, Dry winter, Warm summer; Cfa = Temperate, Without dry season, Hot summer; Cfb = Temperate, Without dry season, Warm summer

Supplementary Table 4 - Wood anatomical differences. *Summary of the wood anatomical measurements of the different mangrove species (M) as compared to their non-mangrove relatives (NM) within the same family. P-values are the result of Mann-Whitney U tests, with trees as replicates and a significance level of 0.05 (two-tailed).*



Family	Tree type	Vessel density (# mm^{-2})			*p*-value	Vessel diameter (µm)			*p*-value	*n*		
		Median	Min	Max		Median	Min	Max		Genera	Species	Trees
Rhizophoraceae	M	26	17	54	<0.0001	73	34	85	<0.0001	3	7	55
	NM	12	4	21		107	76	192		4	8	12
Combretaceae	M	12	9	94	0.1500	58	39	112	0.0193	2	2	5
	NM	11	2	23		87	59	186		8	12	23
Lythraceae	M	50	47	54	0.2453	65	62	67	1.0000	1	1	2
	NM	14	4	23		112	48	176		1	2	2
Meliaceae	M	22	16	25	0.0138	64	52	68	0.0138	1	2	3
	NM	8	3	17		138	53	193		5	9	18
Malvaceae	M	9	7	19	0.0069	75	54	88	0.0253	1	1	4
	NM	4	2	5		134	55	191		2	6	9
Acanthaceae	M	67	48	113	0.0413	40	26	53	0.7473	1	1	10
	NM	35	33	38		38	36	40		1	1	2
All families	**M**	**26**	**7**	**113**	**<0.0001**	**71**	**26**	**112**	**<0.0001**	**9**	**14**	**79**
	NM	**8**	**2**	**38**		**113**	**36**	**193**		**21**	**38**	**66**

A safe hydraulic architecture as wood anatomical explanation for the difference in distribution of the mangroves *Avicennia* and *Rhizophora*

Published in: Functional Ecology 23: 649-657

Summary

1. Wood anatomical characteristics are an important source of information about how trees are coping with the conflicting requirements of optimal hydraulic conductivity and safety. In this study we compared the vessel characteristics of *Avicennia marina* and *Rhizophora mucronata* to contribute to a better understanding of the difference in distribution of these two mangrove species.

2. Density, grouping and diameters of vessels together with vessel element length of *A. marina* trees growing on seven different study sites in Gazi Bay (Kenya) were measured and compared with the vessel characteristics of *R. mucronata* of the same research area. Furthermore, the relation of environmental factors related to the water relations of mangrove trees to the wood anatomy of both species was tested.

3. Vessel density of both species and vessel grouping of *A. marina* are higher with higher salinity. In addition, *A. marina* was shown to have a higher vessel density, a higher vessel grouping, smaller vessel diameters and shorter vessel element lengths as compared to *R. mucronata*.

4. As the vessel characteristics of *A. marina* are related to protection against cavitation or the effects of cavitation, we can infer that this species has a water transport system which can be considered safer under water limiting conditions than that of *R. mucronata*.

5. The contrast in the safety of the water transport system between the two mangrove species studied is reflected in their distribution at local scale, over the land-sea ecotone, and it may also explain the wide latitudinal distribution of *A. marina* as compared to *R. mucronata*.

6. This conclusion emphasizes the importance of the anatomy of the water transport system for survival under diverse environmental conditions. In general it is shown that dominant species of a single ecosystem can deal with the stressful conditions they live in by contrasting ecological strategies.

Introduction

Mangrove forests are a characteristic feature of the intertidal zone of tropical and subtropical coasts. As these forests are regularly flooded with seawater, mangrove trees do not only have to cope with high temperature and low relative air humidity but also with high and changing salt concentrations and hypoxia due to regular inundation. In order to survive under these extreme and continuously changing environmental conditions mangrove trees have developed different types of adaptations. Aerial roots, vivipary, salt exclusion and salt secretion are typical though not exclusive characteristics of mangrove trees (Scholander, 1968; Popp *et al.*, 1993; Tomlinson, 1994; Shi *et al.*, 2005) that partially explain their presence in this peculiar environment. *Avicennia* L. and *Rhizophora* L., the most characteristic genera of mangrove systems worldwide (Figure 1), have different solutions to deal with the extreme ecological conditions under which they thrive: *Avicennia* spp. have cable roots with pneumatophores and can exclude and secrete salt, while *Rhizophora* spp. are growing on stilt roots and can exclude but not secrete salt (Tomlinson, 1994).

The most limiting factor for trees growing in a saline environment is the risk for cavitation – that is air bubble formation in the xylem sap (Cochard, 2006) – blocking water transport. Since cavitation is already highly influencing the functionality of the hydraulic system under mesophilic conditions (*e.g.* Zimmermann & Brown, 1971), the survival of mangrove trees in their extreme environment is even more surprising. Plants, and especially mangroves, face a trade off between the protection of their water transport system against cavitation and conducting capacity (*e.g.* Mauseth & Stevenson, 2004; Hacke *et al.*, 2006). High vessel density, high vessel grouping, small vessels and short vessel members have already been mentioned as adaptations to avoid reduction of hydraulic capacity after cavitation and subsequent air-filling of vessels (*e.g.* Baas *et al.*, 1983). But does the high cavitation risk in the mangrove environment lead to a general mangrove hydraulic structure or are there different alternative strategies between mangrove species to deal with the threats for the water transport, in view of their distinctive ecology?

Although *Avicennia* and *Rhizophora* are the two dominant mangrove genera, their geographical distribution is different: the range of *Avicennia* is wider than that of *Rhizophora*, southward as well as northward, in both the Eastern and the Western biogeographic mangrove regions (Duke, 1991; Spalding *et al.*, 1997). In its habitat preference, *Avicennia* can be observed near the sea as well as in the more inland parts of the mangrove area, while *Rhizophora* only grows at the seaward end of the mangrove forest (MacNae, 1963; Duke, 1991; Dahdouh-Guebas *et al.*, 2004). Mangrove forests near the sea are inundated twice daily by seawater with almost constant salinity levels. This limits the range of salinity seashore trees are exposed to. More land-inward parts of the forests, in contrast, are exposed to a wider range of salinity values due to less frequent inundation and evaporation (Yáñez-Espinosa *et al.*, 2001; Naidoo, 2006). So *Avicennia* spp., growing in both the border parts of the mangrove forest, must be more eurytopic than *Rhizophora* spp. for salinity and inund-

Figure 1 - Avicennia marina *(above) and* Rhizophora mucronata *(below) in Gazi Bay, Kenya.* Avicennia *spp. have cable roots with pneumatophores, while* Rhizophora *spp. are growing on stilt roots.*

ation. What are possible explanations for both the co-occurrence and the local and global differential distribution of *Avicennia* spp. and *Rhizophora* spp.? Are there wood anatomical factors at the base of the wider ecological success of *Avicennia* spp.?

Stuart *et al.* (2007) addressed the role of freezing in the distributions of mangrove trees, but information about the underlying factors of the ecological success and wider distribution of *Avicennia* spp. as compared to *Rhizophora* spp. remains scarce. Vessel anatomy can tell how plants are dealing with the conflicting interests of safety and efficiency. As this balance on its turn is of high importance in where trees can grow, studying the vessel anatomy of mangrove trees can help to clarify mangrove distribution ranges. In the present article, we addressed the question of the ecological significance of wood anatomical features by the study of the hydraulic architecture of *Avicennia marina* (Forssk.) Vierh. and *Rhizophora mucronata* Lamk. from Gazi Bay (Kenya). The objectives were (i) to document the differences in vessel characteristics (vessel density, vessel grouping, vessel diameters and vessel element length) between both species and (ii) to explain the difference in local distribution of both species by these differences in characteristics of the water transport system. The hypothesis is that *A. marina* has a wider local distribution as compared to *R. mucronata* due to a water transport system that is better adapted to a broad range in environmental conditions.

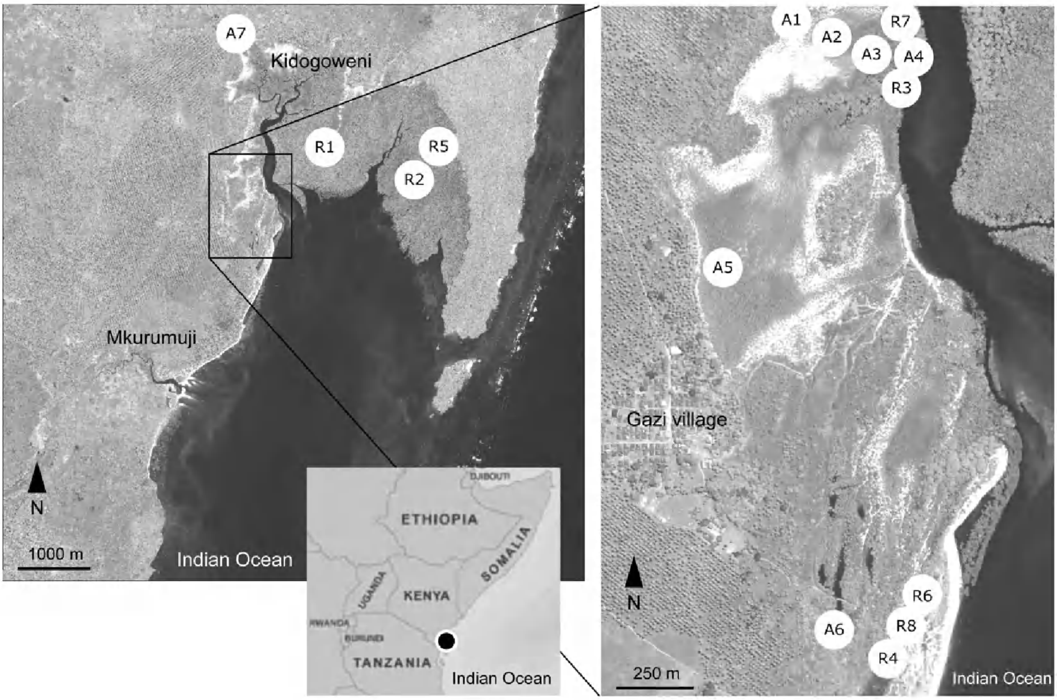


Figure 2 *- QuickBird satellite image of Gazi Bay acquired in 2002 (Neukermans* et al.*, 2008) and map of east Africa situating Gazi Bay on the Kenyan east coast (black dot). The seven study sites where stem discs of* Avicennia marina *were taken are indicated (A1–A7) together with the eight study sites of* Rhizophora mucronata *(R1–R8) (Schmitz* et al.*, 2006). The Mkurumuji and the Kidogoweni, two seasonal rivers that provide freshwater to the mangrove forest in Gazi Bay, are indicated on the left satellite image.*

Material and Methods

Study area

The research was conducted in the mangrove forest of Gazi Bay (4°25’ S, 39°30’ E) situated at the east coast of Kenya (Figure 2), at about 50 km south of Mombasa. The forest covers an area of about 600 ha (UNEP, 2001; Neukermans *et al.*, 2008) and is dominated by *R. mucronata*, *A. marina* and *Ceriops tagal* (Matthijs *et al.*, 1999; Dahdouh-Guebas *et al.*, 2004). The climate of the area is tropical, with a bimodal precipitation distribution: a distinct dry season (December–March) is followed by a long rainy season (April–July) and another short rainy season (October–November). Mean annual temperatures range from 22 to 30 °C, while mean relative humidity varies between 65% to 81% (annual averages of minima and maxima for Mombasa for the period 1972–2001, data from the Kenyan Meterological Department, Mombasa, Kenya). During the wet season, the Mkurumuji and the Kidogoweni, two seasonal rivers (Figure 2), are important sources of freshwater for the surrounding mangrove forests (Kitheka, 1997).

Sample collection and preparation

Wood discs of 35 *A. marina* trees were collected in June 2006. Before sampling, tree height of each tree was determined trigonometrically and stem diameter at the base of the tree was measured. The samples, with a diameter range of 2–7 cm, were all taken at a height of 1.3 m and originate from trees growing in seven different sites (five samples per site) (A1–A7, Figure 2). The sites were selected for their difference in environmental conditions. All wood samples are now part of the collection of the Royal Museum for Central Africa in Tervuren (Belgium) as are the samples of *R. mucronata* already collected in 1999 and 2002 in eight different study sites (R1–R8, Figure 2) (Schmitz *et al.*, 2006). Wood samples were air-dried and sanded using a series of sandpaper from 100 to 1200 grit.

Wood characteristics

Vessel features were measured on the sanded stem discs of *A. marina* under optical magnification of 12.5 times using digital image analysis software (AnalySIS Pro version 3.2, Soft Imaging System GmbH, Münster, Germany). On every sample in the two or three outermost growth segments, that is units of xylem and phloem originating from the same cambium, the radial and tangential diameter of at least 190 vessels as well as the xylem and phloem surface area of the growth segments were measured. For each number of vessels grouped together, the amount of vessel groups was counted. From these data we calculated the vessel density (number of vessels per mm^2), the proportion of phloem to xylem, the percentage of solitary vessels for each segment (number of solitary vessels per total number of vessels), the vessel grouping index (mean number of vessels per vessel group), the vessel surface area of each vessel and the ratio of radial to tangential diameter per vessel. For comparison with *R. mucronata* we used vessel density and vessel diameter data from our earlier study (Schmitz *et al.*, 2006). Data for vessel grouping in *R. mucronata* were obtained by counting the amount of vessel groups for each number of grouped vessels in the same measuring fields as used in Schmitz *et al.* (2006).

Vessel element length of *A. marina* and *R. mucronata* from nine trees out of three study sites for each species (A3, A4, A6, R3, R5 and R7, Figure 2 – three trees per study site) was studied after maceration with hydrogen peroxide and acetic acid. A minimum of hundred vessel elements per tree was measured at an optical magnification of fifty times using digital image analysis software (as above). The vessel element length was measured from perforation plate to perforation plate, tails not included. Wood samples for maceration were taken at 1 cm from the pith on wood discs taken at 1.4 m height.

Site variables

For all 14 sites, height above datum was calculated using tracing and local tide tables. Inundation classes were then calculated according to Tomlinson (1994). Inundation classes I, II, III and IV are attributed to sites inundated by respectively 100–76%, 75–51%, 50–26%, 25–5% of the high tides. One to three salinity measurements of the soil water of each study site were conducted between 1998 and 2007 by use of a WTW P4 multiline conductivity meter or a hand-held refractometer, as described in Schmitz *et al.* (2006, 2008b). Hemispherical images were taken at nine sites (A1–A7, R3, R8, Figure 2) to calculate the Leaf Area Index integrated over 0–75° with the software programme Gap Light Analyser version 2·0 (Simon Fraser University, British Columbia and the Institute of Ecosystem Studies, New York). Soil texture was determined for eight sites (A1–A7, R3, Figure 2) by standard field characterization methods (GLOBE, 2005). Nutrient concentrations (NO_3^-, NH_4^+, and soluble reactive P) were measured by standard procedures (APHA-AWWA-WEF, 1995) from water samples taken 1 day after spring tide in February 2007 at the seven *A. marina* sites.

Statistical analysis

The non-normal distribution of categorised (by study site) wood characteristics of *A. marina* did not allow for parametric tests, so Kruskal–Wallis tests were carried out for each variable (vessel density, proportion of phloem to xylem, vessel grouping index, radial diameter, tangential diameter, ratio of radial to tangential diameter, vessel surface area and vessel element length) with each factor (mean salinity, maximum salinity, minimum salinity, salinity range, inundation, mean NO_3^- concentration, mean soluble reactive P concentration, Leaf Area Index, tree height, stem diameter at 1.3 m and stem diameter at the tree base) with the exception of the mean NH_4^+ concentration for which the data set was considered insufficient. For *R. mucronata* Kruskal–Wallis tests for vessel density, vessel grouping, radial and tangential diameter and vessel element length were conducted for mean salinity, inundation, tree height, stem diameter at 1.3 m and stem diameter at the tree base. Mann–Whitney U tests were done to test for significance of differences in non-normal distributed wood characteristics between groups of *A. marina* study sites. In order to evaluate if the site effect is considerable (variation between the study sites is bigger than the variation within the study sites) variability plots were made for all wood characteristics of both species. All statistical analyses were conducted using Statistica 7·0 (StatSoft Inc., Tulsa, OK).

Results

A wood anatomical description of *A. marina* and *R. mucronata* can be found in Table 1. An overview of the collected environmental data can be found in Schmitz *et al.* (2008b) and in Table S1 of the Supporting Information. All environmental factors tested had a significant effect on the wood variables of *A. marina* and *R. mucronata* – P-values of Kruskal–Wallis tests at least lower than 0.05 but often lower than 0.001 – in contrast to the non-systematic effect of tree factors on the wood variables – Kruskal–Wallis tests often non-significant (see

Table 1 *- Wood anatomical description of the water transport system of* Avicennia marina *and* Rhizophora mucronata *based on the measurements for all trees from all study sites (A1–A7 and R1–R8, see Figure 2)*

	Median	Q1	Q3	Minimum	Maximum	*n*
Avicennia marina						
Vessel density – xylem (number of vessels mm^{-2} xylem area)	78·68	66·12	98·25	26·88	147·67	82
Vessel density – xylem + phloem (number of vessels mm^{-2} stems area)	57·95	46·49	70·73	17·38	110·82	82
Phloem : xylem ratio	0·38	0·31	0·43	0·22	0·63	82
Solitary vessels (%)	28·78	20·99	38·78	3·22	57·55	82
Vessel grouping index*	4·07	3·73	4·58	3·17	6·62	82
Radial diameter (μm)	36·47	23·74	51·67	5·78	115·50	8692
Tangential diameter (μm)	39·00	26·24	52·48	5·57	107·87	8692
Radial diameter : tangential diameter ratio	0·96	0·80	1·14	0·27	3·75	8692
Vessel surface area ($μm^2$)	1085·74	501·11	2043·41	37·94	8463·18	8692
Vessel element length (μm)	186·22	147·32	221·66	33·89	380·47	1127
Rhizophora mucronata						
Vessel density – xylem (number of vessels mm^{-2} xylem area)	25·65	21·51	32·34	12·51	66·02	279
% solitary vessels	78·57	71·05	83·78	42·11	100·00	279
Vessel grouping index*	1·26	1·18	1·36	1·00	1·80	279
Radial diameter (μm)	79·89	71·63	90·91	24·79	134·99	7447
Tangential diameter (μm)	71·63	63·36	77·13	24·79	115·70	7513
Vessel element length (μm)	682·51	593·02	781·65	285·04	1171·25	900

**Mean number of vessels per vessel group; Q1, lower quartile; Q3, upper quartile; n, total number of measurements.*

Table 2 *- Comparison of the wood anatomical characteristics of* Avicennia marina *and* Rhizophora mucronata *at a study site where both species grow together (A4 and R3, see Figure 2)*

	A. marina				***R. mucronata***			
	Median	Q1	Q3	*n*	Median	Q1	Q3	*n*
Vessel density – xylem (number of vessels mm^{-2} xylem area)	74·44	51·70	98·73	11	22·85	20·02	26·14	24
Solitary vessels (%)	31·73	17·28	44·33	11	78·66	71·39	86·10	24
Vessel grouping index*	4·13	3·74	5·05	11	1·26	1·17	1·33	24
Radial diameter (μm)	38·58	23·74	57·07	1192	79·89	71·63	88·15	485
Tangential diameter (μm)	42·98	28·65	58·30	1192	68·87	63·36	74·38	480
Vessel element length (μm)	172·21	132·43	205·97	360	719·94	638·60	769·24	300

**Mean number of vessels per vessel group; Q1, lower quartile; Q3, upper quartile; n, total number of measurements.*

Tables S2 and S3 in Supporting Information).

Variability plots of vessel density and vessel grouping showed a considerable site effect in both *A. marina* and *R. mucronata*. There was a significant difference in vessel density of *A. marina* between the study sites with a mean salinity below 51.0‰ and above 57.6‰ (U = 347.00, P < 0.001, Mann– Whitney U test, Figure 3), a minimum salinity below 20.9‰ and above 38.0‰ (U = 347.00, P < 0.001, Mann–Whitney U test) and a maximum salinity below 68.2‰ and above 70.0‰ (U = 270.00, P < 0.001, Mann–Whitney U test), the latter with exception of the study site with a maximum salinity of 90.0‰. In all three cases higher

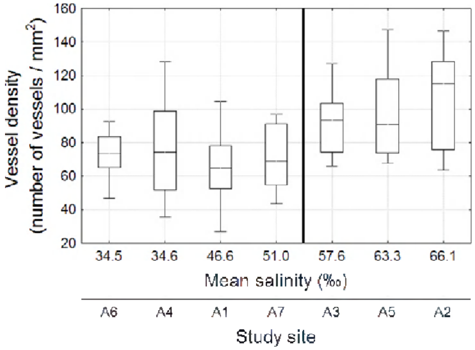


Figure 3 - *Median of the vessel density in relation to mean salinity for* Avicennia marina. *Each salinity value represents one study site. The vertical line separates a group of lower vessel densities from one of higher vessel densities (U = 347.00, P < 0.001, Mann–Whitney U test). line (median), box (25–75%), whiskers (non-outlier range).*

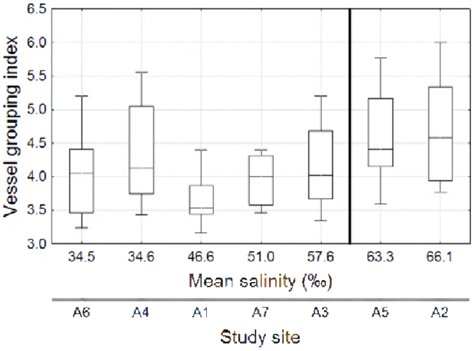


Figure 4 - *Median of the vessel grouping index (mean number of vessels per vessel group) in relation to mean salinity for* Avicennia marina. *Each salinity value represents one study site. The vertical line separates a group of lower vessel grouping from one of higher vessel grouping (U = 352.00, P < 0.001, Mann–Whitney U test). line (median), box (25–75%), whiskers (non-outlier range).*

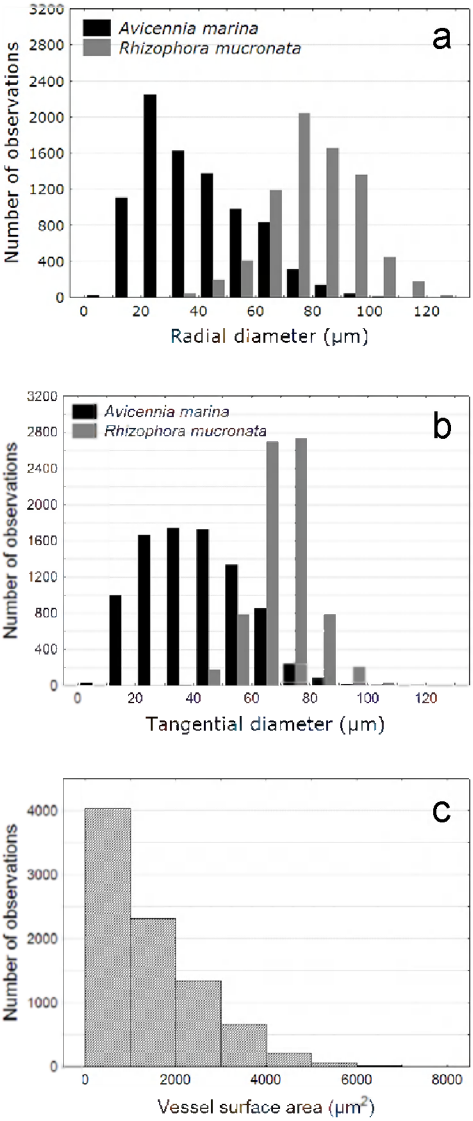


Figure 5 - *Frequency distribution of the radial (a) and tangential (b) diameters in* Avicennia marina *and* Rhizophora mucronata *and of vessel area in* A. marina *(c).*

vessel density was found in sites with higher salinity values. Furthermore the vessel grouping index of *A. marina* in the two study sites with the highest mean salinity values were significantly higher than this of the study sites with a mean salinity equal to or lower than

63.3‰ (U = 352.00, P < 0.001, Mann–Whitney U test, Figure 4). Increasing vessel density with increasing salinity was also found in *R. mucronata* (also see Schmitz *et al.*, 2006), vessel grouping however was not higher in sites with higher mean salinity.

In spite of a high variability in radial and tangential diameter in *Avicennia* trees of the same study site, a study site effect can still be observed. Although the smallest radial diameters and the smallest vessel surface areas can be found in the study sites with the highest mean salinity, vessel diameters varied little with mean salinity in *A. marina*. Frequency distributions of vessel dimensions in *A. marina* show a definite right skewed curve for radial vessel diameter (Figure 5a) and vessel surface area (Figure 5c) and a less pronounced right skewed distribution for tangential diameter (Figure 5b). In *R. mucronata* on the contrary radial (Figure 5a) as well as tangential diameters (Figure 5b) have tendency to the normal distribution. The ratio of radial to tangential diameter in *A. marina* is close (Table 1) and highly centred to one, meaning that most vessels in this species are nearly circular.

Discussion

Strategies of mangrove trees to survive

All environmental factors tested had a significant effect on the wood anatomy of *A. marina* in Gazi Bay, but only salinity gave rise to visually observable trends in different vessel characteristics. The same direct and important relationship between salinity and wood anatomy is reported for *R. mucronata* (Schmitz *et al.*, 2006) and also growth layer width in *A. marina* is depending on salinity conditions (Schmitz *et al.*, 2008b). In this regard, it can be concluded that salinity is the dominant factor of impact on the wood anatomy of mangroves, and this on tissue as well as on cellular level.

Vessel density and not vessel dimension is found to be the most responsive wood characteristic to varying salinity conditions in both *A. marina* and *R. mucronata*. The osmotic stress due to high salinity causes low water potential (causing a high tension or negative pressure) in the xylem sap of mangrove species (Scholander *et al.*, 1965; Scholander, 1968). This tension on turn causes a higher probability for cavitation events (Tyree & Sperry, 1989; Hacke & Sperry, 2001; Naidoo, 2006). Even though vessel diameters are found to be smaller in various tree species under more stressful conditions for the water transport (Lo Gullo *et al.*, 1995; Villagra & Juñent, 1997; Corcuera *et al.*, 2004; Choat *et al.*, 2005a), these two mangrove species seem to deal with the higher cavitation risk at higher salinity values mainly by an increased vessel density. The same trend has been observed in the mangrove *Laguncularia racemosa* in Mexico (Yáñez-Espinosa *et al.*, 2004). Furthermore, Melcher *et al.* (2001) found that *Rhizophora mangle* trees growing at sites of higher soil water salinity were more vulnerable to cavitation then those from sites with lower salinity levels. So the mechanism of higher vessel density under physiological conditions with higher cavitation risk via a gradual density increase in *R. mucronata* (Schmitz *et al.*, 2006) and an abrupt increase around a salinity of 55‰ in *A. marina* (Figure 3), is present in phylogenetically distant mangrove species (Schwarzbach & Ricklefs ,2001; Shi *et al.*, 2005). As Dahdouh-Guebas *et al.* (2004) indicated a genetic difference between landward and seaward *A. marina* stands in Gazi Bay, we cannot confirm whether either plasticity within a genotype or adaptive variation among genotypes is contributing more to the differences in vessel density between the different study sites. Despite the uncertainty about its underlying cause, a higher vessel density under more saline conditions can be considered a common mechanism in trees in the mangrove ecosystem in order to deal with the severe and dynamic conditions of their environment. These results are reinforcing the findings of Verheyden *et al.* (2005) who discovered a within-tree variation in the vessel density of *R. mucronata* depending on the growth season.

Although vessel density reflects the overall salinity of the site, the maximum salinity of study site A1 (Figure 2) is not reflected in a high vessel density in A. marina. The soil water salinity of this most land inward site of *A. marina* has an extremely high maximum salinity due to a low inundation frequency – only a few days of flooding a month – together with high evaporation during the dry season (Naidoo, 2006). But during the rainy season high amounts of intermittent freshwater run-off leads to a serious decline in soil water salinity. This rather low salinity after periods of rain most probably compensates for the high cavitation risk during drier periods leading to trees that have a much lower vessel density than expected. A high vessel density optimizes the water transport under stressful conditions by allowing a more efficient bypass of air-filled vessels and by leaving more vessels functional for the same number of embolized vessels, as was already suggested by Schmitz *et al.* (2006). In non-stressful conditions such as high freshwater availability, this safety solution is however unnecessary and weighed against more advantageous characteristics of the vessel network.

It is remarkable that the vessel density of trees from the same species growing in one forest, so on a very small geographical scale, is different according to differences in environmental conditions. Moreover, this vascular trait variation happens in a parallel way in the two main species of the mangrove ecosystem. Vessel characteristics, especially adaptively-changing vessel densities, only partially explain how mangrove trees survive in their environment. Intervessel pit characteristics also appear to play a role in safeguarding the water transport in the dynamic mangrove environment (Lopez-Portillo *et al.*, 2005; Schmitz *et al.*, 2007a; Schmitz *et al.*, 2008a). We are however far from understanding the full spectrum of the mangrove anatomy and functioning in relation to the mangrove environment. But what can the wood anatomy of *A. marina* and *R. mucronata* throw in to the explanation of the ecological distribution and the biogeography of these mangrove species?

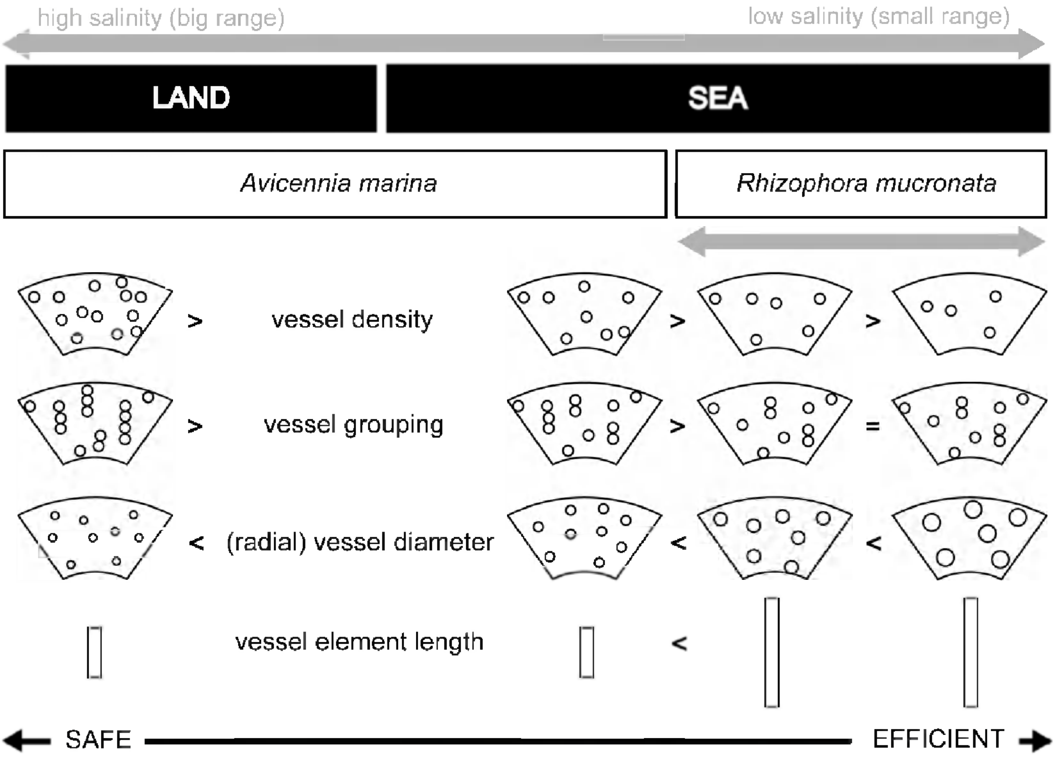


***Figure 6** - Overview of the observed wood anatomical characteristics of* Avicennia marina *and* Rhizophora mucronata *trees in function of the location in the mangrove forest. Trees growing more landward, under environmental conditions that are more challenging for the water transport system, such as high salinity and low inundation, are characterised by a water transport system composed of high vessel density, high vessel grouping, small vessel diameters and short vessel elements. Taking the functional interpretation of all these wood anatomical characteristics together, the water transport system of trees at this side of the mangrove forest is considered safer. On the other hand, trees growing on the seashore seem to have a more efficient water transport system, rather lacking adaptations to avoid air in the water column. Arrows are showing ecological gradients (grey) and a gradient from safe to efficient water transport systems (black).*

Avicennia*: the safer one*

From the comparison between *A. marina* and *R. mucronata* based on all vessel characteristics studied (Table 1) can be inferred that the water transport systems of both species are highly different. This is also true in the study site where both species grow together (A4 and R3, Figure 2, Table 2). Former studies on wood characteristics in *R. mucronata* have already proven the plasticity potential of vessel density and radial vessel diameter in this species (Verheyden *et al.*, 2005; Schmitz *et al.*, 2006). Furthermore, the present study and other studies on different mangrove species (*e.g.* Sun & Suzuki, 2000; Yáñez-Espinosa *et al.*, 2001; Sobrado, 2007) provide additional evidence for adaptation of hydraulic characteristics to the environment mangrove trees live in. However, one should be aware that also the different genetic background of the species could be expressed in their hydraulic structure. Yet even when the phenotypic plasticity of the studied wood characteristics turns out to be low, the highly different structure of both species living in the same habitat can have a major effect on the tolerance towards environmental factors, thus defining the species distribution. In *A. marina* we observed a dominance of smaller vessel diameters, in contrast to *R. mucronata* where vessels of intermediate size were more frequent (Figure 5). Although a link between vessel diameter and cavitation sensitivity is not generally accepted, other studies show (species with) smaller vessels to be less prone to cavitation (Lo Gullo & Salleo, 1991; Hargrave *et al.*, 1994; Lo Gullo *et al.*, 1995; Choat *et al.*, 2003). As *A. marina* not only has smaller vessels than *R. mucronata* but also has a larger proportion of the tiniest vessels, a link between small vessel diameter and low vulnerability to cavitation would include that the water transport system of *A. marina* is double safe. Intervessel pit characteristics are however supposed to explain differences in vulnerability to cavitation better than vessel diameters (Jarbeau *et al.*, 1995; Hacke & Sperry, 2001; Wheeler *et al.*, 2005; Hacke *et al.*, 2006; Choat *et al.*, 2008). A safety difference between *A. marina* and *R. mucronata* is found in the morphology of their intervessel pits. Schmitz *et al.* (2007a) observed that several pit characteristics in *A. marina* – smaller pitfield fractions, smaller inner and outer pit apertures, smaller individual pit size, thicker pit membranes and the presence of vestures – could be linked to a safer water transport system if compared to *R. mucronata*.

Avicennia marina does not only have small but also nearly circular vessel areas (Table 1). Observations of more circular vessels in different *Rhizophora* species growing on locations with lower annual rainfall and longer dry seasons (Nele Schmitz, unpublished data) and in *Aesculus hippocastanum* growing on sites with high salt concentrations due to de-icing salts (Eckstein *et al.*, 1976) give a clear indication that more circular vessels offer an additional advantage in stressful conditions, though further data would be needed to explain this observation.

Vessel grouping and vessel density, other important xylem characteristics, are also found to differ between *A. marina* and *R. mucronata* with the vessels of the latter being much less grouped and less dense. Within *A. marina* vessel grouping, as vessel density, is higher in conditions of higher salinity (Figure 4). High vessel density does not only increase hydraulic efficiency, it also safeguards the water transport system as, compared to a less dense vessel network, a larger proportion of vessel stay functional for the same number of vessels embolized (Baas *et al.*, 1983; Mauseth & Plemons-Rodriguez, 1997; Villar-Salvador *et al.*, 1997). High vessel grouping, more probable with high vessel density, can also bring a functional advantage because it allows water to bypass air-filled vessels by alternative pathways created by the intervessel pits of touching vessels in a vessel group (Baas *et al.*, 1983; Zimmermann, 1983; Yáñez-Espinosa *et al.*, 2001; Lopez *et al.*, 2005). On the contrary, a higher vessel grouping can also increase cavitation probability as vessel contact, influencing the spread of embolisms, is more intense (Wheeler *et al.*, 2005; Hacke *et al.*, 2006; Choat *et al.*, 2008). We, however, do not know whether either the safety aspect or the cavitation spread is the more important. As we observed higher vessel grouping in more stressful conditions and lower pit field fractions in A. marina (Schmitz *et al.*, 2007a), both safety and cavitation spread are maybe balanced, giving *A. marina* a functional advantage.

Apart from vessel diameter, vessel shape, vessel grouping and vessel density also vessel element length is different between the two species studied: *A. marina* has much shorter vessel elements than *R. mucronata* (Table 1). Furthermore, a preliminary study in different sites showed significantly shorter vessel elements in the higher parts of *R. mucronata* trees, where pressures get more negative (Scholander *et al.*, 1965), compared to longer vessel elements at breast height (Elisabeth Robert, unpublished data, $U = 28226{\cdot}00$, $P < 0.001$, Mann–Whitney U test). Although vessel length and not vessel element length is generally considered the more important hydraulic characteristic (Baas, 1986), further research has to be conducted to find out if smaller vessel elements (i) hinder the spreading of embolisms by their perforation plates and hence are a functional advantage, (ii) are, on the contrary, a functional disadvantage by increasing embolism spread due to higher resistance to xylem sap flow (Ellerby & Ennos, 1998; Schulte, 1999) and thus larger pressure differences across intervessel pits, or (iii) do not have a functional significance.

Although validation is needed, the interpretation of all wood anatomical characteristics studied does not contradict the following statement: the water transport system of *A. marina* is most probably safer than that of *R. mucronata*. In that respect the ecological success of *A. marina* can be related to the characteristics of its water transport system. As this safety of the water transport is reflected in the local distribution of the mangrove species studied (Figure 6), we can conclude that the vessel characteristics of *A. marina* and *R. mucronata* explain at least partially the local distribution of both species.

Not only locally but also globally *A. marina* has a wider distribution than *R. mucronata* (Spalding *et al.*, 1997). We could therefore expect that the highly different vessel anatomy of these two mangrove species can also contribute to the explanation of their distribution in the Eastern biogeographical mangrove region. Parallel to the ecotone of environmental conditions from sea to land we can expect a transect of environmental conditions that are less and less suitable for mangrove species from the equator to higher latitude, both north and south. *A. marina* with its safer water transport system could support conditions in which *R. mucronata* cannot survive anymore, logically leading to a more southward and more northward expanded distribution. Moreover the same difference in distribution between *Avicennia* and *Rhizophora* can be observed in the Western biogeographical mangrove region (Spalding *et al.*, 1997). We expect that the same results found for *A. marina* and *R. mucronata* can be found for *Avicennia germinans* and *R. mangle*, the most important species of this biogeographical region. Supposing a general difference in wood anatomy between the two mangrove genera studied, the safety of the water transport system of mangrove trees has a high potential to explain the global distribution of mangrove genera at least partially.

We can conclude that the water transport system of *A. marina* in Gazi Bay (Kenya), built of a high vessel density, a high vessel grouping, small vessel diameters and short vessel elements, is contrasting with the water transport system of *R. mucronata* and is expected to be safer based on a physiological interpretation of the combination of observed vessel characteristics. This contrast is reflected in the local distribution of the species and most likely contributes to the explanation of the global distribution of *Avicennia* and *Rhizophora*. Because vessel characteristics are only part of the tree system, patterns in other anatomical and functional factors have to be studied to complete these results. Furthermore, we hypothesize other mangrove species in other localities, in the Eastern as well as in the Western biogeographical mangrove regions, to reflect the same trends, though this still has to be investigated.

Acknowledgements

We thank James Gitundu Kairo and Jared Bosire of the Kenyan Marine and Fisheries Research Institute for the logistic help during the field expedition, Hamisi Ali Kirauni and Elisha M'rabu Jenoh for the help during the fieldwork and Samuel Teissier for the assistance with the nutrient analyses. We are grateful to both referees for their clear and helpful comments on the manuscript. The study was financially supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT–Vlaanderen), the Flemish Interuniversity Council (VLIR), the Schure-Beijerinck-Popping Fonds (Koninklijke Nederlandse Akademie van Wetenschappen, Nederland) and the National Fund for Scientific Research (FWO, Belgium).

Supporting Information

Table S1 *- Stand characteristics of* Avicennia marina *(from Schmitz* et al.*, 2008b) and* Rhizophora mucronata *study sites in Gazi Bay, Kenya. Soil water for nutrient and salinity analyses was taken at about 25 cm depth. Inundation classes are according to Tomlinson (1994). Values for soil water nutrients are means with range between brackets.*

Location	Soil Water Nutrients (10^{-3} µmol/cm^3 soil)			LAI	Soil texture	Soil Water Salinity (‰)			Inundation class
	NO_3^-	NH_4^+	P			mean	min	max	
Avicennia marina									
A1	1 (0-4)	0.5 (0.1-1.1)	0.1 (0-0.3)	0.62	sandy loam	46.6	10.0	90.0	IV
A2	0.15 (0.10-0.24)	*	0.2 (0.1-0.3)	0.23	loamy sand	66.1	38.0	86.0	III
A3	0.3 (0.2-0.5)	*	0 (0-0)	0.25	sandy loam	57.6	40.0	70.0	II
A4	0.5 (0.2-0.6)	3 (0-5)	1.4 (0.9-1.8)	1.42	silty clay	34.6	20.9	46.0	I
A5	2 (0-6)	10 (7-14)	0.1 (0-0.3)	1.18	clay loam	63.3	40.0	79.9	III
A6	23 (0-68)	*	2 (0-4)	1.73	loamy sand – sandy loam	34.5	10.0	48.0	IV
A7	0.3 (0.0-0.4)	1 (0-4)	2 (0-4)	1.29	loamy sand – sandy loam	51.1	5.0	68.2	III
Rhizophora mucronata									
R1	*	*	*	*	*	26.4	-	-	III
R2	*	*	*	*	*	31.9	-	-	III
R3	0.5 (0.2-0.6)	3 (0-5)	1.4 (0.9-1.8)	1.42	silty clay	33.6	-	-	I
R4	*	*	*	*	*	35.2	-	-	II
R5	*	*	*	*	*	35.4	-	-	III
R6	*	*	*	*	*	38.2	-	-	II
R7	*	*	*	*	*	42.7	-	-	IV
R8	*	*	*	1.27	*	49.2	-	-	II

*P (soluble reactive phosphor), LAI (Leaf Area Index), min (minimum), max (maximum), * (no data records), - (minimum and maximum values are not reported as salinity is measured in one year only)*

Table S2 *- Results of Kruskal-Wallis tests of the wood characteristics for* Avicennia marina.

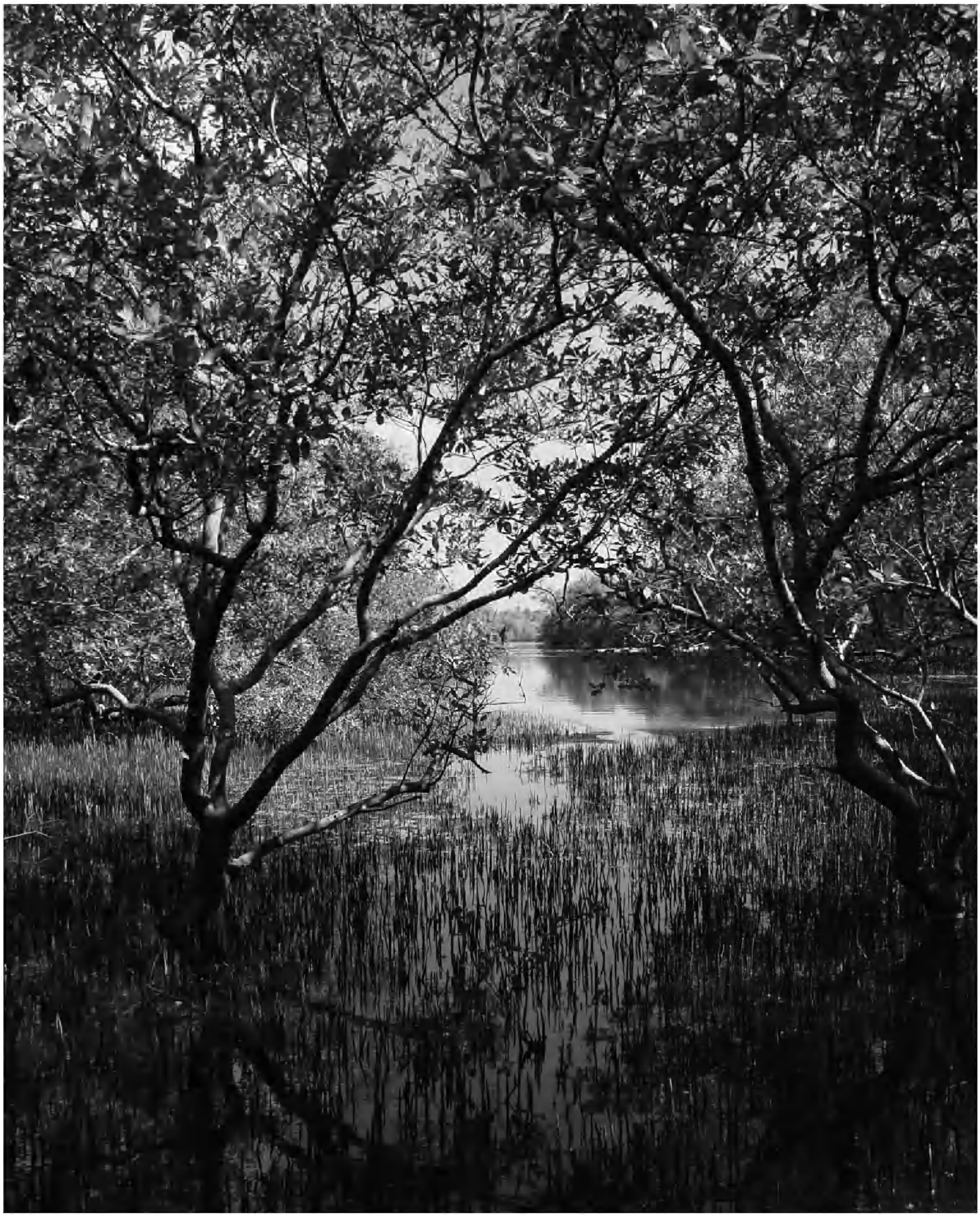
	H	p	n	H	p	n	H	p	n
	vessel density – xylem			*vessel grouping index**			*ratio of phloem to xylem*		
**	21.36	< 0.01	82	18.93	< 0.01	82	18.09	< 0.05	82
minimum salinity	20.85	< 0.001	82	13.36	< 0.01	82	9.05	< 0.05	82
inundation	12.34	< 0.01	82	12.62	< 0.01	82	11.25	< 0.05	82
soil texture	14.09	< 0.01	82	15.02	< 0.01	82	11.81	< 0.05	82
tree height	15.09	< 0.01	82	3.32	n.s.	82	6.80	n.s.	82
stem diameter (1.3 m)	8.48	< 0.05	82	1.61	n.s.	82	5.43	n.s.	82
stem diameter (0 m)	1.08	n.s.	82	1.86	n.s.	82	4.87	n.s.	82
	tangential diameter			*radial diameter*			*ratio of radial to tangential diameter*		
**	316.46	< 0.001	8692	125.38	< 0.001	8692	225.54	< 0.001	8692
minimum salinity	93.74	< 0.001	8692	50.78	< 0.001	8692	57.28	< 0.001	8692
inundation	230.11	< 0.001	8692	73.66	< 0.001	8692	111.25	< 0.001	8692
soil texture	252.99	< 0.001	8692	103.18	< 0.001	8692	196.84	< 0.001	8692
tree height	70.18	< 0.001	8692	58.02	< 0.001	8692	15.18	< 0.01	8692
stem diameter (1.3 m)	70.37	< 0.001	8692	38.54	< 0.001	8692	5.71	n.s.	8692
stem diameter (0 m)	105.86	< 0.001	8692	32.27	< 0.001	8692	57.47	< 0.001	8692
	vessel surface area			*vessel element length*					
**	207.20	< 0.001	8692	111.22	< 0.001	1127			
minimum salinity	74.91	< 0.001	8692	111.22	< 0.001	1127			
inundation	148.98	< 0.001	8692	111.22	< 0.001	1127			
soil texture	168.49	< 0.001	8692	111.22	< 0.001	1127			
tree height	65.51	< 0.001	8692	1.73	n.s.	1127			
stem diameter (1.3 m)	58.19	< 0.001	8692	0.68	n.s.	1127			
stem diameter (0 m)	68.34	< 0.001	8692	40.88	< 0.001	1127			

*n (total number of measurements), * (mean number of vessels per vessel group), ** (mean salinity / maximum salinity / salinity range / mean NO3- concentration / mean soluble reactive P concentration / Leaf Area Index) n.s. (non significant)*

Table S3 - _Results of Kruskal-Wallis tests of the vessel characteristics for_ Rhizophora mucronata.

	H	p	n	H	p	n
	vessel density – xylem			_vessel grouping index*_		
mean salinity	78.78	< 0.001	279	25.27	< 0.001	279
inundation	70.24	< 0.001	279	19.99	< 0.001	279
tree height	15.07	< 0.001	255	8.52	< 0.05	255
stem diameter (1.3 m)	6.96	< 0.05	279	9.52	< 0.01	279
	tangential diameter			_radial diameter_		
mean salinity	321.42	< 0.001	7447	524.10	< 0.001	7513
inundation	108.44	< 0.001	7447	358.55	< 0.001	7514
tree height	173.05	< 0.001	6592	487.94	< 0.001	6659
stem diameter (1.3 m)	59.33	< 0.001	7447	241.31	< 0.001	7514
	vessel element length					
mean salinity	136.56	< 0.001	900			
inundation	136.56	< 0.001	900			
tree height	48.13	< 0.001	900			
stem diameter (1.3 m)	3.74	n.s.	900			

n (total number of measurements), * (mean number of vessels per vessel group), n.s. (non significant)



Ik heb ervaren, geloof mij:
in de wouden vindt gij meer dan in boeken.
Hout en stenen zullen u over dingen leren
waarvan gij bij de leraars niets kunt horen.

BERNARDUS VAN CLAIRVAUX [5]

Part 2

Successive cambia at the basis of ecological success

Chapter 3 - Successive cambia: A Developmental Oddity or an Adaptive Structure?
> Robert et al. (2012) - PLoS ONE 6(1): e16558 - p. 91

Chapter 4 - How to catch the patch? A dendrometer study on the radial increment through successive cambia in the mangrove *Avicennia*. - - - - - - - - - p. 111

Chapter 5 - Visualisation of the stem water content of two species with internal phloem through Magnetic Resonance Imaging (MRI). - - - - - - - - p. 149

Summary

Are successive cambia contributing to ecological success when water access is limited?

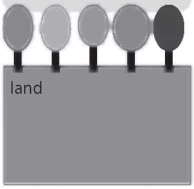
Chapter 3

Chapter 5

APPROACH 1: INTERNAL PHLOEM TISSUE

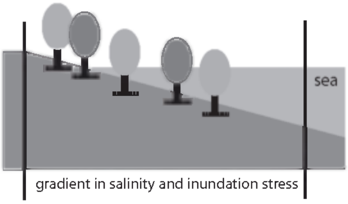
Ecological setting

non-mangrove trees



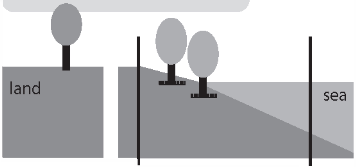
limited water availibility

Avicennia trees



gradient in salinity and inundation stress

Bougainvillea & *Avicennia* tree



▶ transported to Wageningen NMR Centre

Research questions

Do woody species with internal phloem tissue mainly occur in conditions of limited water access?

How is the amount of internal phloem in *Avicennia* trees adapted to the gradient in salinity and inundation stress within the mangrove forest?

Does the internal phloem tissue of *Bougainvillea* and *Avicennia*, two species with successive cambia, contain a considerable amount of water throughout the entire stem?

Methodology

A) Literature analysis to study the habitats in which species with internal phloem mainly occur.

B) Visualisation of the internal structure of the vascular tissues in *Avicennia* trees through (micro-)CT-scanning and comparison of the amount of internal phloem in different *Avicennia* species and different trees of one species along the intertidal gradient.

MRI-scanning of one *Bougainvillea* and two *Avicennia* trees to obtain images of the internal stem showing the water content of the different vascular tissues.

Results & Discussion

> Trees and shrubs with internal phloem occur for 84.9% in habitats characterized by (periodical) water shortage.

> *Avicennia* has a complex network of non-cylindrical wood and phloem patches, the complexity of which increased with more stressful ecological conditions.

The internal phloem tissue of the studied trees showed high water content over the entire stem.

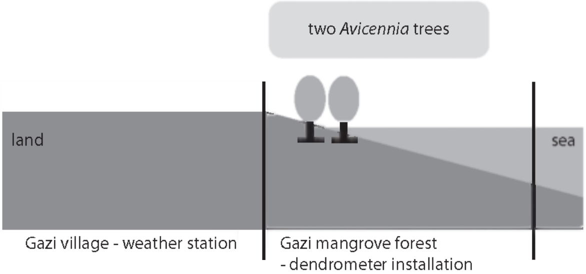
Successive cambia are an ecologically important characteristic, which seems strongly related with waterlimited environments.

The extra availability of water storage locations inside the trees stem could be responsible for ecological advantage in conditions of water shortage

Conclusion

Having successive cambia brings *Avicennia* two ecological advantages in the mangrove environment where conditions are dynamic and often characterized by physiological drought: (i) internal phloem tissue can store water and (ii) patchiness allows for a temporally and spatially dynamic reaction on prevailing environmental conditions.

APPROACH 2: PATCHY GROWTH



Do *Avicennia* spp. have a patchy growth system with different locations of the stem growing at different moments in time?

Hourly measurement of:
- rainfall
- temperature (shadow & sun)
- relative humidity
- wind speed
- wind direction

a) Measurement of tree diameter variations at four positions on each tree with point dendrometers.

b) Measurement of local
- soil water conductivity
- soil temperature
- air temperature
- relative air humidity

c) Determination of local inundation level and frequency

d) Study of tree phenology

> *Avicennia* trees show patchiness in their radial growth as well as in the shrinking and swelling patterns of their stem. The patchiness however seems a potential rather than a systematic feature.

> Radial growth increment is strongly linked to rainfall and rain events had an instant effect on the daily shrinking and swelling patterns of tree stems.

Potential patchy growth and patchy reaction of the vascular tissues to the environmental conditions of the mangrove forest could allow *Avicennia* trees to optimally react on the prevailing environmental conditions, leading to ecological success in the highly dynamic mangrove environment.

Successive cambia: a developmental oddity or an adaptive structure?

Published in: PLoS ONE 6(1): e16558

Abstract

Background: Secondary growth by successive cambia is a rare phenomenon in woody plant species. Only few plant species, within different phylogenetic clades, have secondary growth by more than one vascular cambium. Often, these successive cambia are organised concentrically. In the mangrove genus *Avicennia* however, the successive cambia seem to have a more complex organisation. This study aimed (i) at understanding the development of successive cambia by giving a three-dimensional description of the hydraulic architecture of *Avicennia* and (ii) at unveiling the possible adaptive nature of growth by successive cambia through a study of the ecological distribution of plant species with concentric internal phloem.

Results: Avicennia had a complex network of non-cylindrical wood patches, the complexity of which increased with more stressful ecological conditions. As internal phloem has been suggested to play a role in water storage and embolism repair, the spatial organisation of *Avicennia* wood could provide advantages in the ecologically stressful conditions species of this mangrove genus are growing in. Furthermore, we could observe that 84.9% of the woody shrub and tree species with concentric internal phloem occurred in either dry or saline environments strengthening the hypothesis that successive cambia provide the necessary advantages for survival in harsh environmental conditions.

Conclusions: Successive cambia are an ecologically important characteristic, which seems strongly related with waterlimited environments.

Introduction

Expansion in girth in most vascular plant species is the result of the meristematic activity of one cylindrical vascular cambium, producing xylem towards the inner and phloem towards the outer part of the plant stem (Figure 1a) (Carlquist, 2001). However, some plant species (out of 34 families according to the taxonomy used (Carlquist, 2001)) have not one but several successive cambia causing the secondary growth and the resulting wood anatomy to be different from that of plants with only one vascular cambium (Figure 1b). In the stem of plants with successive cambia a sequence of vascular cambia can be found, each responsible for the production of secondary xylem inwards and secondary phloem outwards (Figure 1b) (Carlquist, 2007a). The cambia can literally develop successively but several cambia can also develop simultaneously (Schmitz *et al.*, 2008b). This results in plants from which stem discs show a succession of dark coloured xylem tissue bands and pale coloured phloem tissue bands.

Special forms of secondary wood formation are typical for lianas and vines (*e.g.* Dobbins & Fisher, 1986; McDonald, 1992; Nair, 1993; Carlquist, 2001; Araujo & Costa, 2006), though the phenomenon of successive cambia is also found in a select group of herb, shrub and tree species (*e.g.* Fahn & Shchori, 1967; Fahn & Zimmermann, 1982; Rajput & Rao, 2000; Carlquist, 2001; InsideWood, 2004; Rajput *et al.*, 2006) coming from different phylogenetic clades (Carlquist, 2001; InsideWood, 2004; Carlquist, 2007a). The genus *Avicennia* is the only mangrove tree genus showing the developmental oddity of secondary growth by successive cambia. Xylem and phloem tissue in *Avicennia* are found to be non-concentric (Figure 1c) (*e.g.* (Baker, 1915; Chapman, 1947; Studholme & Philipson, 1966; Zamski, 1979; Zamski, 1981; Schmitz *et al.*, 2007b; Schmitz *et al.*, 2008b) and form an intricate three-dimensional network instead of separate concentric cylinders (Zamski, 1979; Schmitz *et al.*, 2007b; Schmitz *et al.*, 2008b). Detailed and four dimensional - the three spatial dimensions and time - observations of these xylem and phloem networks are lacking, though highly necessary to understand *Avicennia*'s growth pattern and the secondary growth of trees with successive cambia in general.

From a study in two dimensions, the nature of secondary growth in *Avicennia marina* has been suggested to be patch-like, with active growth displacing around the stem circumference with time (Schmitz *et al.*, 2008b). In *Avicennia*, cambium differentiation was found to be internally controlled (Gill, 1971; Zamski, 1981; Schmitz *et al.*, 2007b), with indications for an interaction with the micro-environmental conditions such as soil water salinity (Schmitz *et al.*, 2008b). What triggers the development of new vascular cambia however remains unclear. In the harsh and changing environmental conditions of the mangrove habitat, the secondary growth of *Avicennia marina* and the resulting pattern of vascular tissue have been proposed to offer a functional advantage for the water transport. While the non-lignified and more thin-walled phloem tissue in between the more rigid xylem tissue increases the mechanical flexibility of lianas (Bhambie, 1972; Carlquist, 2001; Carlquist, 2007a), it can also provide water and photosynthate supply (Salleo *et al.*, 2004; Salleo *et al.*, 2006; Schmitz *et al.*, 2007b; Schmitz *et al.*, 2008b; Salleo *et al.*, 2009; Zwieniecki & Holbrook, 2009) for plants growing under water stress. The living phloem tissue in at least the outer part of the stem could play an important role in embolism repair (Mauseth & Plemons-Rodriguez, 1997; Salleo *et al.*, 2004; Salleo *et al.*, 2006; Scholz *et al.*, 2007; Salleo *et al.*, 2009; Zwieniecki & Holbrook, 2009). In addition, beneficial growth conditions can be exploited by simultaneous activity of more than one cambium or by alternating cambial activity around the stem disc. In this way, wood segments with different wood anatomical characteristics – and thus different physiological possibilities - can be created around the stem disc.

In this study we aimed at clarifying the three-dimensional structure of the xylem and phloem tissue of the mangrove genus *Avicennia* through (micro-)CT-scanning. X-ray (micro-)CT-scanning allows a non-destructive visualization of the internal structures at high resolution. In order to differentiate between connected structures a difference in X-ray density is required. Since xylem is denser than phloem tissue, this technique is appropriate to study the 3D structure. We further investigated the effect of the environment on the spatial organisation of this structure to unveil aspects of the remarkable adaptation of *Avicennia* to the mangrove ecosystem. In order to widen the scope of our conclusions, we tested the relation between habitats that are susceptible to water stress and the presence of woody species with concentric internal phloem. As successive cambia and the resulting internal phloem rings are thought to bring physiological advantage in harsh environmental conditions, we hypothesized that the major part of the woody species with internal concentric phloem are growing in habitats that are physiologically harsh either by drought or salt stress.

Results

The Three-Dimensional Structure of Avicennia*'s Transport System*

A reticulate xylem structure, consisting of xylem patches joined in horizontal as well as in vertical direction (Figure 1c–k), was found consistently in all species and tree samples studied. Within this reticulate system, the connections between xylem zones (patches) changed rapidly with height (Figure 1h–k) creating a complex network of xylem patches that fused at certain heights of the tree but separated to join different patches in other parts of the tree stem (Figure 1d–e). In small zones of wood, it could be observed that vessels, although in the same organisation with respect to each other, were more or less scattered depending on height. The remaining space was filled with fibre cells. Moreover, xylem volumes with their corresponding phloem could be found to belong to one growth patch at a certain tree height, while being part of another one at another height (Figure 1h–k). Therefore phloem

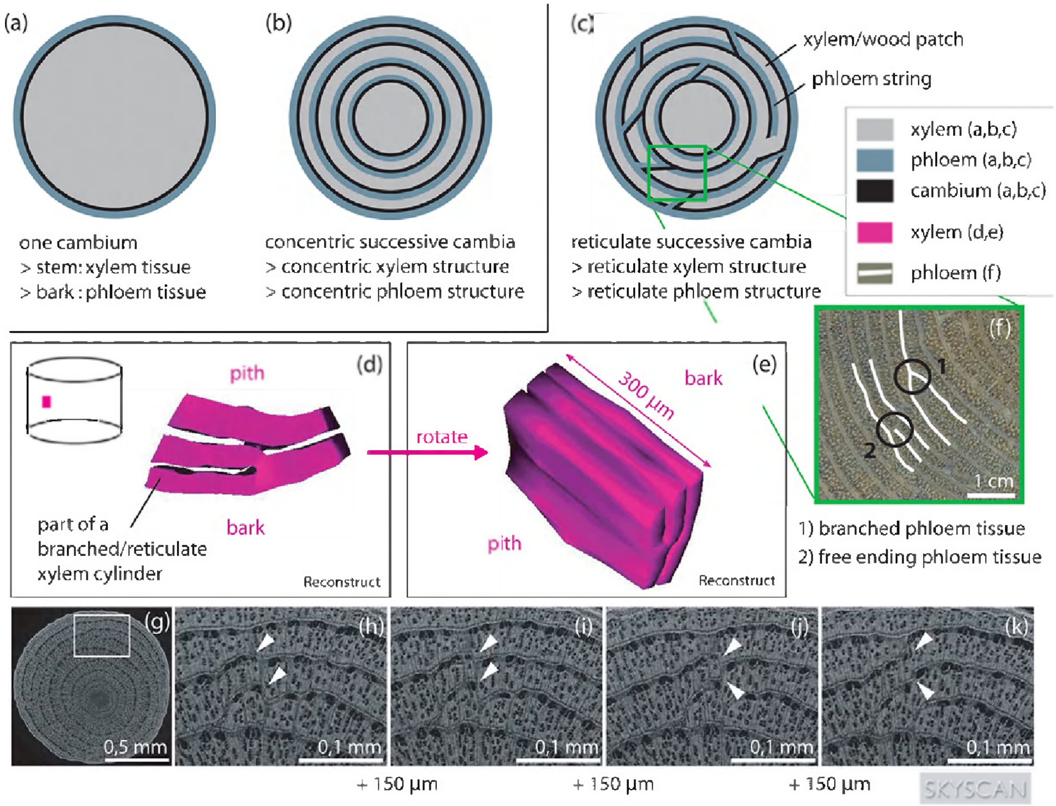


Figure 1 - Spatial structure of the xylem and phloem tissue in* Avicennia *in comparison to other trees. *Schematic view of a stem disc from (a) a tree with only one cambium, (b) a tree with successive cambia organised in concentric cylinders, giving rise to a stem disc with concentric circles of xylem tissue, phloem tissue and cambium circles and (c)* Avicennia*, having a reticulate organisation of its cambia and transport structure. The spatial structure of* Avicennia *is depicted by smoothed surface images after three-dimensional reconstruction in Reconstruct (Fiala, 2005) (d,e). Only a small part of the xylem tissue has been visualised. It can be seen that* Avicennia *had a complex network of xylem patches that fused at certain heights of the tree and joined different patches at other parts of the stem. The connections between xylem patches changed rapidly with vertical distance. This is shown by the white arrowheads in the four serial micro-CT-scan images of an outside zone of an* Avicennia marina *stem disc, produced by a SkyScan 1172 scanner (g-k). Each section is 150 µm apart from the previous. On the micro-CT-images (g-k) the wide light grey bands are xylem tissue while the dark small strings are phloem tissue. The complexity of the internal structure can be expressed by the sum of the locations of free ending and branched phloem tissue (f) per stem disc surface area.*

and xylem parts that were not connected and part of different growth patches at a certain place in the tree stem lined up lower or higher in the tree.

Phloem was observed to have the same reticulate structure as the xylem network: in most parts of the stem discs studied phloem did not form concentric rings but an assembly of free ending strings and branched circles (Figure 1f–k). Considering this in three dimen-

Table 1 *- Overview of the* Avicennia *samples used for the CT-analysis and micro-CT- analysis.*

species	collection number	country	location	biogeographical region	analysis
A. marina	Tw58927-9	Kenya	Gazi Bay – site 1	eastern	CT
	Tw58916, 18, 19	Kenya	Gazi Bay – site 2	eastern	CT
	Tw58937, 38, 41	Kenya	Gazi Bay – site 3	eastern	CT
	Tw60819	Kenya	Gazi Bay – site 3	eastern	CT*
	Tw60820	Kenya	Gazi Bay – site 2	eastern	CT**
	Tw60821	Kenya	Gazi Bay	eastern	micro-CT
	Tw42907	Union of the Comoros	Ngasidja	eastern	CT
	Tw57257	Sri Lanka	Rekawa	eastern	CT
A. officinalis	Tw57246-8	Sri Lanka	Pambala	eastern	CT
	Tw57255	Sri Lanka	Rekawa	eastern	CT
A. germinans	Tw50689	Benin	Cotonou	western	CT
	Tw57679	Democratic Republic of Congo	Ile Bula	western	CT
	Tw55845	Democratic Republic of São Tomé and Principe	Lagoa Azul	western	CT

Samples were selected from the xylarium of the Royal Museum of Central Africa in Tervuren (Belgium) or collected in the field (Gazi Bay – Kenya).
** (root sample),*
*** (root sample and samples at different heights).*

sions, we could recognize free ending sheets and branched cylinders, the three-dimensional equivalents of free ending tissue and branched circles respectively (Figure 1d,e). At certain heights of the tree stems, phloem was found to exist in very small portions that were not connected with the surrounding phloem network at that same height. In these parts of the tree a continuous circular band of phloem around the stem circumference was lacking. In a part of the stem of the *A. germinans* individual from Benin (Tw50689, Table 1) small isolated patches of xylem completely surrounded by phloem tissue were observed on the transverse CT-images. They were at least 4.56 cm in height.

Vessels were found to be rather straight at distances of less than 6.5 mm. However, we observed, at zones of the stem disc with a highly reticulate transport system, that some vessels were part of a certain wood patch at a certain height of the tree but were part of wood volumes that were non-existing at this height, only a few hundred micrometers higher in the tree.

The ratio of ending to branched phloem (Figure 1f) depended on species ($H = 19.64$, $p<0.001$, Kruskal-Wallis test) but not on the ecological conditions of the growing site (*A. marina* – Kenya; $H= 2.04$, $p>0.05$, Kruskal-Wallis test). The species of the eastern biogeographic mangrove region (Indo-West Pacific and East Africa) had a significantly lower ratio than *A. germinans* of the western biogeographic mangrove region (America and West Africa) ($U = 32.50$, $p<0.001$, Mann-Whitney U test, Figure 2a). On the contrary, the level of branching of the xylem and phloem network, expressed as the number of points were the growth segments are not concentric per surface area, did not depend on species ($H = 1.99$, $p>0.05$, Kruskal-Wallis test) but on growing site ($H = 16.67$, $p<0.001$, Kruskal-Wallis test,

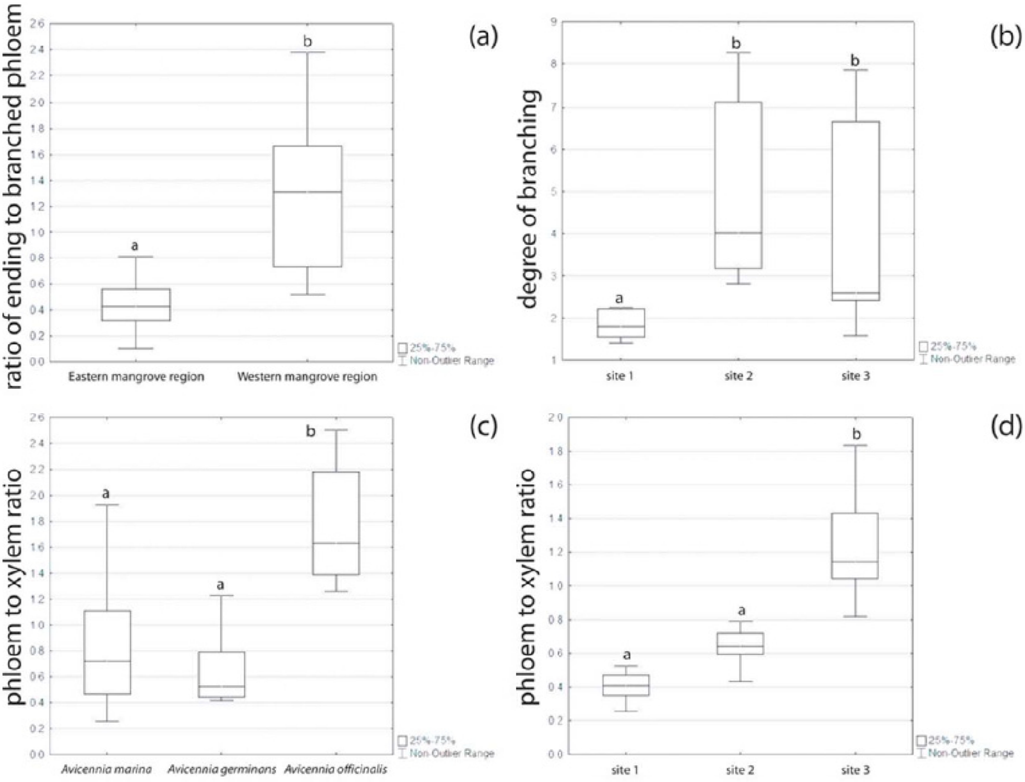


***Figure 2 - Characteristics of the spatial network of the transport system in* Avicennia.** *(a) Ratio of ending to branched phloem in function of the biogeographical mangrove regions; (b) Degree of branching of the transport network of* Avicennia marina *in function of the study site (Table 2); (c–d) Ratio of phloem surface area to wood surface area in function of the mangrove species (c) and in function of the study site (Table 2) for* A. marina *(d). Lines: medians. Different letters indicate significant differences.*

Figure 2b). The lowest values were found for trees growing in the study site with the lowest salinity and the highest inundation frequency (Table 2). There was a significant difference in ratio of phloem surface area to wood surface area in *A. officinalis* as compared to *A. marina* and *A. germinans* (H = 21.49, p<0.001, Kruskal-Wallis test, Figure 2c). Within *A. marina* the growing site had a significant effect on the phloem to xylem ratio (H = 19.83, p<0.001, Kruskal-Wallis test, Figure 2d).

Furthermore, tree height had a significant effect on the branching of the xylem and phloem tissue (H =38.63, p<0.001, Kruskal-Wallis test): from the base to the upper crown of the tree, the degree of branching of the xylem and phloem tissue was increasing (Figure 3). In the roots however, the degree of branching was similar to that in the crown of the tree (Figure 3). In contrast, no trend with tree height could be found in the ratio of ending to branched phloem or in the phloem to xylem ratio.

Table 2 - *Stand characteristics of* Avicennia marina *study sites in Gazi Bay, Kenya (Schmitz et al., 2008b).*

Location			Soil Water Nutrients (10^{-3} µmol/cm³ soil)									LAI	Soil texture	Soil Water Salinity (‰)			Inundation class
	°S	°E	NO_3^-			NH_4^+			P					mean	min	max	
			mean	min	max	mean	min	max	mean	min	max						
site 1	4°24'54,0"	39°30'42,0"	0.5	0.2	0.6	3	0	5	1.4	0.9	1.8	1.42	silty clay	34.6	20.9	46.0	I
site 2	4°25'55,5"	39°30'34,5"	0.15	0.10	0.24	*	*	*	0.2	0.1	0.3	0.23	loamy sand	66.1	38.0	86.0	III
site 3	4°25'16,0"	39°30'27,0"	2	0	6	10	7	14	0.1	0	0.3	1.18	clay loam	63.3	40.0	79.9	III

*Soil water for nutrient and salinity analyses was taken at about 25 cm depth. Inundation classes are defined according to Tomlinson (1994). P (soluble reactive phosphor), LAI (Leaf Area Index), * (no data records).*

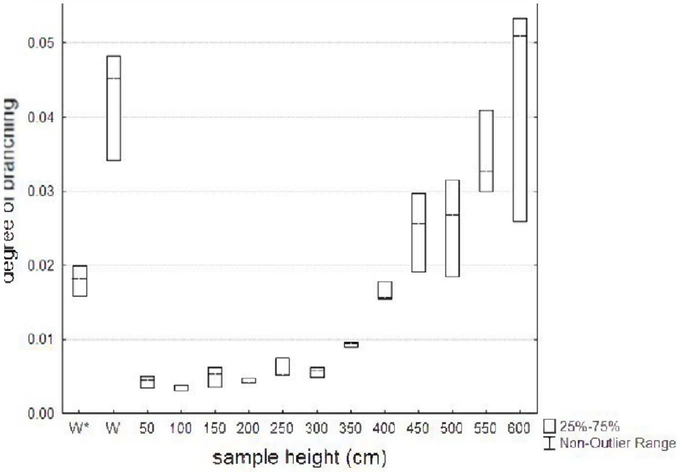


Figure 3 - Degree of branching of the internal transport structure of* Avicennia *in function of tree height. *Data are from one* Avicennia marina *tree from Gazi Bay (Kenya). Lines: medians; W: main root of the same tree; W*: main root of another* A. marina *tree from a different study site in the same mangrove forest.*

Table 3 *- Table showing the number of woody species with concentric internal phloem (Table S1) that occur in (periodically) dry and/or saline environments (stress) and in wet, non-saline environments (no stress) categorized by habit (lianas, shrubs and trees).*

	Stress	No stress
Lianas	9 (4.1%)	110 (49.5%)
Shrubs	48 (21.6%)	14 (6.3%)
Trees	31 (14.0%)	10 (4.5%)
	88 (39.6%)	134 (60.4%)

Absolute numbers with percentage between brackets.

Distribution of concentric internal phloem species according to drought stress intensity

From the investigated woody species with concentric internal phloem, 39.6% could be found in habitats characterised with drought or salt stress while 60.4% could be found in rather wet habitats (Table 3). Then, again, the abundance of lianas was much higher in the non-stressed compared to the drought stressed category (49.5% versus 4.1% respectively) (Table 3). If lianas were excluded, 76.7% of the species was growing in dry conditions while 23.3% was growing in a wet, non-saline environment (Table 3). Ten of the fourteen shrub species growing without drought stress could also have a liana habitus. Excluding them was giving a balance of 84.9% of species in a dry and/or saline environment versus 15.1% in wet, non-saline conditions.

Discussion

The three-dimensional structure of Avicennia *wood*

Seen in three dimensions, the transport tissues in *Avicennia* had a network-like structure (Figure 1c–k). This network of xylem and phloem tissue, extensively connected in horizontal and vertical direction, confirmed the existence of the structure proposed by Schmitz *et al.* (2007b; 2008b) for *A. marina* as well as with the structure proposed by Zamski (1979) who studied young *A. resinifera* and *A. germinans* trees. Our study however, added that a similar threedimensional structure systematically occurred in stems of different *Avicennia* species from different origins, and in roots, branches and stems from *A. marina* (Table 1). Therefore, the structure could be considered a general characteristic of the mangrove genus *Avicennia*. Furhtermore we added that the observed network was highly variable with tree height and position in the tree for all species studied as the connection pattern could change within a few micrometers only (Figure 1d–e, h–k).

Similar reticulate three-dimensional structures have, as far as we are aware, been proposed (i) in small stems of *Anabasis articulata* and *Kochia indica* (Fahn & Shchori, 1967), (ii) in roots of *Beta vulgaris* (Krishnamurthy & Venugopla, 1984) and (iii) in branches and stems of *Dalbergia paniculata* (Nair & Ram, 1990) having concentric rings of successive cambia. Furthermore, this structure has been observed in branches of *Bougainvillea spectabilis* (Zamski, 1980), in stems of *Phytolacca dioica* (Wheat, 1977) and in small stems of *Atriplex halimus* (Fahn & Shchori, 1967; Fahn & Zimmermann, 1982) and *Haloxylon salicornicium* (Fahn & Shchori, 1967) having concentric rings of collateral vascular bundles. With the exception of Fahn *et al.* (1982; 1967) and Zamski 1979, three-dimensional visualisations and characterisations of these structures are lacking. In general, a threedimensional way of interpreting internal phloem is not yet developed, although the articles of Fahn and Zamski were published in 1967, 1979 and 1986 already. This lack of spatial insight is obstructing in depth discussions on the ecological and developmental implications of the observed reticulate structures.

Observation of transversal sections of other species in which successive cambia have been described as concentric rings (*e.g.* Krishnamurthy & Venugopla, 1984; Bhambie & Sharma, 1985; Terrazas, 1991) showed that many more species potentially have a complex network of transport tissues. Connections between rings could already be seen in two-dimensional observations. Furthermore, in the stem of *Combretum nigricans*, the diffuse internal phloem, seen as scattered patches on transversal stem sections, was also observed to be organised as a network if watched in three dimensions (den Outer & van Veenendaal, 1995). This only strengthened the demand for threedimensional observations of plant anatomy and morphology and for wood anatomical studies with three-dimensional approaches of scientific questions.

Within the observed structure, vessels were criss-crossing different xylem patches along the tree stem. Since each xylem patch is formed by another (part of the) cambium, vessel elements of the same vessel were not always derived from one cambial zone only. To assure the longitudinal alignment of vessel elements, a xylogenic signal might be given by the developing vessel to an undifferentiated cell in the cambial zone of a contiguous xylem patch via parenchyma-vessel pits (Murakami *et al.*, 1999; Kitin *et al.,* 2009).

The complex reticulate structure raised questions about its formation and functioning. What type of cambium can give rise to this kind of spatial structure? How does such a cambium originate? Is there a different system of molecular signalling in these non-continuous tissues than in a cylindrical cambium? Has this kind of organisation a functional significance? Answers to these questions touch upon our fundamental understanding of the hydraulic architecture and its ontogeny in plants and in trees in particular and may reveal unknown mechanisms of plant growth and functioning.

Looking at the visualized three-dimensional structure of the xylem and phloem tissue in *Avicennia*, we would expect the cambia in *Avicennia* to have the same reticulate structure as the xylem and phloem tissue, and, in that sense, be non-continuous, either in space or in time. Speculating about the nature of such cambia, we could define two possibilities: cambia that look like fishnet stockings or cambia that are broken cylinders (Figure 4). In the first model (Figure 4a), we imagined multiple layers of cambium sheets that are discontinuous along as well as around the tree stem and that are possibly connected, while in the second one (Figure 4b), continuous cambium sheets have the ability to break up longitudinally and/or radially at certain locations, after formation. Both models have to be considered together with the possibility of cambia to line up radially and longitudinally once active and of different growth speeds in distinct parts of the tree as has been already proposed by Schmitz *et al.* (2008b).

The first as well as the second model on the three-dimensional structure and functioning of *Avicennia*'s cambia are both compatible with the proposed parenchymatic origin

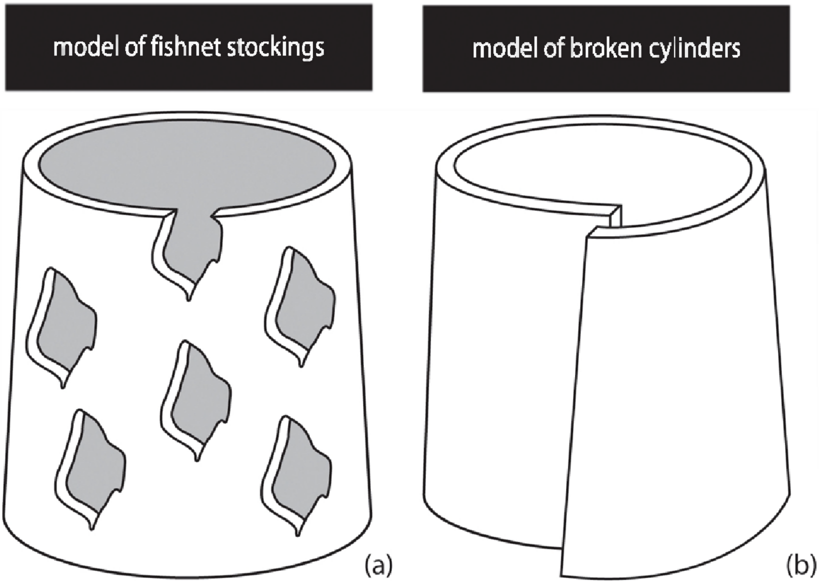


Figure 4 - Nature of the successive cambia in* Avicennia*: two models. *(a) Model of the fishnet stockings: multiple non-continuous cambial sheets give rise to the reticulate transport system. (b) Model of the broken cylinders: complete cambium shields have the ability to break at certain locations and can eventually line up in a later state of the development of the transport structure. Only one cambial layer is shown in both visualisations.*

of new cambia in *Avicennia* (Studholme & Philipson, 1966; Philipson *et al.*, 1971; Wheat, 1977; Zamski, 1997; Zamski, 1981; Schmitz *et al.*, 2007b; Schmitz *et al.*, 2008b). In the fishnet stockings model the parenchyma cells of the assumed cambial cylinder do not all dedifferentiate to meristematic cells, giving rise to the suggested reticulate structure. This has already been proposed for *Dalbergia paniculata*, a tree species with successive cambia (Nair & Ram, 1990). In the broken cylinders model on the other hand, modifications of the cambia, initially organised in concentric cylinders, would occur after dedifferentiation. In reality, a combination of the two proposed models is not excluded. In that case, cambia would be reticulate while formed as well as able to break at certain locations. However, only growth experiments on high spatial and temporal resolution can illuminate the exact ontology of the transport system in *Avicennia*.

Several authors report a number of simultaneously active cambia in plant species with successive cambia (Zamski, 1997; Terrazas, 1991; Carlquist, 2007b; Schmitz *et al.*, 2007b; Schmitz *et al.*, 2008b). We should not picture different cambial zones as separate unities only because they seem to be unconnected on transverse (thin) sections. With the three-dimensional structure in mind, different zones of the cambium in different rings might be part of one and the same connected cambial zone. In that sense, the cambia, as well as the resulting transporting tissues have to be considered as co-functioning in a unit, as has been proposed for the rings of transport tissue in *B. spectabilis* by Zamski (1980).

The Ecological Advantage of Successive Cambia

Within *Avicennia* we observed a higher degree of branching in the spatial structure of the transport system in trees growing in ecological conditions that are more demanding for the water transport, *i.e.* higher salt concentrations in the soil water (mean soil water salinity and soil water salinity range) and low inundation frequency (Schmitz *et al.*, 2008b; Robert *et al.*, 2009) (Figure 2b, Table 2). Furthermore, a higher phloem to xylem tissue ratio has been measured in the trees of the ecologically most stressful study site (Figure 2d, Table 2). These observations corroborated the current idea for a role of phloem tissue in embolism repair (Mauseth & Plemons-Rodrigues, 1997; Salleo *et al.*, 2004; Salleo *et al.*, 2006; Scholz *et al.*, 2007; Salleo *et al.*, 2009; Zwieniecki & Holbrook, 2009). The more extensive branching of the phloem tissue, going with a higher absolute amount of internal phloem, as well as the higher phloem to xylem ratio might thus provide a safer water transport system, next to the enhanced water storage potential (Salleo *et al.*, 2004; Salleo *et al.*, 2006; Salleo *et al.*, 2009; Zwieniecki & Holbrook, 2009).

With increasing height aboveground, the degree of branching of the transport system within an *Avicennia* tree became higher in absolute value and bigger in range. In the roots, the degree of branching was similar to that of the crown (Figure 3). This means that in the crown and in the roots, the phloem tissue was more entangled within the xylem, leading to closer contact between the two transport tissues. In the crown an increased tension on the water column exists, and thus putting a higher demand on the water transport system requiring a higher safety level. Although the tension is lowest in the roots, securing water flow is as essential at the start of the pipeline as it is in the higher parts of the crown.

Surprisingly, the ratio of ending to branched phloem differed between species, with a lower ratio in species from the eastern biogeographical mangrove region than in *A. germinans* from the western biogeographical mangrove region (Figure 2a). This characteristic seemed to be species-specific and therefore part of the evolutionary background of the species rather than a flexible response to environmental drivers.

Supporting the findings in *Avicennia* spp. and the functional role for the phloem in safeguarding the water transport was that 84.9% of trees and shrubs with concentric internal phloem grow in dry or saline habitats. This relationship, however, held only when lianas were excluded from the database since they were far more distributed in wet habitats (Table 3). Concerning the functional advantage concentric internal phloem - often deriving from successive cambia - might offer, a distinction has to be made between trees and shrubs on one side and lianas on the other side. In trees and shrubs, the main functional advantage of internal phloem is thought to be water and photosynthate storage as well as the role in embolism repair. For scandent lianas however, the succession of the rigid xylem tissue with the thin-walled phloem tissue provides the necessary flexibility (Bhambie, 1972; Carlquist, 2001; Carlquist, 2007a). Besides, the additional starch storage capability of the successive

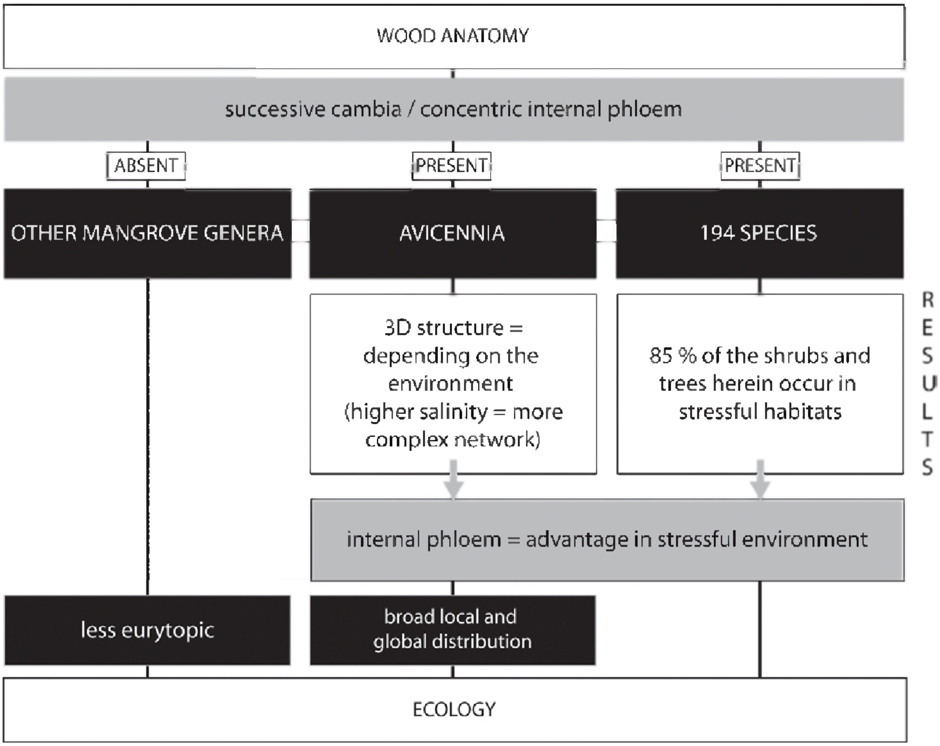


Figure 5 - From wood anatomy to ecology: overview of the study. *The flow chart is showing the organisms involved (black boxes, capital text) and the results of the study of successive cambia and concentric internal phloem in these organisms (white boxes, normal text) establishing that internal phloem could offer advantages in ecological stressful conditions (grey box with black frame). In this way, the wood anatomical features of* Avicennia *can help in explaining its broad distribution compared to other mangrove genera.*

phloem layers offers an advantage for quick growth to the top of the canopy (Carlquist, 2007a). In terms of our hypothesis, it means that concentric internal phloem in lianas is not per se related to drought or salt stress conditions, whereas it is in trees and shrubs.

Apart from the majority of trees and shrubs with concentric internal phloem growing under drought stress conditions, there was still 15.1% related to habitats with adequate water supply. Although speculative, this can be explained by the wide range of habitats these species occur in, or by the evolutionary evolvement of these species from species in dryer habitats together with the neutral effect of growth via successive cambia on the survival of trees and shrubs growing under wet conditions. Another explanation is the higher flexibility and regeneration capacity offered by successive cambia. Flexibility can be advantageous for trees and shrubs confronted with storms and/or heavy wind while a good regeneration capacity can be due to the presence of more than one cambium. Lastly, the increased storage of photosynthates is not only advantageous for lianas in their sprint to the canopy in order to receive light (Carlquist, 2007a), but also for smaller trees growing in dense tropical forests.

Conclusions and Perspectives

Growth via successive cambia offers an ecological advantage under water stress conditions. Woody species showing concentric internal phloem were predominant in salt or drought prone habitats, and the networking of the phloem tissue was more pronounced under harsher environmental conditions for the water transport (Figure 5). Physiological experiments - *e.g.* those proving that the internal phloem tissue is still active (Fahn & Shchori, 1967) - should strengthen the hypothesis that the advantage is most probably related to the water storage capacity and the role in embolism repair of the internal phloem. Concentric internal phloem and thus growth via successive cambia was found to offer several ecological benefits for different groups of species growing in different habitats: under humid-wet conditions this anatomy offers ecological advantage for woody plants with a scandent growth, while under drought or salt stress conditions it can be beneficial for trees and shrubs. If this type of secondary growth is so beneficial, we can wonder why the commonly occurring growth through a single cambium is the main growth form of woody plants. The cost-benefit ratio of having more than one cambium might be the answer, although this has not been calculated to date, unless disadvantages as yet unknown to us would impede evolutionary spread of growth by more than one cambium.

The ecological benefits of successive cambia, in combination with the specialised vessel characteristics (Robert *et al.*, 2009), help explaining the broad local and global distribution of *Avicennia* and its occurrence in extremely high salinity conditions. Generating data on the growth of species with network-like successive cambia at high temporal and spatial resolution with the laser-scanning method (Dünisch *et al.*, 2003) or point dendrometers is needed to obtain more insight in the precise functioning of those cambia and the way the complex structure of the wood develops. The complexity of the water transport system structure that can originate from successive cambia is extending our current insight in plant secondary growth, as these types of structure can only be generated from cambia that are anatomically different from those generally observed in trees.

In conclusion, we can state that our study indicates that successive cambia are an important wood anatomical characteristic explaining ecological species distribution at least partially. It moreover stressed the importance of three-dimensional visualisations and interpretations of plant structures.

Materials and Methods

X-ray Computed Tomography (CT): CT-scanning

Wood Samples. In order to study the three-dimensional wood structure of the mangrove genus *Avicennia* L., we used (i) stems available in the xylarium of the Royal Museum of Central Africa in Tervuren (Belgium) and (ii) root samples and serial stem parts of one tree collected in the mangrove forest of Gazi Bay (Kenya).

Eighteen air-dried wood samples were selected from the Tervuren wood collection (Table 1). The samples were selected to allow comparison between (i) *Avicennia* species (*A. marina*, *A. officinalis* and *A. germinans*), (ii) biogeographical regions (eastern region: Indo-West Pacific and East Africa and western region: America and West Africa) and (iii) study sites mainly differing in degree of soil water salinity. All selected stems were at least 3 cm in diameter.

In addition to the stem samples out of the Tervuren wood collection, two cable root samples of *Avicennia marina* (Forssk.) Vierh. were collected in the mangrove forest of Gazi Bay, Kenya, in February 2009 at two different study sites (Table 1). Study of the variation in wood structure by height was done with twelve samples of the same tree (distance between samples: 50 cm) sampled from study site 2 of which a root sample also has been taken (Table 1). All collected samples were been air-dried before analysis and now are part of the Tervuren wood collection (Tw60819-20 – Table 1).

Environmental data from the different study sites in Gazi Bay, Kenya, were determined during former studies (Table 2) (Schmitz *et al.*, 2008b; Robert *et al.*, 2009).

Scanning Procedure. X-ray tomographical images have been made for all samples using a multi-slice spiral CT-scanner (CT-scan Brilliance 64 slice, Philips, The Netherlands) with the following characteristics: collimation: 20 x 0.625 mm; slice thickness: 0.7 mm; reconstructed slice interval: 0.35 mm; intensity: 330 mAs; tension: 140 kV; X-tube rotation time: 0.75 s; reconstruction filter: B-standard. The field of view and the pixel spacing were adapted to the object diameter, all with 512 x 512 image matrix. Radial and three-dimensional reconstructions were made on the Philips CT-scan with dedicated software.

Data Collection and Analysis. In order to investigate the degree of branching of the *Avicennia* vascular network, points of ending and branched phloem tissue (Figure 2c–g) were counted on the CT-images using ImageJ 1.41k (Wayne Rasband, National Institute of Health, Bethesda, Maryland, USA). For every sample, measurements were made at three different heights at 30 mm distance. On every image the stem surface area was determined by the ellips tools in eFilm Lite 2.1.2 (Merge OEM, Mississauge, Canada) or after measuring the stem diameters in iQ-VIEW 2.5.0 (IMAGE Information Systems, London, UK). From these measurements we calculated (i) the degree of branching, defined as the sum of ending and branched phloem tissue *i.e.* the number of points were the growth segments are not concentric per surface area, giving an indication of the complexity of the network of transport tissue and (ii) the ratio of ending to branched phloem. On the same images the internal phloem surface area was determined semi-automatically (Color Range tool in Adobe Photoshop CS3 – version 10.0.1, Adobe Systems Inc., San Jose, California, USA).

From these measurements, the xylem surface area (total stem surface area minus internal phloem surface area) as well as the ratio of internal phloem surface area to xylem surface area could be calculated.

Degree of branching, ratio of ending to branched phloem and ratio of phloem to xylem surface area were compared within and between species, biogeographical regions and study sites using Mann-Whitney U and Kruskal-Wallis tests. All statistical analyses were conducted using Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA).

X-ray Computed Tomography (CT): micro-CT-scanning

The samples used for micro-CT-scanning were representative *A. marina* samples of Gazi Bay, Kenya, out of the Tervuren wood collection. The pictures showed in this article are from the Tw60821 *A. marina* sample.

A high resolution desktop X-ray micro-CT-system (SkyScan 1172, SkyScan, Belgium) with closed X-ray micro-focus source, was used for non-destructive visualization of the internal structure of *A. marina*. It was possible to obtain an isotropic pixel resolution of 7.5 mm using a camera binning mode of 2 by 2 pixels resulting in a projection image of 1000 x 2000 pixels. A filter of 0.5 mm aluminium was chosen in order to get a better contrast and to reduce the beam hardening effect. The peak voltage of the source was set at 90 kV. Projection images were taken with a rotation step of 0.4° over 180° and the signal to noise of the projection images was improved by using a frame averaging of 3. After acquiring the projection images the reconstruction was done using a modified Feldkamp cone-beam algorithm (Feldkamp *et al.*, 1997). Two-dimensional crosssectional images of the sample were obtained in consecutive slices throughout the object in order to obtain a three-dimensional dataset that can be viewed in any direction.

Three-dimensional smoothed surface images were made using the Reconstruct software (version 1.1.0.0 - John C. Fiala, Austin, Texas, USA) (Fiala, 2005).

Database Analysis

In order to upscale and verify the hypothesis that concentric internal phloem is an adaptive characteristic of species growing under drought stress, either by a lack of water or by the presence of salt, we conducted a database analysis in which we analysed 198 woody species with concentric internal phloem. Species were selected from the modern wood database of the Inside Wood Database (InsideWood, 2004) (species with IAWA (International Association ofWood Anatomists) characteristic 133: included phloem, concentric) or from scientific literature. For each species the growth habit, the habitat and the (bio)geography were searched for in the Inside Wood Database or in scientific articles and websites (Table

S1). In all, 194 species (87 genera out of 25 families) could been taken into the analysis (Table S1).

For statistical analysis (descriptive statistics) habitats were classified according to Walter's zonobiomes (Table S1) (Breckle, 2002) based on both habitat and (bio)geography of each species. Zonobiomes II – IV and VII – IX have been associated with water stress as a result of drought during at least one period of the year and also saline habitats were considered physiologically dry. In contrast, zonobiomes I, V and VI as well as mountain areas were considered to be without extensive drought stress. Zonobiome II has been divided into II a (semi-evergreen and wet season green forests) and II b (savannas, grassland and dry woodlands) according to Walters's vegetational zones (Breckle, 2002). Habitat descriptions were decisive to divide species into stress categories (drought and/or salt stress versus no drought stress). Species with two growth habits (tree/shrub or shrub/liana) were assigned to both groups.

Acknowledgements

We thank James Gitundu Kairo of the Kenya Marine and Fisheries Research Institute for the logistic help during the field expedition, Abudhabi Jambia for the help during sampling in the field, Karel Robert for the development of the measuring method in Adobe Photoshop and the assistance with the figure creation, Maarten De Munter for his help during the CT-scanning and Wim Tavernier for his contribution concerning the Tervuren wood collection. We are very grateful to the Department of Radiology of the Universitair Ziekenhuis Brussel for offering free CT-scanning and to an anonymous reviewer for the critical comments on an earlier version of the manuscript. The study was financially supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen), the National Fund for Scientific Research (FWO, Belgium), the King Leopold III Fund for Nature Exploration and Conservation, Belgium, and the AFRODENDRO project of the Royal Museum for Central Africa (RMCA), Tervuren. Elke Van de Casteele was, at the time the study was conducted, employed by SkyScan NV, Kontich, Belgium, which offered free micro-CT-scanning.

Supporting Information

Table S1. List of species with concentric internal phloem taken into account in the presented study.

Species names are according to the reference article while families are along the APG (Stevens, 2001). Clear synonyms are removed from the list. For each species habit (L: liana, S: shrub or T: tree), habitat and (bio)geography have been searched for in scientific articles and websites. Based on this information, species have been categorised non salt tolerant (0) or at least salt tolerant (1) and have been classified to the different zonobiomes described in Walter's Vegetation of the Earth (Breckle, 2002). Species from coastal areas have been classified as azonal (A), while species from mountain areas have been been indicted with mountain (M). Genera that were found to have internal concentric phloem are not taken into account in the analysis but only mentionend in this list. Number of species accoring to Mabberley's Plant-Book (Mabberley, 2008) are mentioned between brackets.

The table can be found at:
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0016558#s5

Alphabetic list of species included in the study:
Abuta brevifolia, A. colombiana, A. fluminum, A. grandifolia, A. grisebachii, A. imene, A. rufescens, A. sandwithiana, Albertisia exelliana, A. papuana, A. villosa, Allenrolfea vaginata, Anabasis articulata, Anamirta cocculus, Anisocycla cymosa, Anomospermum chloranthum, A. reticulatum, Arcangelisia flava, Arthraerua leubnitziae, Arthrocnemum macrostachyum, A. perenne, Atriplex halimus, A. nummularia, Avicennia alba, A. bicolor, A. germinans, A. integra, A. marina, A. officinalis, A. rumphiana, A. schauriana, Azima tetracantha, Barbeuia madagascariensis, Beirnaertia cabindensis, Bosea yervamora, Bougainvillea spectabilis, Bredemeyera sp., *Cadaba glandulosa, C. rotundifolia, Caryomene olivascens, Casimirella ampla, Celosia floribunda, Ceratoides lanata, Charpentiera densiflora, C. elliptica, C. obovata, Cheiloclinium* spp., *C. anomalum, C. belizense, C. hippocrateoides, C. serratum, Chenopodium sandwicheum, Chondrodendron tomentosum, Cissampelos pareira, Cocculus hirsutus, C. laurifolius, C. pendulus, Colignonia scandens, Combretum nigricans, Curarea candicans, C. tecunarum, C. toxicofera, Cycas circinalis, C. rumphii, C. thuarsii, Dalbergia lanceolaria, D. paniculata, Dicranostyles guianensis, D. mildbraediana, D. villosus, Dioscoreophyllum cumminsii, Diploclisia glaucescens, Doliocarpus* spp., *D. coriaceus, Elephantomene eburnea, Forchhammeria longifolia, Gallesia integrifolia, G. scorododendrum, Gnetum ula, Haematocarpus subpeltatus, Haloxylon persicum, H. salicornicum, Hyperbaena domingensis, H. winzerlingii, Hypserpa nitida, Ipomoea arborescens, I. murucoides, I. pauciflora, I. pentaphylla, I. pes-caprae, I. praecana, I. populina, I. wolcottiana, Iresine* sp., *Kochia sedifolia, Koompassia malaccensis, Legnephora minutiflora, Machaerium cobanense, M. floribundum, Macrococculus pomiferus, Maerua angolensis, M. filliformis, M. oblongifolia,*

M. rigida, M. rosmarinoides, Mestoklema tuberosum, Nototrichum sandwicense, Orthomene schomburgkii, Pachygone dasycarpa, Pera bicolor, Peritassa spp., *P. bullata, P. calypsoides, P. huanucana, P. pruinosa, Petiveria alliacea, Pfaffia grandiflora, Phytolacca dioica, P. dodecandra, P. weberbaueri, Pisonia brunoniana, Polygala* sp., *Pycnarrhena cauliflora, P. celebica, P. lucida, P. tumefacta, Rhabdodendron amazonicum, Salacia* spp., *S. adolfo-friderici, S. alwynii, S. amplectens, S. cauliflora, S. cerasifera, S. chinensis, S. chlorantha, S. crassifolia, S. debilis, S. disepala, S. duckei, S. elegans, S. erecta, S. germainii, S. impressifolia, S. juruana, S. kanukuensis, S. lateritia, S. letestui, S. macrantha, S. miqueliana, S. multiflora, S. nitida, S. opacifolia, S. prinoides, S. pynaertii, S. reticulata, S. solimoesensis, S. staudtiana, S. whytei, Salsola baryosma, Salvadora persica, Sarcobatus vermiculatus, Sciadotenia eichleriana, S. sprucei, S. toxicofera, Securidaca diversifolia, S. philippinensis, S. virgata, Seguieria americana, S. paraguayensis, Simmondsia chinensis, Sleumeria auriculata, Solenostemma argel, Spatholobus roxburghii, Stegnosperma cubense, S. halimifolium, S. watsonii, Strychnos glabra, Suaeda fruticosa, S. monoica, Syrrheonema fasciculatum, Telitoxicum glaziovii, T. krukovii, T. minutiflorum, T. peruvianum, Tetracera volubilis, Tetrastigma voinierianum, Tiliacora acuminata, T. chrysobotrya, T. dielsiana, T. funifera, T. glycosmantha, T. laurentii, Tontelea corymbosa, T. mauritioides, T. micrantha, T. nectandrifolia, Triclisia dictyophylla, T. jumelleana, T. patens, T. sacleuxii, Wisteria floribunda*

How to catch the patch? A dendrometer study on the radial increment through successive cambia in the mangrove *Avicennia*

Abstract

Patchy growth has been put forward in the mangrove *Avicennia*, *i.e.* the positions of active secondary growth change around and along the tree stems with time. In this study, we aim to clarify the process of secondary growth of *Avicennia* trees by monitoring tree stem variations by automatic point dendrometers at four different positions around and along the stem of two *Avicennia marina* trees in the mangrove forest of Gazi Bay (Kenya) during one year. We hypothesize that, at every moment in time, stem radial increment does not occur at all sides of the tree circumference, if one height is considered, corresponding to the patchy structure of the successive cambia. We found that patchiness is a prominent characteristic of the hydraulic system of *Avicennia* trees. It does not only occur in the structure of their hydraulic tissues but also in their radial growth and in the shrinking and swelling patterns of their stems. Patchiness is however a potentially rather than systematically present feature. By this potential patchiness, *Avicennia* trees seem to have an extra tool to optimally react on the prevailing environmental conditions, leading to ecological success in the highly dynamic mangrove environment. Further study should address the alternation of patches reactive to the environment within one tree and the variation in patchy and concentric radial growth between different trees in one forest stand.

Introduction

Patchy growth has been put forward in the mangrove *Avicennia* (Schmitz *et al.*, 2007b; Schmitz *et al.*, 2008b), *i.e.* the positions of active secondary growth change around and along the tree stems with time. Point dendrometers, registering radial changes in tree stems at high temporal and spatial resolution, can help in clarifying the secondary growth mechanism through successive cambia of *Avicennia*. They do not only allow for estimation of secondary growth in relation to environmental variables measured concomitantly, but they also allow the study of daily and seasonal tree stem variations.

Due to temporal differences in water loss by transpiration and water uptake by roots, the internal water reserves of trees are depleted during the day and subsequently refilled during the night (*e.g.* Améglio & Cruiziat, 1992; Herzog *et al.*, 1995; Zweifel *et al.*, 2000; Zweifel *et al.*, 2001; Steppe *et al.*, 2006; Steppe *et al.*, 2012). This causes recurrent shrinking and swelling of the tree stems. It is mainly the elastic living tissues of the phloem that contribute to these diameter changes but also the xylem undergoes small fluctuations (Brough *et al.*, 1986; Irvine & Grace, 1997; Zweifel *et al.*, 2000; De Schepper & Steppe *et al.*, 2010). It has been suggested that radial transport of water from the bark to the xylem can take place through the cell walls (apoplastic route) as well as from cell to cell across the cell membranes, while water flow from the xylem to the bark primarily depends on the apoplastic route (Steppe *et al.*, 2012). Environmental conditions leading to higher transpiration or sap flow rates are supposed to boost aquaporin abundance and/or activity (Steppe *et al.*, 2012), triggering depletion of the trees' internal water reserves. This leads to stem diameter shrinkage. Changes in the concentration of soluble carbohydrates in the phloem are determining the osmotic flow of water from or towards the phloem (De Schepper *et al.*, 2010; Sevanto *et al.*, 2011). Hence, xylem and phloem in trees can be considered tightly coupled hydraulically, with no real barrier between them (Sevanto *et al.*, 2011), but rather with layers of tight control of water flow.

On top of these daily fluctuations in the stem diameter of trees, irreversible radial stem growth takes place. Radial growth is dependent on the water status as well as on the carbon status of trees, as cell wall expansion depends on turgor pressure and on incorporation of carbohydrates (Zweifel *et al.*, 2006; De Schepper *et al.*, 2010). In order to extract the growth signal from continuous dendrometer measurements, the daily fluctuations of the tree stem have to be extracted from the data. Changes in the diameter of tree stems can be divided into three phases: (i) contraction, (ii) expansion and (iii) stem radius increase (Downes *et al.*, 1999; Deslauriers *et al.*, 2007; Deslauriers *et al.*, 2011) (Figure 1). The duration of each phase can be determined, as well as the maximum daily shrinkage (MDS) and stem radius increment (ΔR) (Figure 1). While ΔR can be used as an estimation of tree growth (Deslauriers *et al.*, 2011), MDS is a robust indicator for water stress (Ortuño *et al.*, 2006; Conejero *et al.*, 2007; Giovannelli *et al.*, 2007). Increasing MDS has been found to be the first detectable

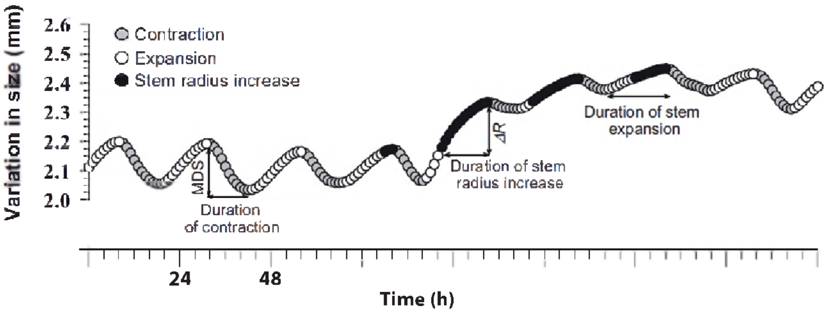


Figure 1 *- Visualisation of the variation in size of a tree stem showing the three different phases: contraction (grey dots), expansion (white and black dots) and stem radius increase (black dots) and their duration, as well as the maximum daily shrinkage (MDS) and stem radius increment (ΔR). Adapted from Deslauriers* et al.*, 2011.*

morpho-physiological signal of changes in the tree's water status, before detectable changes in ΔR (Goldhamer *et al.*, 1999; Naor & Cohen, 2003; Remorini & Massai, 2003; Conejero *et al.*, 2007; Giovannelli *et al.*, 2007).

In this study, we aim to clarify the process of secondary growth of *Avicennia* trees by monitoring tree stem variations at four different positions around and along the stem of two trees in one monospecific natural mangrove forest during one year. As a preparation to this study, we conducted a pinning analysis in which we wounded the cambia of six *Avicennia* tree at monthly basis, at three locations around the stem circumference. We hypothesize that, at every moment in time, stem radial increment does not occur at all sides of the tree circumference, if one height is considered, corresponding to the patchy structure of the successive cambia.

Materials and Methods

Pinning analysis

As a preparation to the dendrometer study, the cambium of six *Avicennia marina* (Forssk.) Vierh. trees of two locations in the mangrove forest of Gazi Bay, Kenya (site 1: 4°25'43"S - 39°30'36"E and site 2: 4°25'15"S - 39°30'27"E) were wounded by pinning with a hypodermic needle of 0.8 mm in diameter. The pinning was done on a monthly basis between February 2006 and January 2007, each time at three different sides of the tree stem at the same height, moving some centimetres downwards every month (Figure 2) to avoid interactions with wound wood formation. As a reference indication, the wind directions of the pinnings were noted. They could differ between different trees. Pinned stem parts were harvested in February 2007 and stored in FAA (formalin - acetic acid - alcohol). Before felling the trees, the stem circumference at 130 cm height and the stem diameter at the base were measured while the height of the trees was calculated trigonometrically.

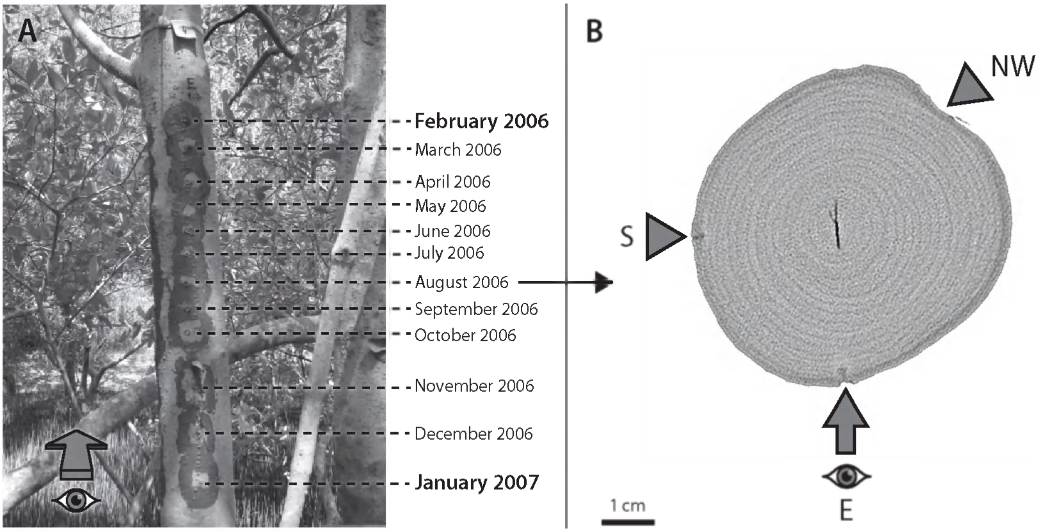


Figure 2 *- Visualisation of the pinning method used to analyse the secondary growth in six* Avicennia marina *trees in Gazi Bay (Kenya). The cambium of the trees was wounded with a hypodermic needle of 0.8 mm in diameter on a monthly basis between February 2006 and January 2007 (A), each time at three sides of the stem circumference (B). As an example, the tree selected for the in-detail study through thin sections is depicted in this figure. This tree was pinned in east (E), south (S) and northwest (NW) directions. Panel B is a CT-image of the tree at the height of the August 2006 pinnings. The eye symbol indicates the direction from which the picture in panel A was taken.*

X-ray tomographical images have been made for all harvested stem parts, using a multi-slice spiral CT-scanner (CT-scan Brilliance 64 slice, Philips, The Netherlands) with the same settings as described in Robert *et al.*, 2011 (Materials and Methods - CT-scanning - Scanning procedure). The number of growth layers from the cambial mark onwards was counted and the increment during the period of the cambial marking experiment was measured at both sides of the wound in iQ-VIEW 2.5.0 (IMAGE Information Systems, London, UK). For one tree (site 1), transverse sections of the pinned wood parts were made. Wood samples were embedded in PEG1500, after which sections were made with a sliding microtome and double stained with safranin–alcian blue. The increment during the period of the cambial marking experiment was measured with the image analysis software AnalySIS Pro 3.2 (Soft Imaging System GmbH, Münster, Germany) via a camera connected to a microscope (Olympus BX60). The increment was measured at about 2 mm distance from the abnormal growth at the wound, which is the furthest position showing a cambial mark. This was done at both sides of the wound and the increment of both sides was averaged.

Dendrometer study

Within the mangrove forest of Gazi Bay (Kenya), two adult *Avicennia marina* (Forssk.) Vierh. trees of 18 cm in diameter at breast height, and *ca.* 8m high were selected for the study (4°25'16.2"S - 39°30'28.50"E) (Figure 3). Both experts and non-experts estimated the visual similarity and health status of both trees, before and after the observations. On each tree, four electronic point dendrometers (Ecomatik, Germany) were placed at the beginning of March 2010. Along the stem circumference of each tree, three dendrometers were placed at 130 cm height (35°N, 175°N and 285°N) and one at 140 cm (285°N) in order to account for the proposed patchy growth of *Avicennia* trees (Schmitz *et al.*, 2008b) (Figure 3). Radial stem changes were registered automatically in 30 minute intervals from March 3, 2010 to March 8, 2011 with a precision of 0.002 mm.

In the same period, local environmental variables were logged (Figure 3):
(i) air temperature and relative air humidity - 10 minute intervals - Hobo U23 pro Temperature/Relative Humidity Data Logger (Onset, Bourne, USA) - at the study site;
(ii) soil water conductivity and soil temperature at *ca.* 25 cm depth - 10 minute intervals - HI 9828 Multiparameter Water Quality Portable Meter - 10 minute intervals (Hanna Instruments, Woonsocket, USA)- at the study site;
(iii) wind speed, wind direction, air temperature (in shadow and in sun), relative air humidity and rainfall - 1 hour intervals - TFA Nexus Weather Station (TFA Dostmann, Hamburg, Germany) - in the near village.
Calibration of the conductivity meter has been executed on a monthly basis with two calibration solutions (80,000 µS/cm and 111,800 µS/cm). Gaps in rainfall data were corrected through data from a manual self-manufactured pluviometer, based on weighed water volumes. Height above datum of the study site was calculated using tracing paper and local tide tables (Kilindini Habour, Mombasa, Kenya). Maximum daily **inundation** was than calculated based on the tide tables and verified in the field to take the local topography into account as well as the possible differences in timing and level of inundation between Mombasa and Gazi. **Vapour pressure deficit (VPD)** has been calculated from temperature and relative humidity data according to Schönwiese (2003).

For both trees, **tree phenology** was followed on a monthly basis. Leaves, fruits and flowers were counted in three litter traps per tree. Average height of the **pen roots** of each tree was calculated based on measurements of 100 pen roots per tree.

At the end of the measuring period, wood samples of all dendrometer positions were taken and stored in alcohol. Transverse sections were made with a sliding microtome and double stained with safranin–alcian blue. Images were made with a camera connected to a microscope (Leica DM LB), after which measurements of the growth segment width and the tissue fractions in the outermost part of the stem at each dendrometer side were conducted in Image J 1.41k (Wayne Rasband, National Institute of Health, Bethesda, Maryland, USA) and Axiovision (Carl Zeiss, Germany). Both growth segment width (the sum of the internal phloem and xylem tissue formed by one cambium) and tissue fractions (bark, xylem and internal phloem tissue) were measured along one radius at each dendrometer position.

Hourly measurements of tree stem variations have been extracted and analysed according to Deslauriers *et al.* (2011). A smoothing level of 2 on a scale from 0 to 10 has been chosen in order to preserve significant variation within a day. During the manual correction phase (Deslauriers *et al.* 2011), cycles of shrinking and swelling based on diameter differences smaller than the detection precision of the dendrometers have been corrected. For the visualization of maximum daily shrinkage (MDS), values have been summed by day if more than one shrinkage phase per day took place. Therefore, in our study, maximum daily shrinkage is defined as the total shrinking of a tree per day, despite possible intermediate swelling.

In order to assess the effects of both inundation (with a tidal pattern and little seasonal aspects) and climate (rainy versus dry season), we chose days with and without rainfall in combination with tidal phases from the extensive datasets. Days were selected as such that the dry or wet signature was as pronounced as possible. Selected days were the ones with the largest amount of rainfall during the study period (the 6th and the 9th of May 2010 - without inundation and the 27th of April and the 27th of May 2010 - with inundation) and the last days without rain after the longest dry period (the 18th of January and the 12th of February

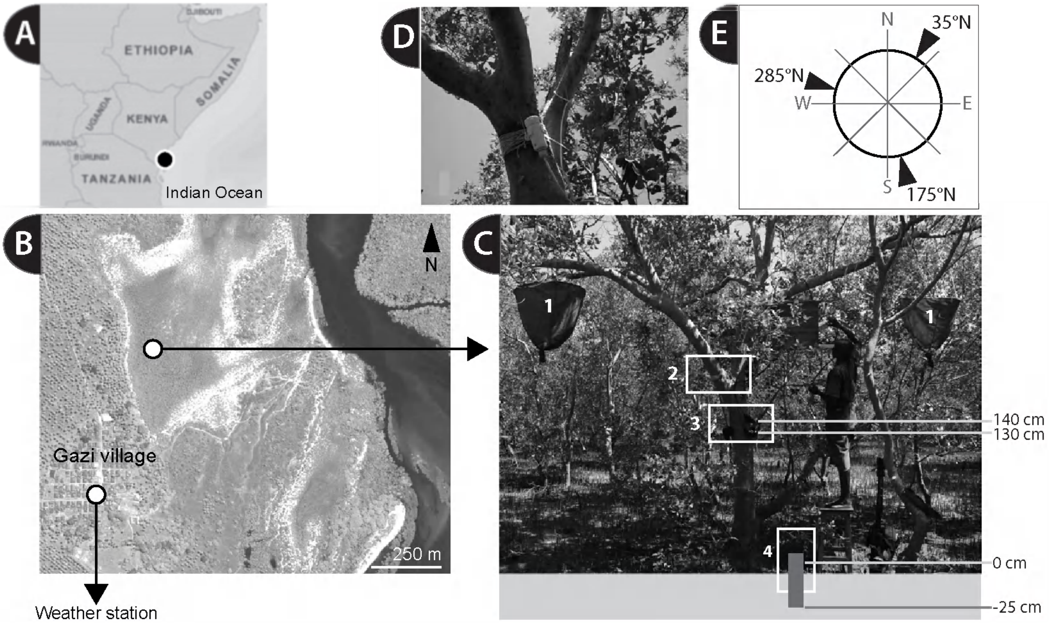


Figure 3 *- (A) Map of east Africa situating Gazi Bay on the Kenyan coast (black dot) and (B) QuickBird satellite image of Gazi Bay acquired in 2002 (Neukermans* et al.*, 2008) showing the locations of the weather station in Gazi village and the dendrometer installation in the mangrove forest (white dots). (C) Picture of the dendrometer installation on one of the studied trees showing the locations of the (1) litter traps, (2 and D) temperature and relative humidity logger, (3) automatic point dendrometers and (4) conductivity logger. (E) Visualization of the locations of the dendrometers on the stem circumference in function of the wind directions.*

2011 - without inundation and the 23rd of October and the 5th of January - with inundation).

Remark 1: The multiparameter used to measure soil water conductivity could only measure if the soil was sufficiently wet. Steep decline and increase in the salinity curves are therefore an indication of soil drought and water supply respectively, rather than an abrupt change in soil water salinity.

Remark 2: Temperature and relative humidity data were measured on both trees standing about 5 meter apart from each other. Figures that concern both trees show the mean value of the two since data were highly similar (Spearman's rank correlation, $r = 0.99$, $p < 0.05$).

Results

Pinning analysis

In the observed tree stems, with a diameter ranging from 5.4 to 9.2 cm at breast height, the pinned positions around and along the stem circumference were, in almost all cases, part of different growth segments in horizontal as well as in vertical direction. At the same height, the three different positions around the stem circumference showed similar amount of radial increment in some periods but not in others. The same is true for the number of growth segments that were formed. The tree from study site 1, selected for detailed observation through thin sections, is representative for the observations made in all six studied trees, *i.e.* during some of the observed periods of growth, one side of the tree was not growing while the other sides were (Table 1). This can be observed for example after the pinning of December 2006 (Figure 4, Table 1) and the one of August 2006 (Table 1).

Dendrometer study

The course of the environmental variables during the year of study is visualized in Figure 5. There was a pronounced rainy season from April to July 2010. Months without rain were December 2010 and January 2011. In February 2011, some days with rainfall interrupted the dry season. In the dry season, wind was faster and had a northern character (northwest to northeast) while in the rest of the year it was generally slower and mostly south-western (south tot west), corresponding to the northeast monsoon (Swahili: kaskazi) and the southwest monsoon (Swahili: kusi) respectively. Maximum wind speed per hour followed the same pattern as the wind speed but with more pronounced maxima during the rainy season (data not shown). Air temperature, soil temperature and soil water salinity were lowest in July and August. Relative air humidity was highest from May to June while vapour pressure deficit was lowest. Temperature ranges measured in the forest were in between those measured by the weather station in a full sun and a shaded position (data not shown). Rainfall differed significantly between Gazi village (data used in this study) and the nearest weather station in Mombasa, especially in its daily amount and pattern (data not shown).

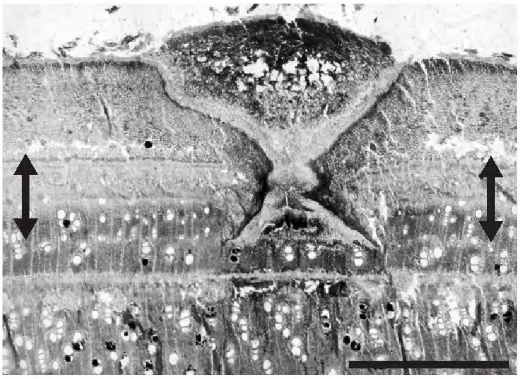
Table 1 *- Average increment in micrometer from the moment of pinning up to February 2007, when the tree was harvested. Increment was measured at both sides of the pinning wound (left and right) and averaged. The tree was pinned monthly at three sides of the stem circumference (south, northwest and east) in order to account for patchy growth (Figure 2).*

Pinning date	Number of months	Increment (µm)								
		SOUTH			NORTHWEST			EAST		
		left	right	average	left	right	average	left	right	average
Jan-07	1	0	0	**0**	0	0	**0**	0	0	**0**
Dec-06	2	0	0	**0**	539	588	**564**	556	582	**569**
Nov-06	3	804	732	**768**	807	958	**882**	1632	1925	**1779**
Oct-06	4	758	861	**809**		793	**793**	1425	1724	**1575**
Sep-06	5	717	704	**711**	1356	2813	**2084**	2115	2153	**2134**
Aug-06	6	997	983	**990**	0	0	**0**	2222	2268	**2245**
Jul-06	7	-	-	**-**	2304	1769	**2036**	2491	2600	**2546**
Jun-06	8	1506	1697	**1602**	3293	2875	**3084**	2393	2451	**2422**
May-06	9	1725	1925	**1825**	3279	3401	**3340**	2408	2336	**2372**
Apr-06	10	2100	2120	**2110**	2743	3261	**3502**	3981	2794	**3388**
Mar-06	11	2828	2909	**2868**	3464	2987	**3226**	-	3180	**3180**
Feb-06	12	2084	2483	**2283**	2602	2543	**2573**	3050	3235	**3142**

- no data could be obtained

Northwest

East



South

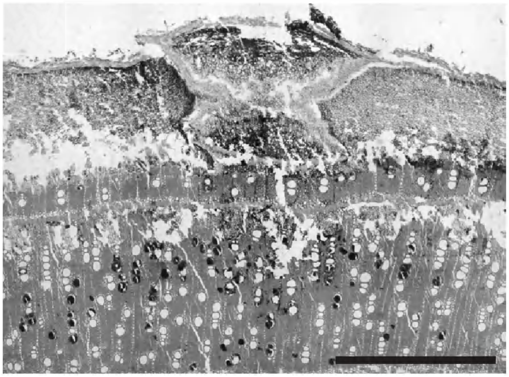


Figure 4 *- Thin sections of the pinned parts of the stem showing the increment at the north-western, the southern and the eastern side of the stem at the height pinned in December 2006 (see also Figure 2). Since the tree was harvested in February 2007, the thin sections show the increment of the tree in the two months after the pinning. The north-western and eastern side of the tree show an average increment of 564 and 569 micrometer respectively while no increment could be observed at the southern side. Scale bars are 1 mm.*

The variation in stem diameter of the two studied trees (tree A and B) showed remarkable differences, as well in total amount of increment after one year (Table 2) as in the pattern of this increment (Figure 6). In tree A, the total increment after one year was much lower than in tree B for all positions around the stem circumference. Furthermore, the increment after one year was the result of a sudden increase in stem diameter at one moment in time, at three of the four positions studied. At the fourth position (130 cm - 285°N), the sudden increase was combined with a more gradual increase in April and May 2010 and from the end of October 2010 to February 2011. In tree B, a gradual stem increment mainly occurred from April to July 2010, with some variation among the different positions around the stem circumference. A second phase of slower but still gradual increment took place from the end of October 2010 at all but one position.

The width of the growth segments in the outermost zone of the tree stems at the locations of the dendrometer measurements ranged from 235 to 1691 micrometer (Figure 7 and 9, Table 3). No remarkable difference in growth segment width or tissue proportions in the outermost stem part was found between the two trees. The different positions around the stem circumference, however, differed in average, minimum and maximum growth segment width (Table 3) as well as in tissue proportions (Figure 8). The phenology of both trees had similar patterns in litter fall, fruit production and flower production, with highest litter fall in July and August and the main fruit production in March to June (Figure 10). Flowers were present almost the whole year, with peaks in April to May and December to February. Average pen root height was 8.0 cm and 8.4 cm for tree A and B respectively, with ranges from 3.0 to 25.0 cm (tree A) and 2.7 to 17.0 cm (tree B).

Detailed graphs of the stem diameter changes and the course of the environmental variables during the days in which tree A showed a sudden increase in its stem diameter at least at one side of the tree can be found in figure 11. The sudden increases in stem diameter systematically took place after rain events. These rain events corresponded with a drop in air temperature, soil temperature and vapour pressure deficit and an increase in relative humidity. Inundation, soil water salinity and wind speed did not show a clear relation to sudden increases of the stem diameter. At the same moment in time, one or two sides of the stem of tree A showed pronounced and sudden increase in diameter. However, a similar growth pattern, though smaller in increment, could sometimes, not systematically, be observed at other positions around the tree stem at the same time. Tree B showed a similar smaller or larger increase in its stem diameter during the same moments in time, at some but not all positions of the stem circumference.

During days without rainfall, the typical pattern in tree stem variation (late morning shrinkage and late afternoon expansion) was found in all but one position around the stem circumference of both trees (Figure 12). Inundation did not interfere this pattern. Tree stems were slowly swelling in the morning up to around 8 am (*ca.* 1.5 h after sunrise),

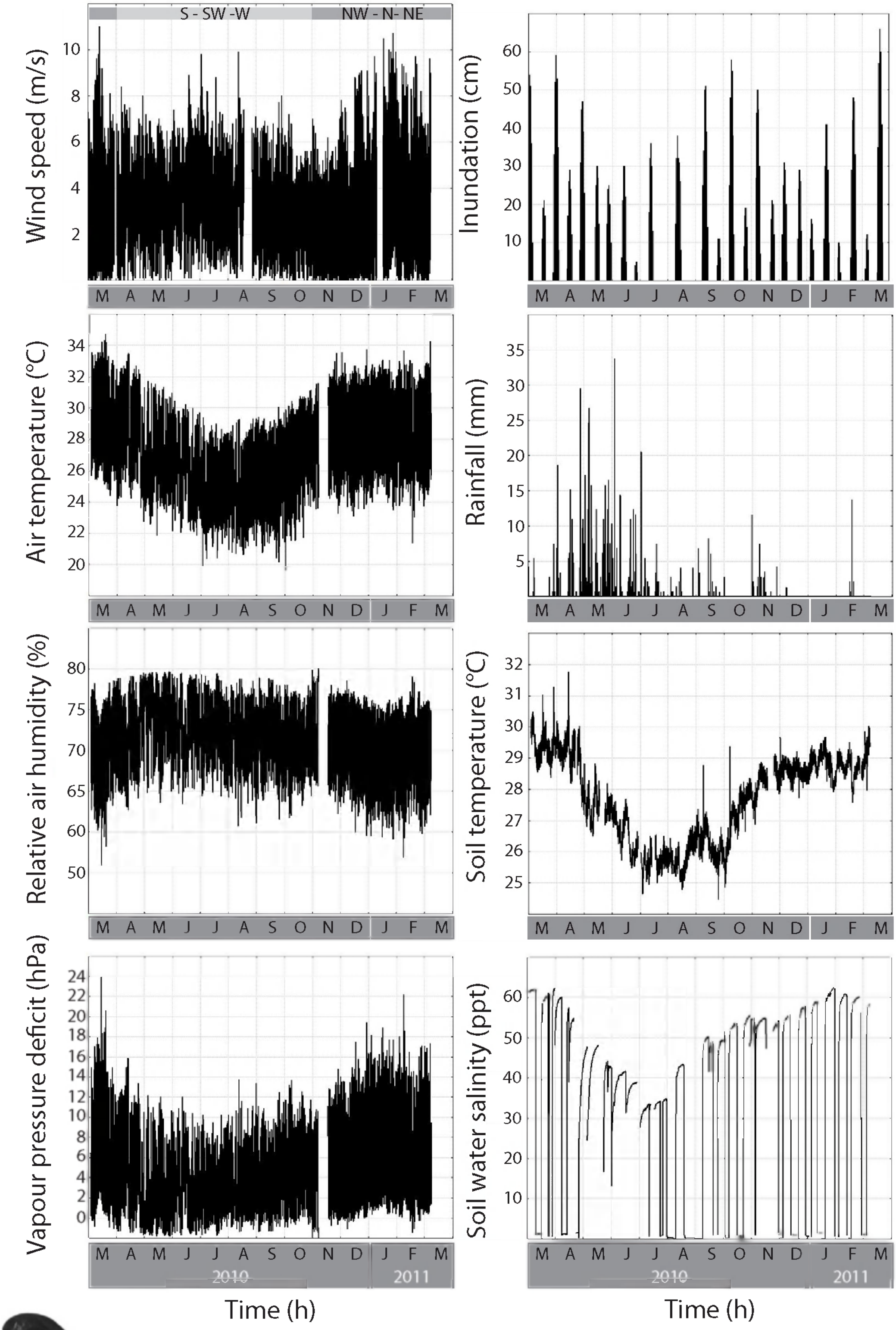

S - SW -W
NW - N- NE
Wind speed (m/s)
2
4
6
8
10
Inundation (cm)
10
20
30
40
50
60
Air temperature (°C)
20
22
24
26
28
30
32
34
Rainfall (mm)
5
10
15
20
25
30
35
Relative air humidity (%)
50
60
65
70
75
80
Soil temperature (°C)
25
26
27
28
29
30
31
32
Vapour pressure deficit (hPa)
0
2
4
6
8
10
12
14
16
18
20
22
24
Soil water salinity (ppt)
10
20
30
40
50
60
M A M J J A S O N D J F M
2010
2011
Time (h)

Table 2 *- Difference in stem diameter (mm) between the beginning (March 2010) and the end (March 2011) of the study period, for all four positions on the stems of tree A and B.*

	TREE A	TREE B
Position 1 – 130cm – 35°N	0.23	1.92
Position 2 – 130cm – 175°N	0.16	2.06
Position 3 – 130cm – 285°N	0.91	1.86
Position 4 – 140cm – 285°N	0.18	1.48

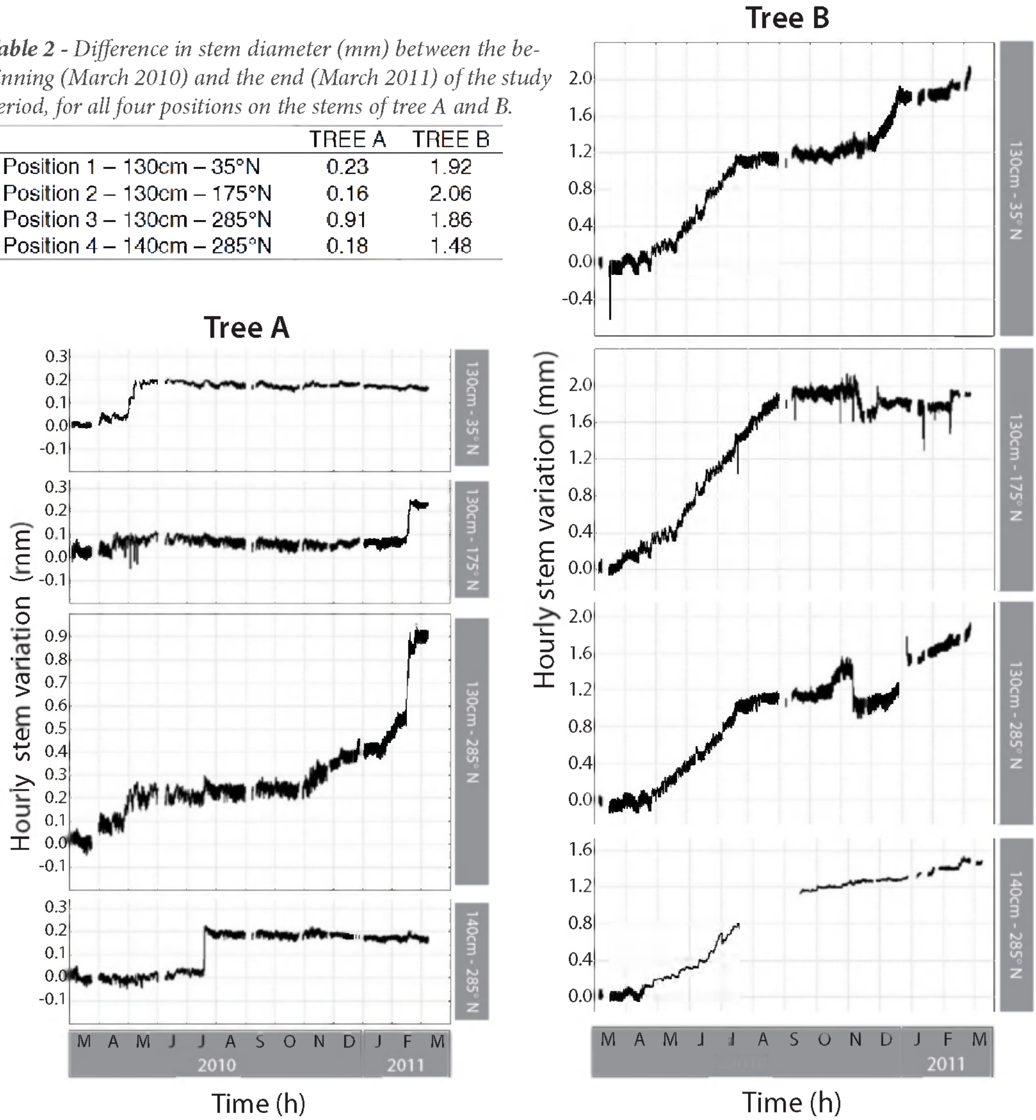


Figure 6 *- Hourly radial stem variation from the 3rd of March 2010 to the 8th of March 2011 in tree A (left) and tree B (right) growing in the mangrove forest of Gazi Bay (Kenya). Measurements have been taken at four different positions around the stem of both trees.*

◀◀ ***Figure 5*** *- Environmental variables measured from the 3rd of March 2010 to the 8th of March 2011 at the study site in the forest of Gazi Bay (Kenya) (air temperature, relative humidity, soil temperature and soil water salinity) and in Gazi village (wind speed, wind direction and rain fall). Wind direction is indicated on the wind speed panel (upper left) as main wind directions for the indicated period. Maximum daily inundation has been calculated from local tide tables after determination of the study sites height above datum while vapour pressure deficit (VPD) has been calculated from air temperature and relative humidity according to Schönwiese (2003). Air temperature, relative humidity and vapour pressure deficit data are mean values from two loggers attached to tree A and tree B, standing* ca. *5m apart from each other. Steep decline and increase in the salinity curves are an indication of soil drought rather than an abrupt change in soil water salinity.*

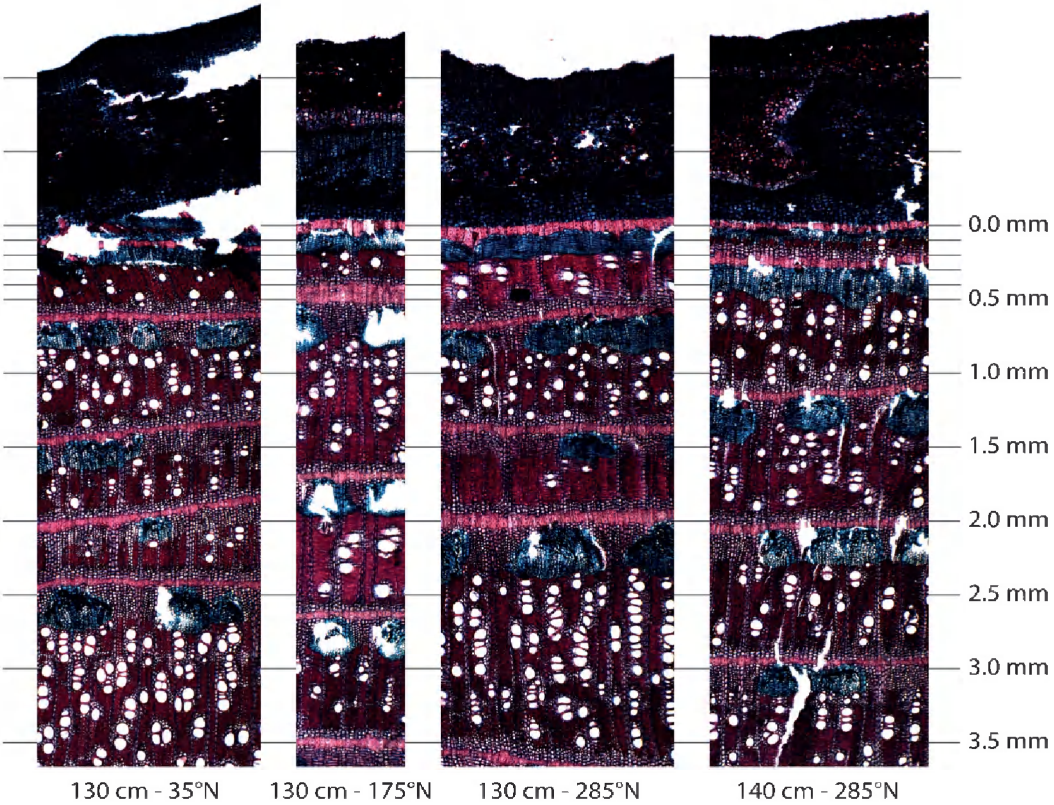


Figure 7 *- Wood anatomy of the outer part of the stem at the dendrometer positions of tree A showing the bark tissue (dark blue), the sclereid layers (pink), the xylem tissue (red) with water conducting vessels, the phloem tissue (blue) and the parenchyma cells of the phloem, the xylem and the rays (purple). Thin sections have been double stained with safranin-alcian blue.*

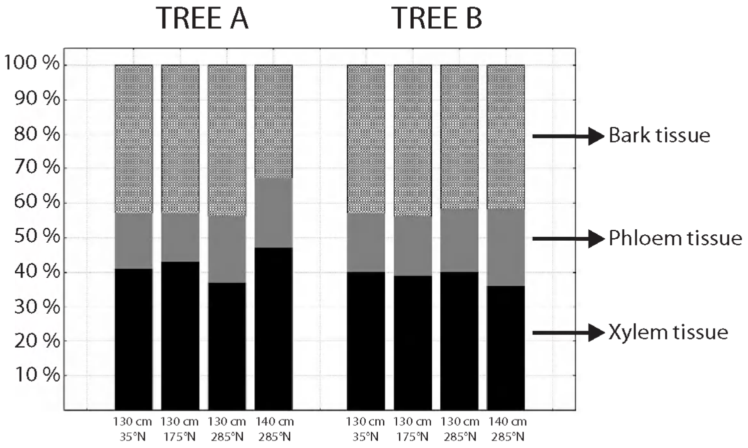


Figure 8 *- Proportion of xylem tissue, phloem tissue and bark tissue in the outermost 3 mm of each stem part at the four dendrometer positions in both tree A and B. Tissue fractions have been measured at the radius of the dendrometer location.*

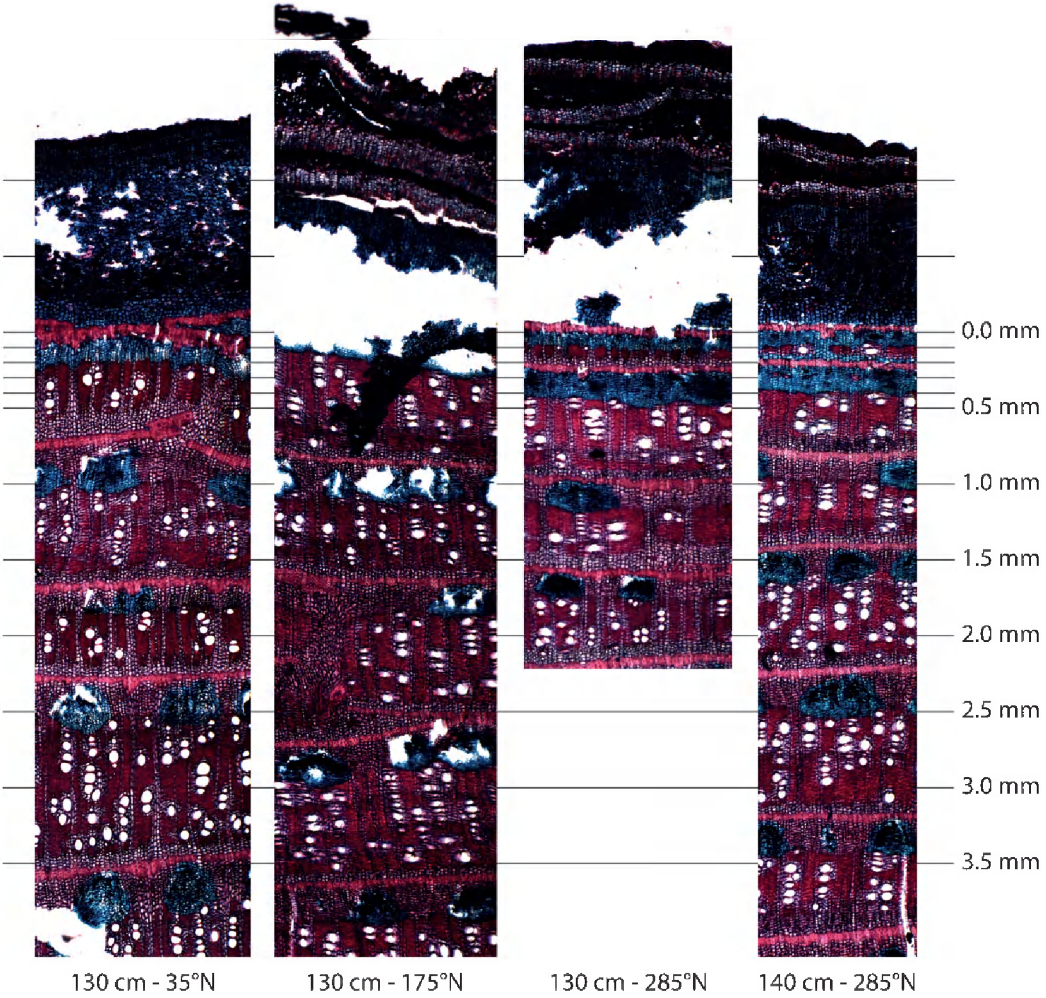


Figure 9 *- Wood anatomy of the outer part of the stem at the dendrometer positions of tree B showing the bark tissue (dark blue), the sclereid layers (pink), the xylem tissue (red) with water conducting vessels, the phloem tissue (blue) and the parenchyma cells of the phloem, the xylem and the rays (purple). Thin sections have been double stained with safranin-alcian blue.*

then started shrinking to reach a minimum between 12 am and 6 pm (sunset is around 6:30 pm), after which they started swelling again at higher speed. The period of shrinking corresponded with an increase in air temperature and vapour pressure deficit and a decrease in relative humidity. Soil temperature increase lagged behind the increase in air temperature but decreased suddenly after inundation. The daily patterns in stem diameter variation did not shown a clear relation to the variations in soil water salinity, wind speed and maximum wind speed. In days with rainfall, the typical pattern in tree stem variation was often disturbed and large variation between the different positions around the stem circumferences of both trees could be observed.

Table 3 *- Width of the growth segments (average, minimum, maximum and range) visible on the wedges of the stem that were sampled for both tree A and B. The number of growth segments (N) and the part of the radius (LENGTH) taken into account for the measurements and calculations are indicated. Values, except the number of growth segments, are in micrometer.*

	N	LENGTH	GROWTH SEGMENT WIDTH			
			average	min	max	range
TREE A						
130cm - 35°N	5	5006	742	469	1411	942
130cm – 175°N	4	4779	875	428	1275	847
130cm – 285°N	5	5176	769	247	944	697
140cm – 285°N	5	5537	910	586	1691	905
TREE B						
130cm - 35°N	4	4863	894	633	1239	606
130cm – 175°N	5	5730	879	741	1066	325
130cm – 285°N	4	3453	547	270	740	470
140cm – 285°N	6	5293	671	235	974	739

Maximum daily shrinkage was lower but with larger variation in the rainy season than in the dry season for tree A at three of the four positions around the stem circumference. In tree B, the same decrease in stem diameter during the rainy season could be observed at three of the four positions around the stem circumference. However, there was no larger variation. For both trees, the total hours of shrinking per day had larger ranges in the rainy season than in the dry season. Besides, the number of distinct shrinkage phases per day was more variable in the rainy season. The position at 140 cm was an exception herein, showing a higher number of distinct shrinkage phases in July and August 2010. At two positions along the stem circumference of tree B (130 cm - 175°N and 285°N) a higher variation in number of distinct shrinkage phases took also place in January to March 2011. The absence of a shrinkage phase is rare and only occurs during the rainy season. No general pattern could be observed in the speed of the tree stem shrinkage.

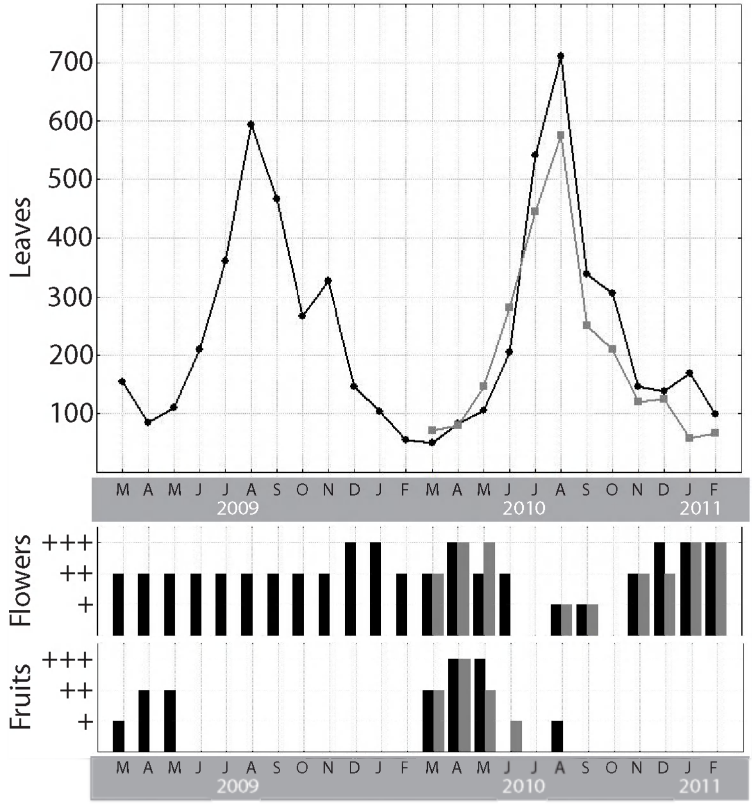
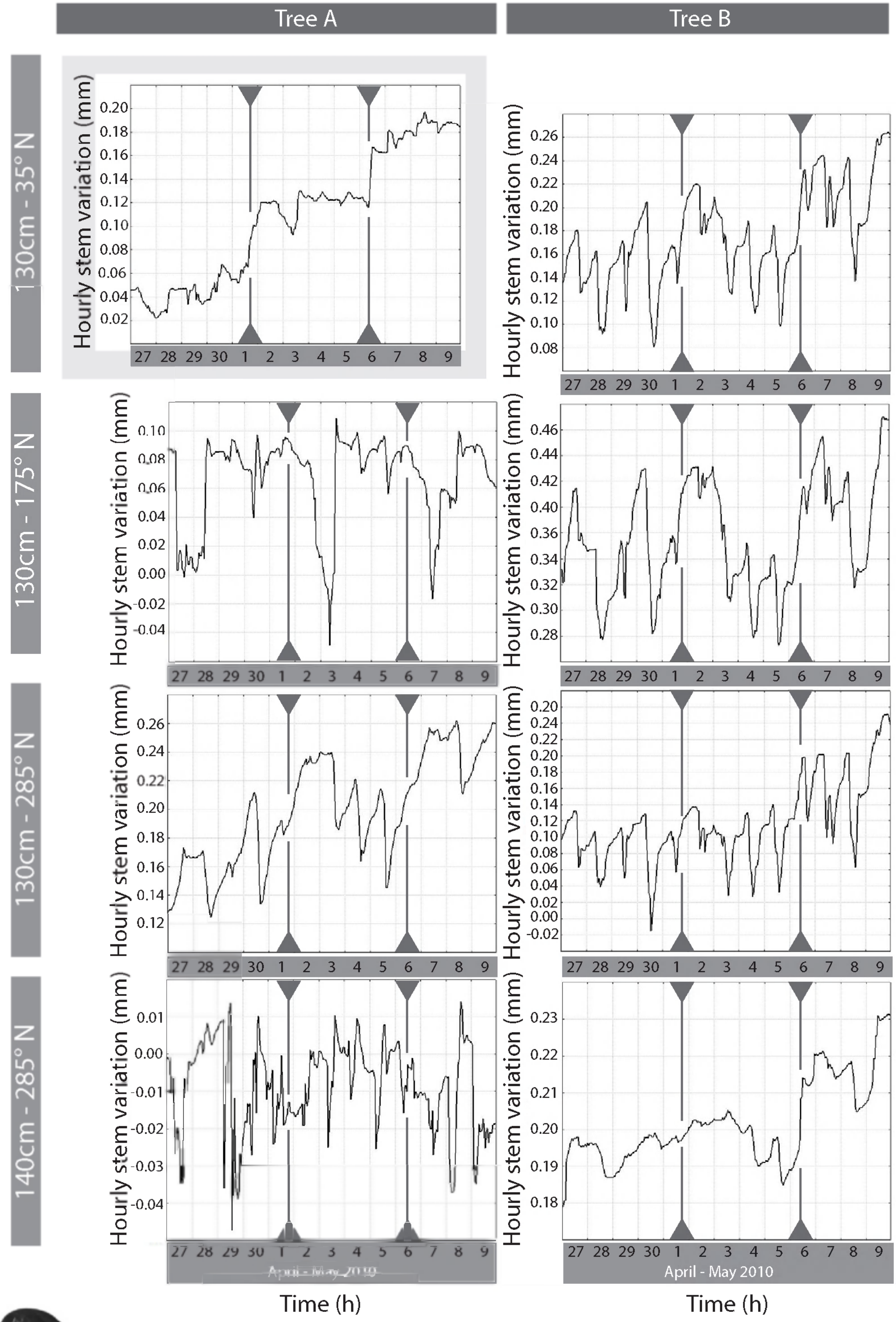
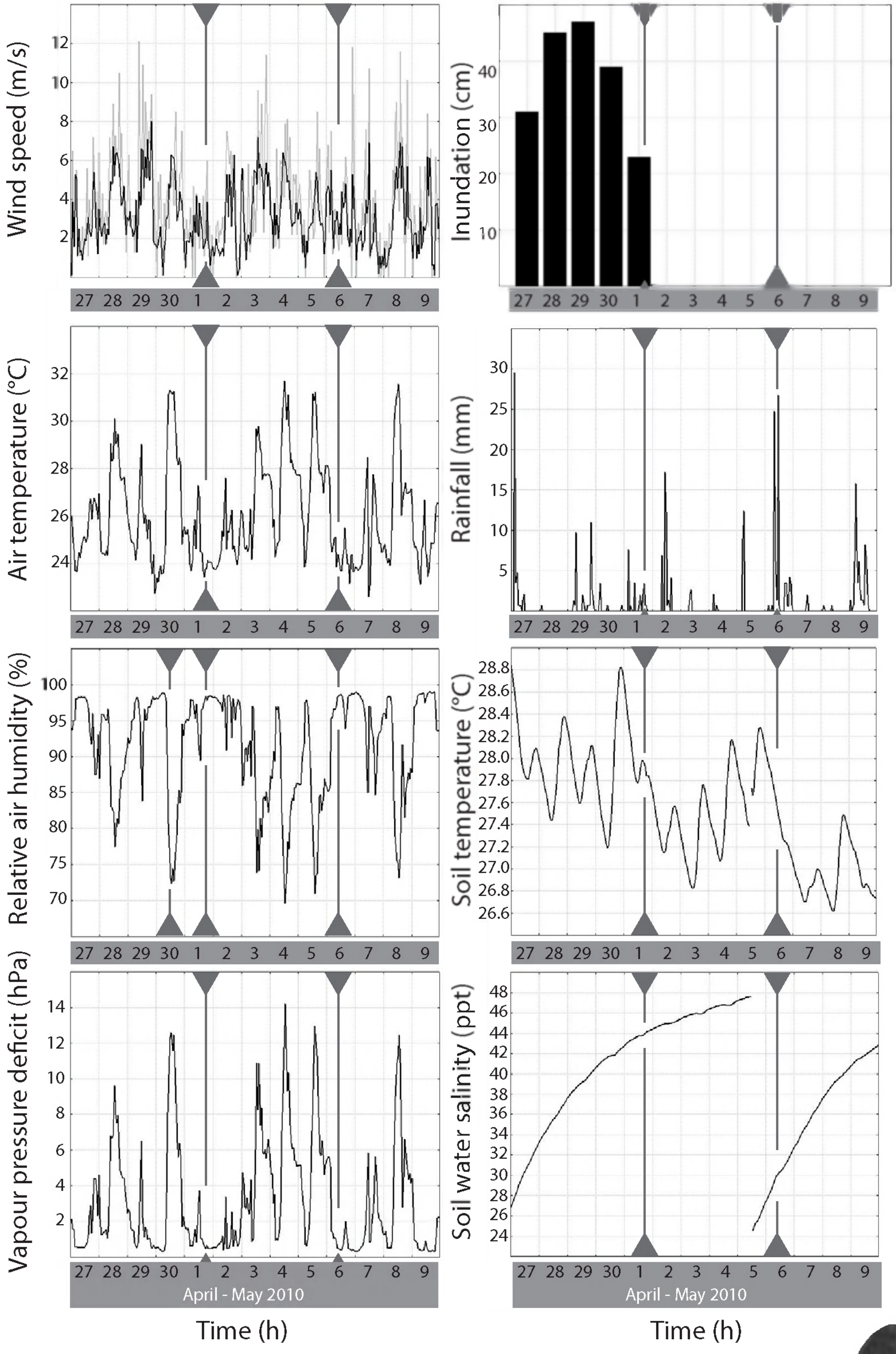
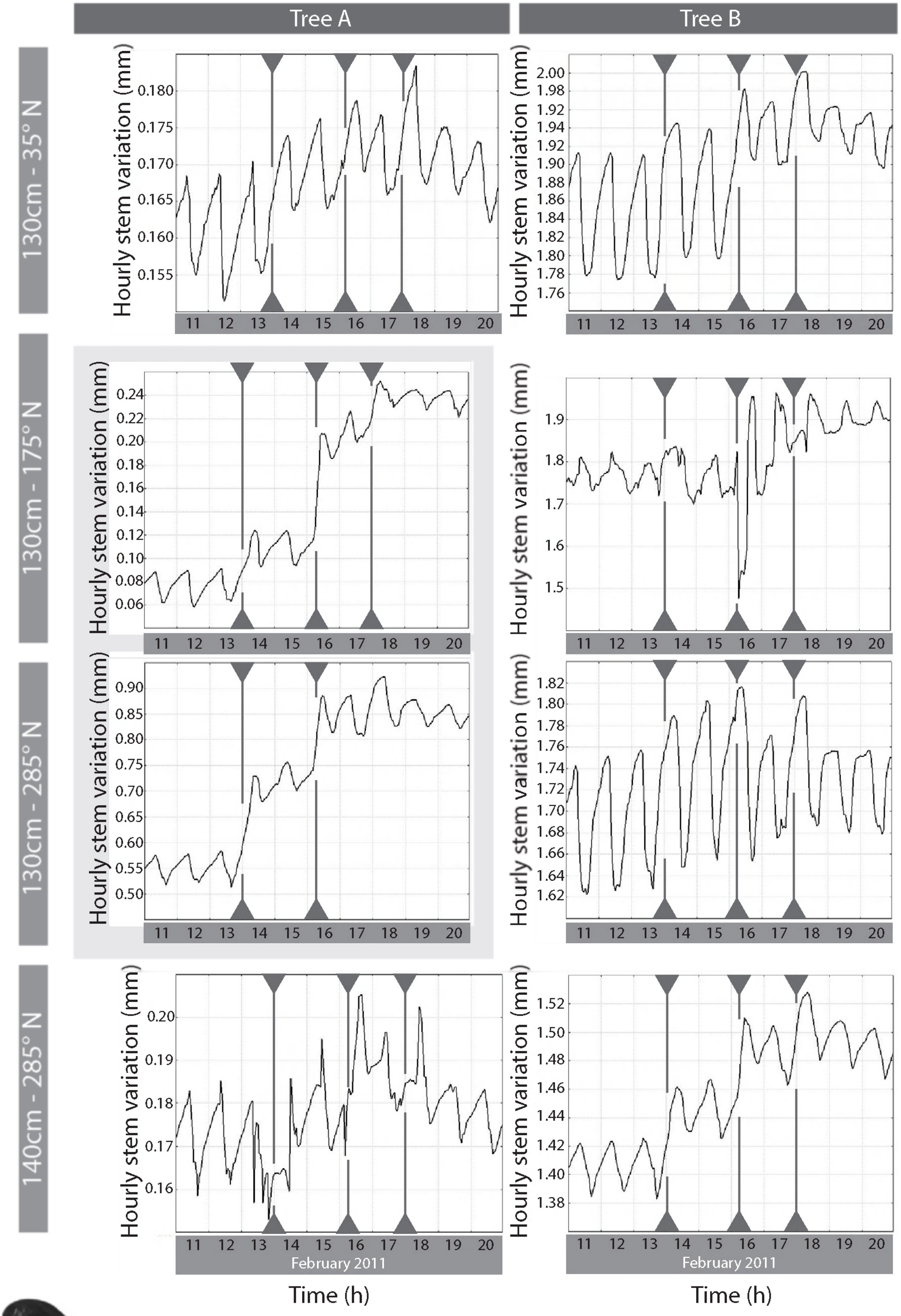


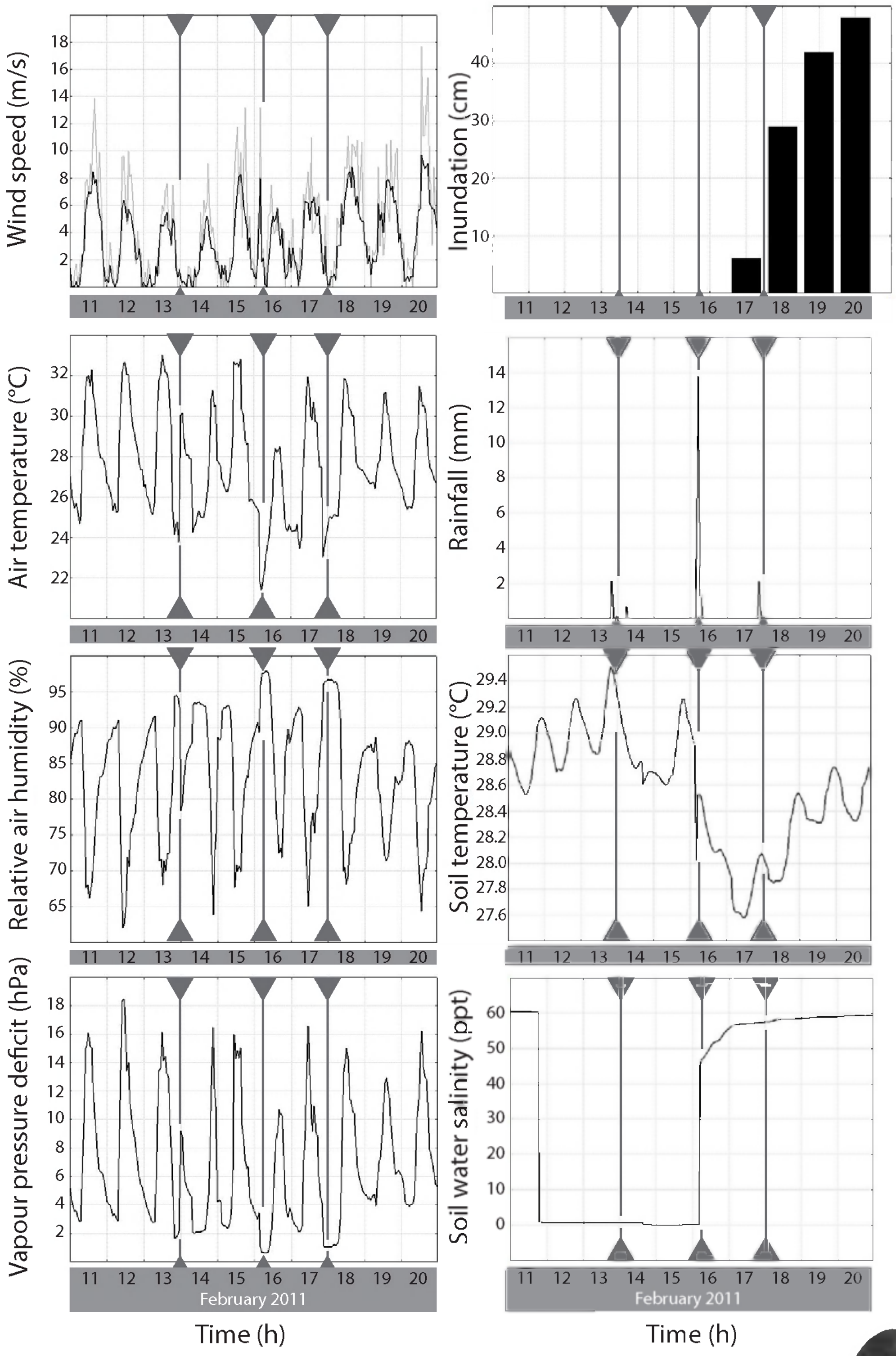
Figure 10 *- Tree phenology data from tree A (black - March 2009 to February 2011) and tree B (grey - March 2010 to February 2011) showing the litter fall (total number of leaves) and the amount of flowers and fruits (in four categories of increasing amount) in three litter traps on each tree.*

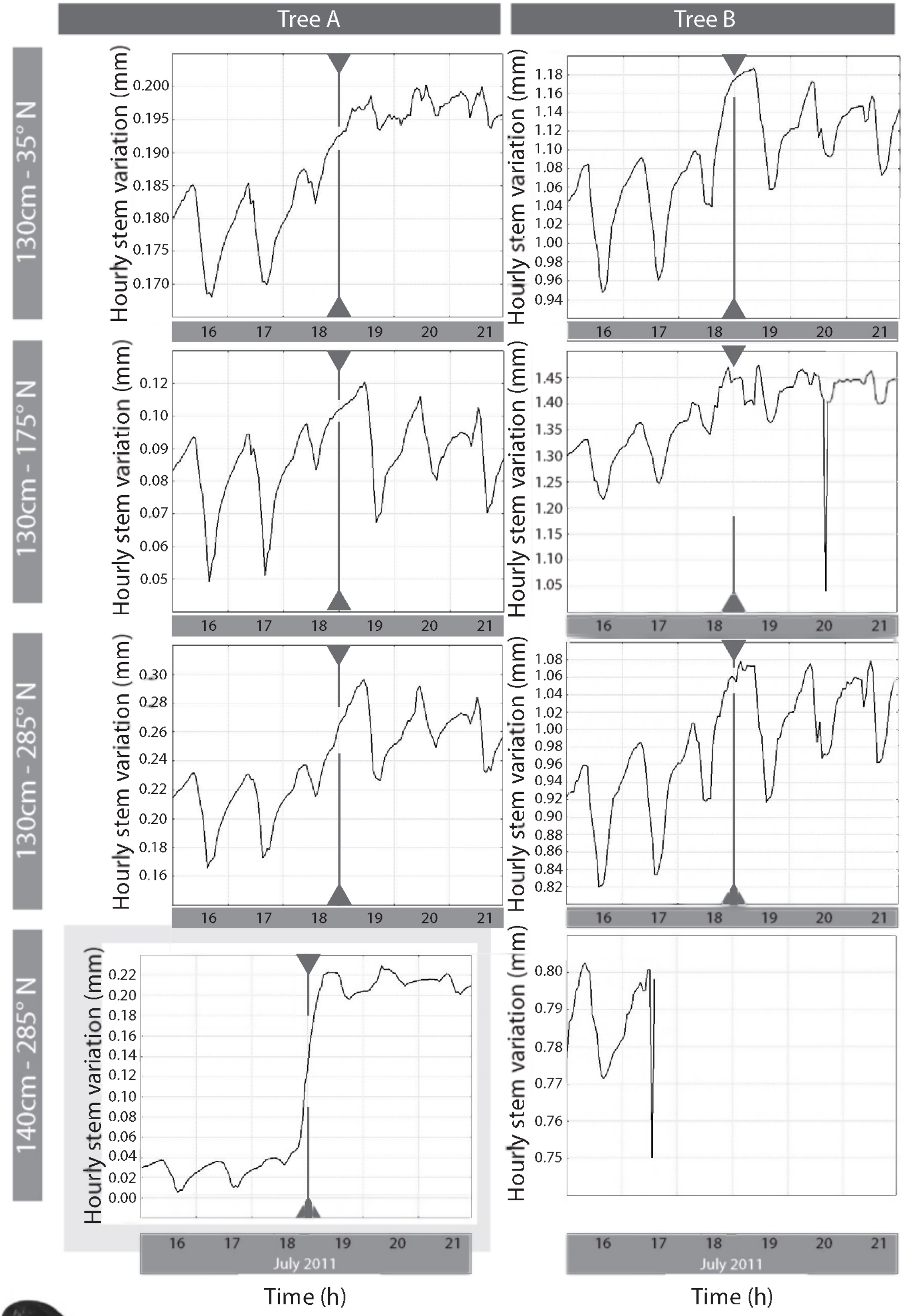
▶▶ ***Figure 11 (following pages) - Left page*** *- Hourly stem variation at all four positions around and along the stem of tree A and B for the periods of abrupt increase of the stem diameter of tree A (grey box). Grey arrow heads and lines mark the specific hours of strong increase, at the base of the abrupt increase in the stem diameter of tree A, observed on the one-year curves (Figure 6).* ***Right page*** *- Wind speed (black) and maximum daily wind speed (grey), air temperature, relative humidity, vapour pressure deficit, inundation, rainfall, soil temperature and soil water salinity of the same period. Air temperature, relative humidity and vapour pressure deficit data are mean values from two loggers attached to tree A and tree B, standing* ca. *5m apart from each other. Steep decline and increase in the salinity curves are an indication of soil drought rather than an abrupt change in soil water salinity. Grey arrow heads and lines mark the specific hours of strong increase, at the base of the abrupt increase in the stem diameter of tree A, observed on the one-year curves (Figure 6).*

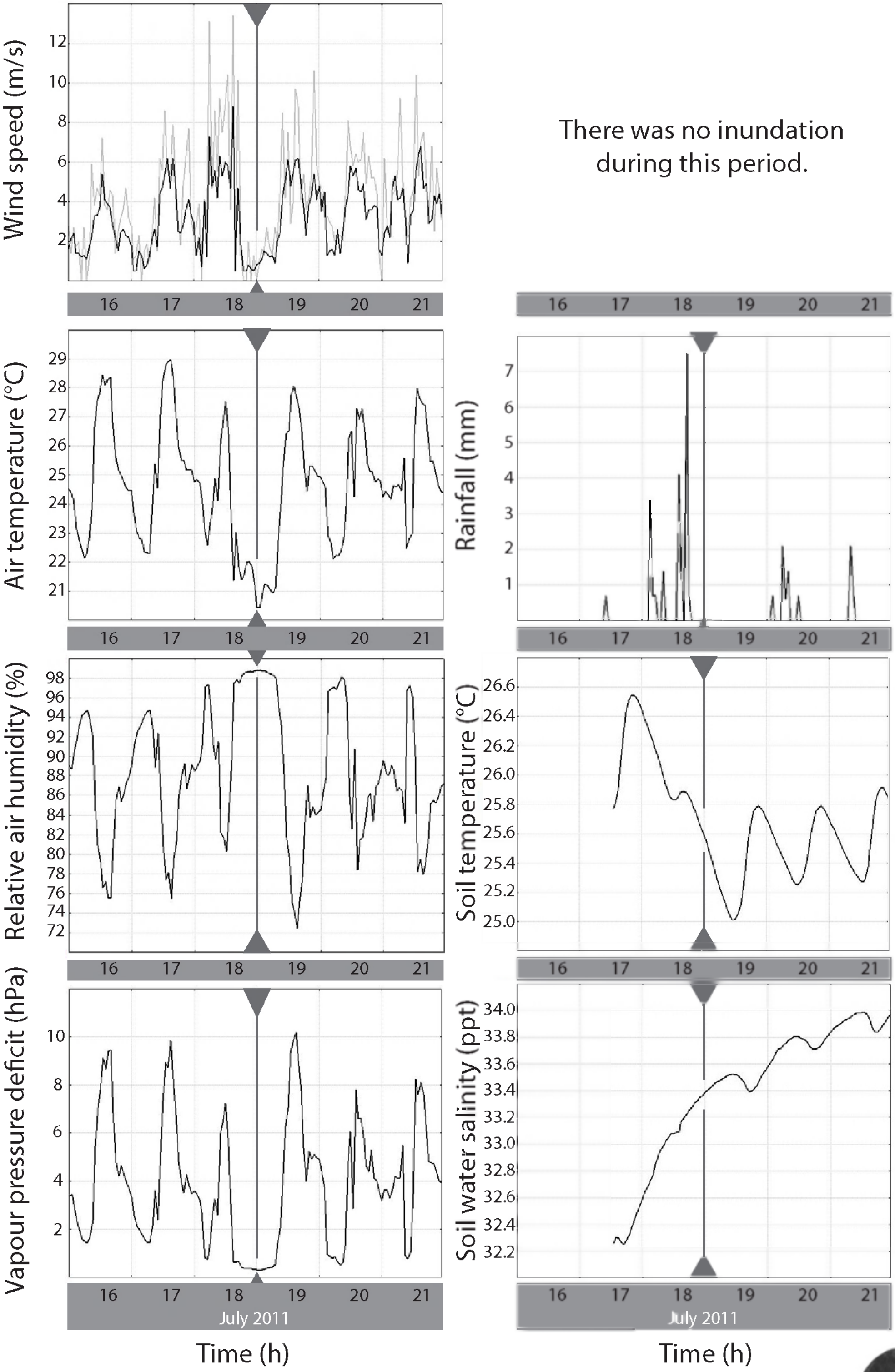

Tree A
Tree B
130cm - 35° N
130cm - 175° N
130cm - 285° N
140cm - 285° N
Hourly stem variation (mm)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
0.46
0.44
0.42
0.40
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.20
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
0.23
0.22
0.21
0.20
0.19
0.18
27 28 29 30 1 2 3 4 5 6 7 8 9
April - May 2010
Time (h)


Wind speed (m/s)
Inundation (cm)
Air temperature (°C)
Rainfall (mm)
Relative air humidity (%)
Soil temperature (°C)
Vapour pressure deficit (hPa)
Soil water salinity (ppt)
27 28 29 30 1 2 3 4 5 6 7 8 9
April - May 2010
Time (h)


Tree A
Tree B
130cm - 35° N
130cm - 175° N
130cm - 285° N
140cm - 285° N
Hourly stem variation (mm)
0.180
0.175
0.170
0.165
0.160
0.155
2.00
1.98
1.92
1.94
1.92
1.90
1.88
1.86
1.84
1.82
1.80
1.78
1.76
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
1.9
1.8
1.7
1.6
1.5
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
1.82
1.80
1.78
1.76
1.74
1.72
1.70
1.68
1.66
1.64
1.62
0.20
0.19
0.18
0.17
0.16
1.52
1.50
1.48
1.46
1.44
1.42
1.40
1.38
11 12 13 14 15 16 17 18 19 20
February 2011
Time (h)


Wind speed (m/s)
2
4
6
8
10
12
14
16
18
Inundation (cm)
10
20
30
40
Air temperature (°C)
22
24
26
28
30
32
Rainfall (mm)
2
4
6
8
10
12
14
Relative air humidity (%)
65
70
75
80
85
90
95
Soil temperature (°C)
27.6
27.8
28.0
28.2
28.4
28.6
28.8
29.0
29.2
29.4
Vapour pressure deficit (hPa)
2
4
6
8
10
12
14
16
18
Soil water salinity (ppt)
0
10
20
30
40
50
60
11
12
13
14
15
16
17
18
19
20
February 2011
Time (h)

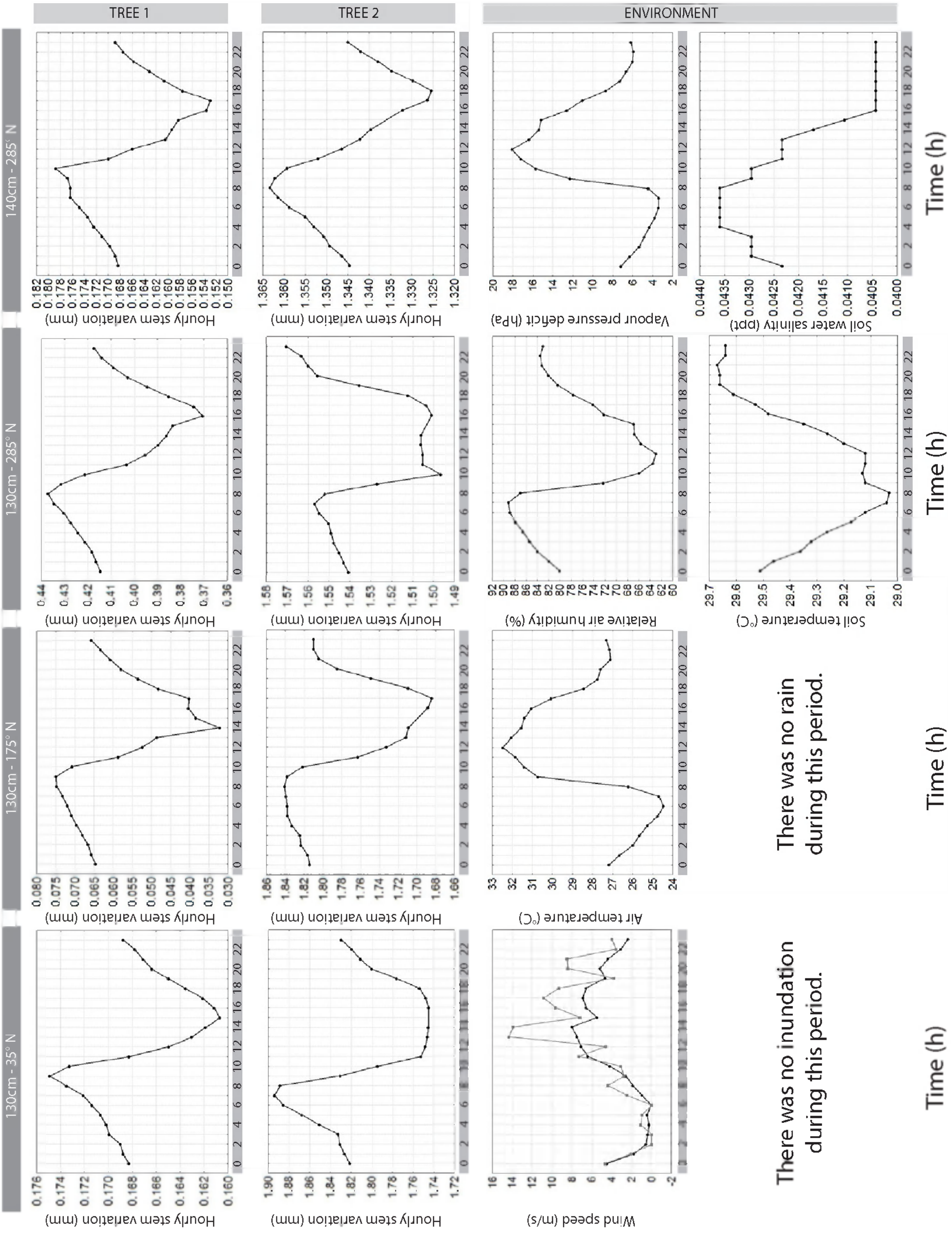

Tree A
Tree B
130cm - 35° N
130cm - 175° N
130cm - 285° N
140cm - 285° N
Hourly stem variation (mm)
0.200
0.195
0.190
0.185
0.180
0.175
0.170
1.18
1.16
1.14
1.12
1.10
1.08
1.06
1.04
1.02
1.00
0.98
0.96
0.94
0.12
0.10
0.09
0.08
0.07
0.06
0.05
1.45
1.40
1.35
1.30
1.25
1.20
1.15
1.10
1.05
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
1.08
1.06
1.04
1.02
1.00
0.98
0.96
0.94
0.92
0.90
0.88
0.86
0.84
0.82
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.80
0.79
0.78
0.77
0.76
0.75
16
17
18
19
20
21
July 2011
Time (h)

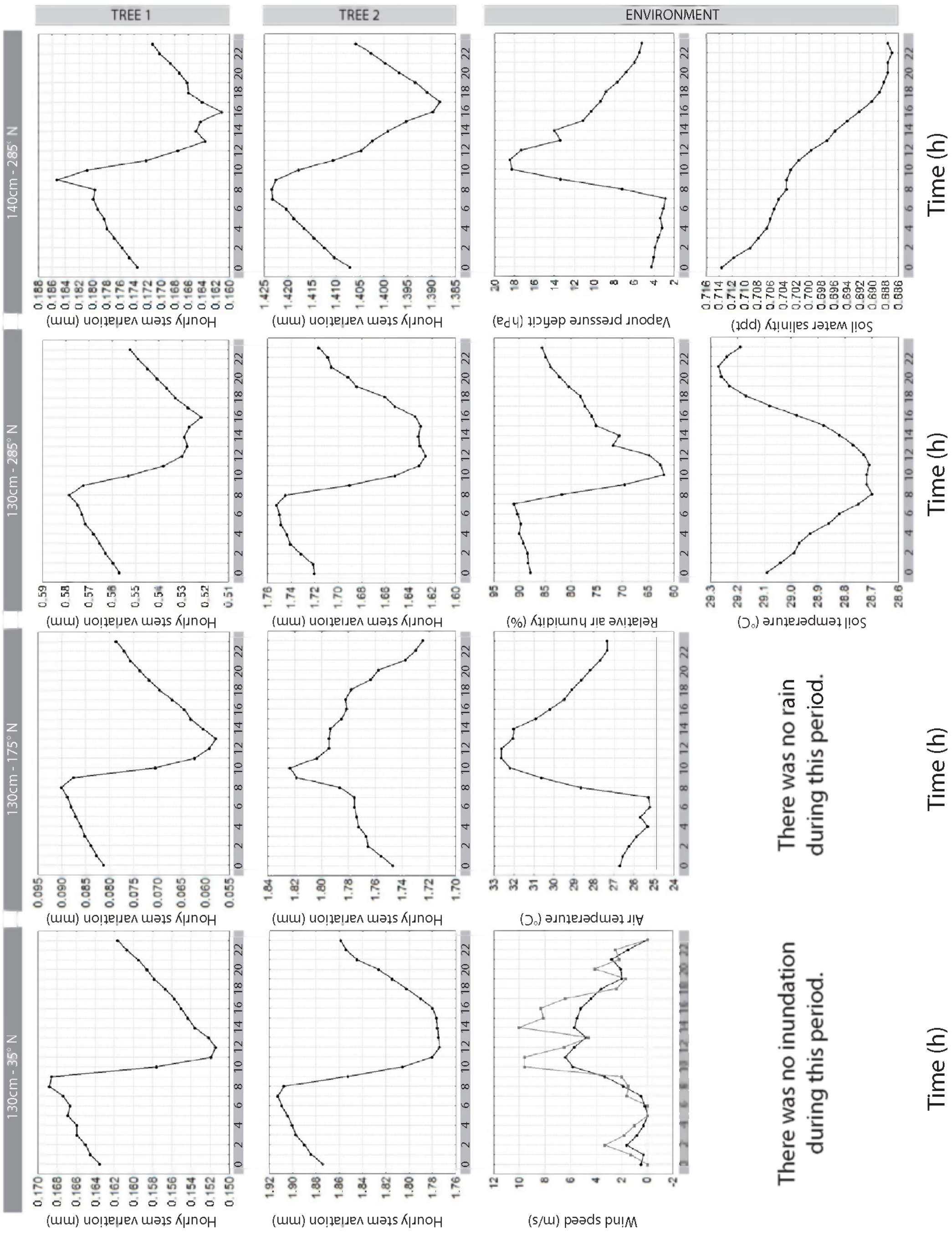


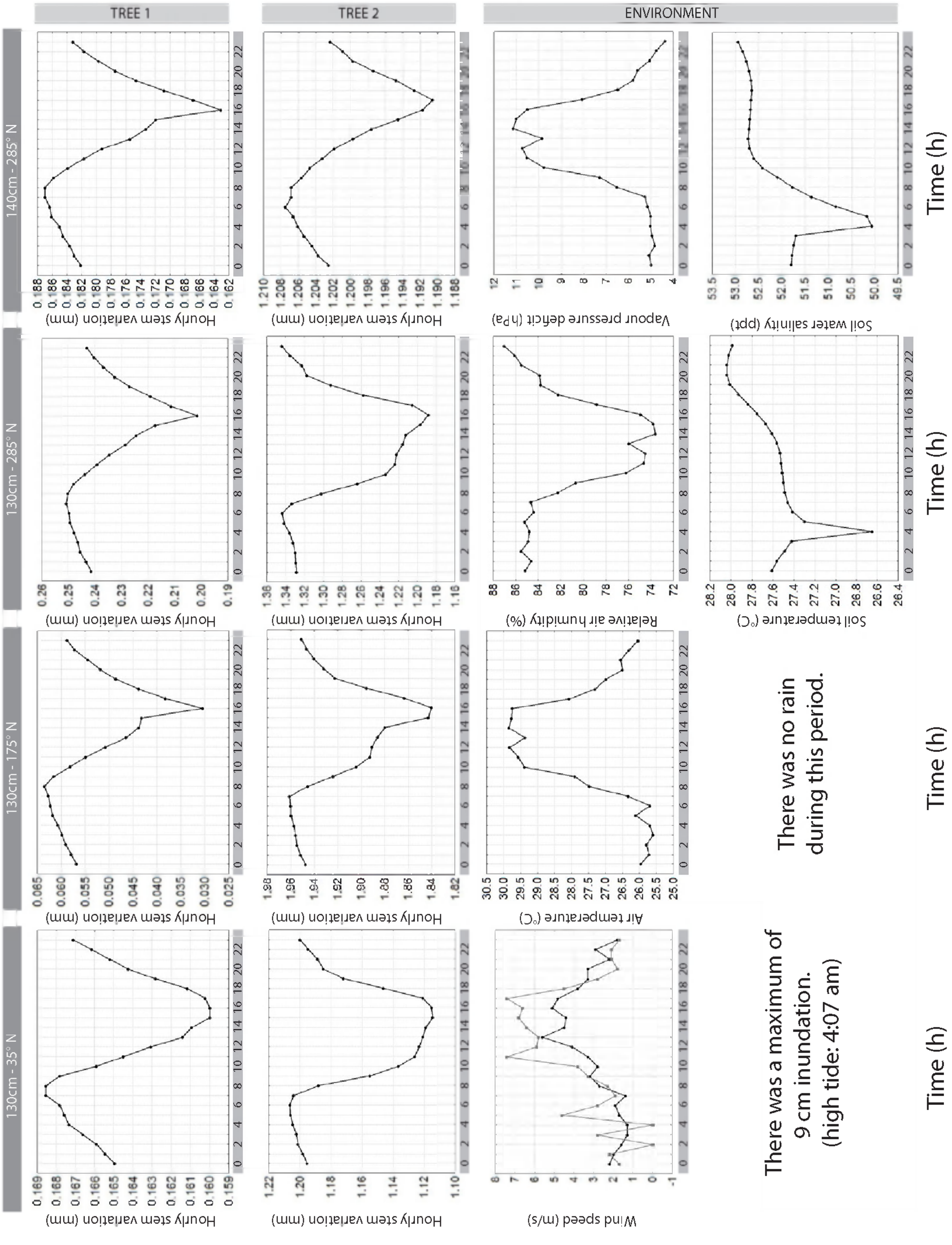
There was no inundation during this period.

▶▶ ***Figure 12 (following pages)*** *- Hourly stem variation at all four positions around and along the stem of tree A and B during eight selected days with or without rain and with or without inundation (page 134: 18/01/2011, page 135: 12/02/2011, page 136: 23/10/2010, page 137: 05/01/2011, page 138: 06/05/2010, page 139: 09/05/2010, page 140: 27/04/2010, page 141: 27/05/2010 - see also table below). Wind speed, air temperature, relative humidity, vapour pressure deficit, inundation, rainfall, soil temperature and soil water salinity of the same days. Air temperature, relative humidity and vapour pressure deficit data are mean values from two loggers attached to tree A and tree B, standing* ca. *5m apart from each other.*

	days without rainfall	**days with rainfall**
days without inundation	18th of January 2011 *(p. 134)* 12th of February 2011 *(p. 135)*	6th of May 2010 *(p. 138)* 9th of May 2010 *(p. 139)*
days with inundation	23rd of October 2010 *(p. 136)* 5th of January 2011 *(p. 137)*	27th of April 2010 *(p. 140)* 27th of May 2010 *(p. 141)*

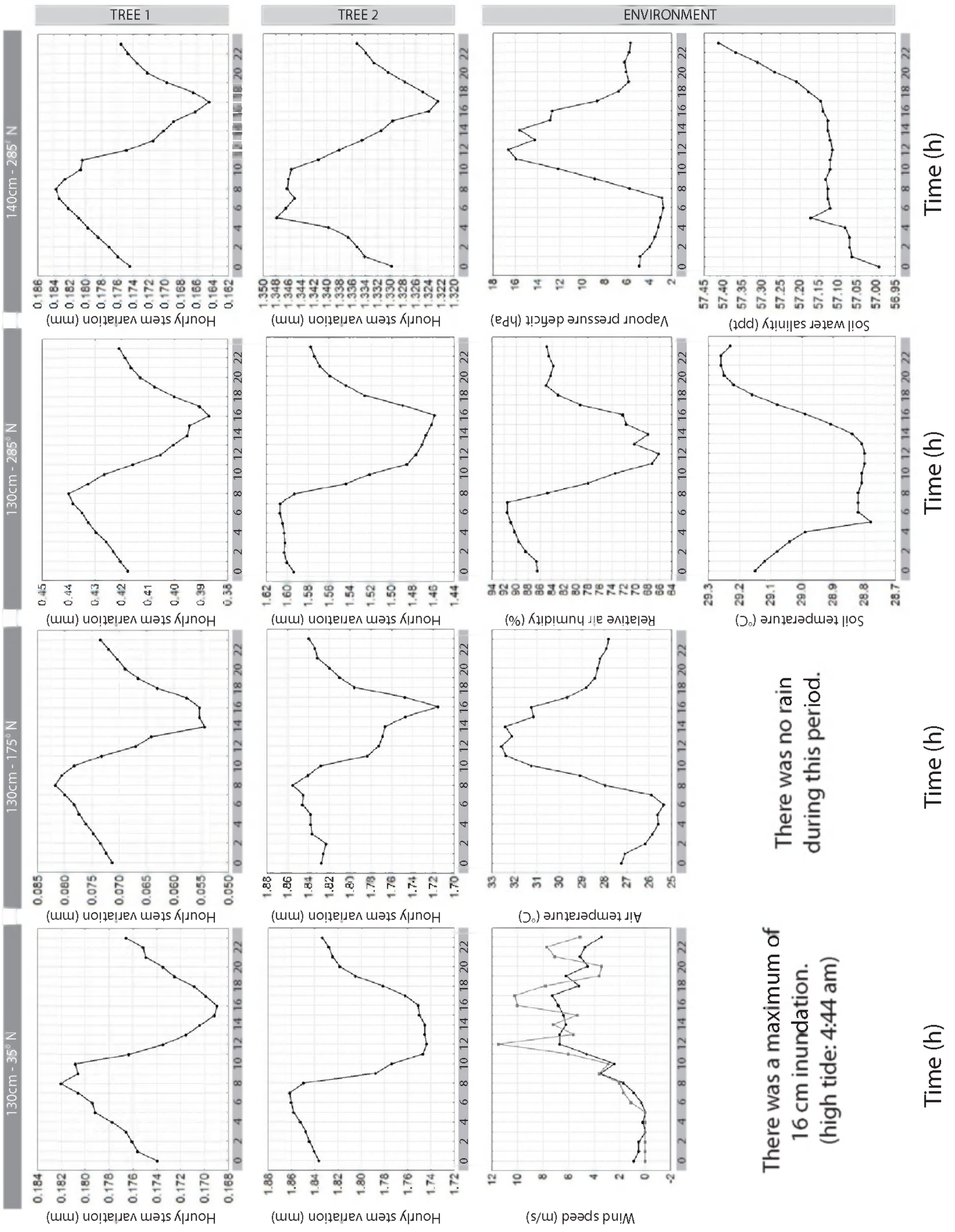

TREE 1
TREE 2
ENVIRONMENT
140cm - 285° N
130cm - 285° N
130cm - 175° N
130cm - 35° N
Hourly stem variation (mm)
Vapour pressure deficit (hPa)
Soil water salinity (ppt)
Relative air humidity (%)
Soil temperature (°C)
Air temperature (°C)
Wind speed (m/s)
0 2 4 6 8 10 12 14 16 18 20 22
Time (h)
There was no rain during this period.
There was no inundation during this period.


TREE 1
TREE 2
ENVIRONMENT
130cm - 35° N
130cm - 175° N
130cm - 285° N
140cm - 285° N
Hourly stem variation (mm)
0.170 0.168 0.166 0.164 0.162 0.160 0.158 0.156 0.154 0.152 0.150
0.095 0.090 0.085 0.080 0.075 0.070 0.065 0.060 0.055
0.59 0.58 0.57 0.56 0.55 0.54 0.53 0.52 0.51
0.188 0.186 0.184 0.182 0.180 0.178 0.176 0.174 0.172 0.170 0.168 0.166 0.164 0.162 0.160
1.92 1.90 1.88 1.86 1.84 1.82 1.80 1.78 1.76
1.84 1.82 1.80 1.78 1.76 1.74 1.72 1.70
1.76 1.74 1.72 1.70 1.68 1.66 1.64 1.62 1.60
1.425 1.420 1.415 1.410 1.405 1.400 1.395 1.390 1.385
Wind speed (m/s)
12 10 8 6 4 2 0 -2
Air temperature (°C)
33 32 31 30 29 28 27 26 25 24
Relative air humidity (%)
95 90 85 80 75 70 65 60
Vapour pressure deficit (hPa)
20 18 16 14 12 10 8 6 4 2
Soil temperature (°C)
29.3 29.2 29.1 29.0 28.9 28.8 28.7 28.6
Soil water salinity (ppt)
0.716 0.714 0.712 0.710 0.708 0.706 0.704 0.702 0.700 0.698 0.696 0.694 0.692 0.690 0.688 0.686
0 2 4 6 8 10 12 14 16 18 20 22
There was no inundation during this period.
There was no rain during this period.
Time (h)
Time (h)
Time (h)
Time (h)



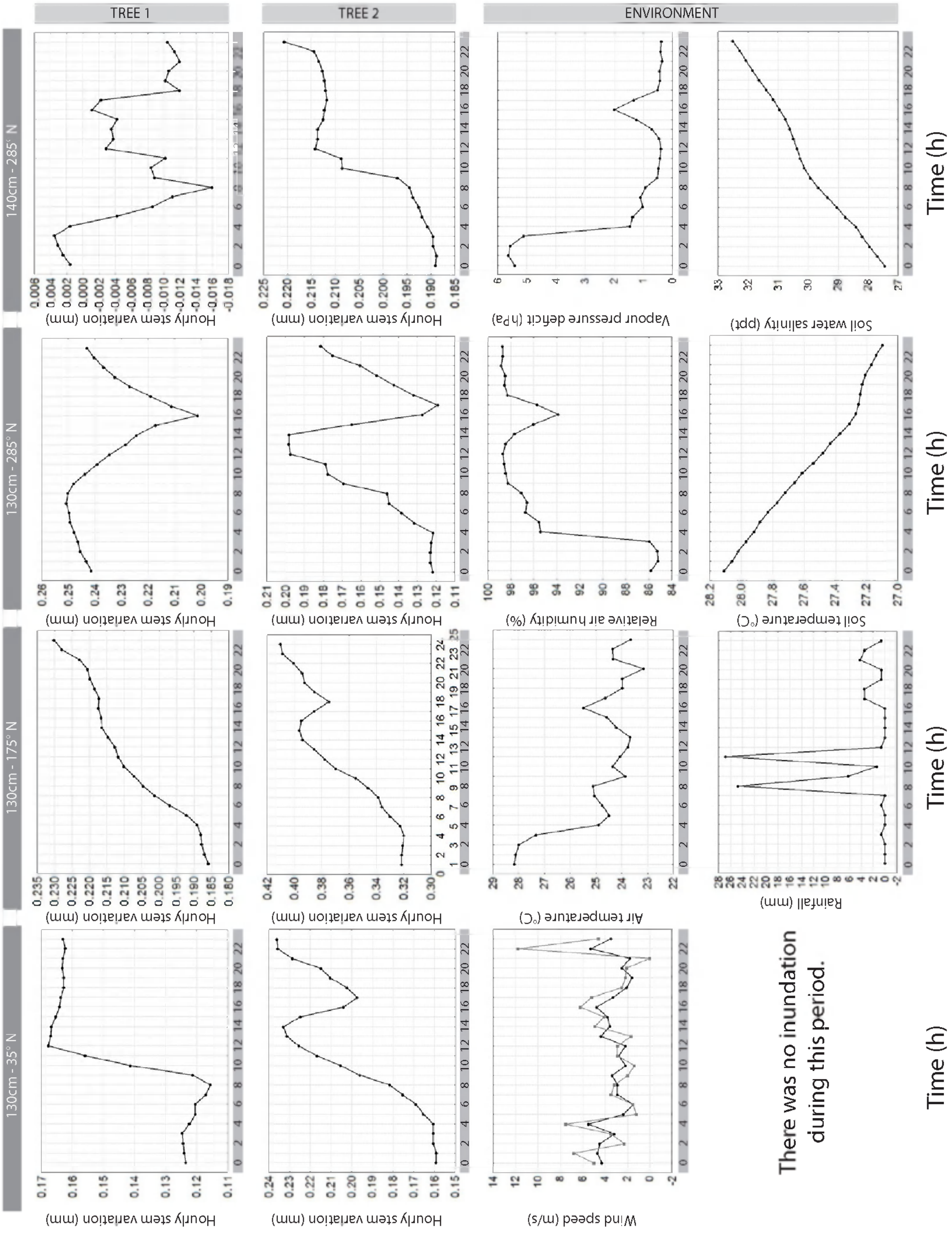
There was a maximum of 9 cm inundation. (high tide: 4:07 am)

There was no rain during this period.

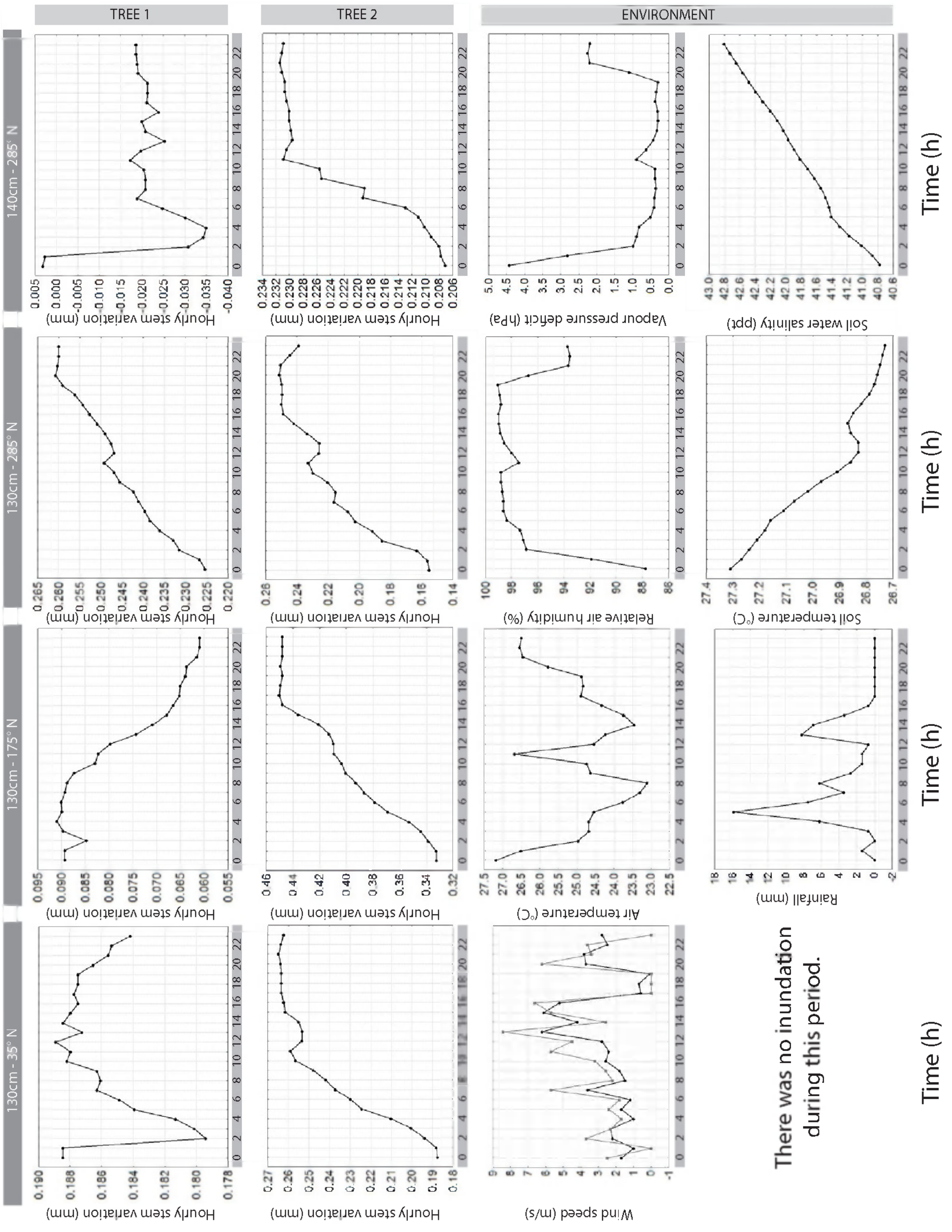


There was a maximum of 16 cm inundation. (high tide: 4:44 am)

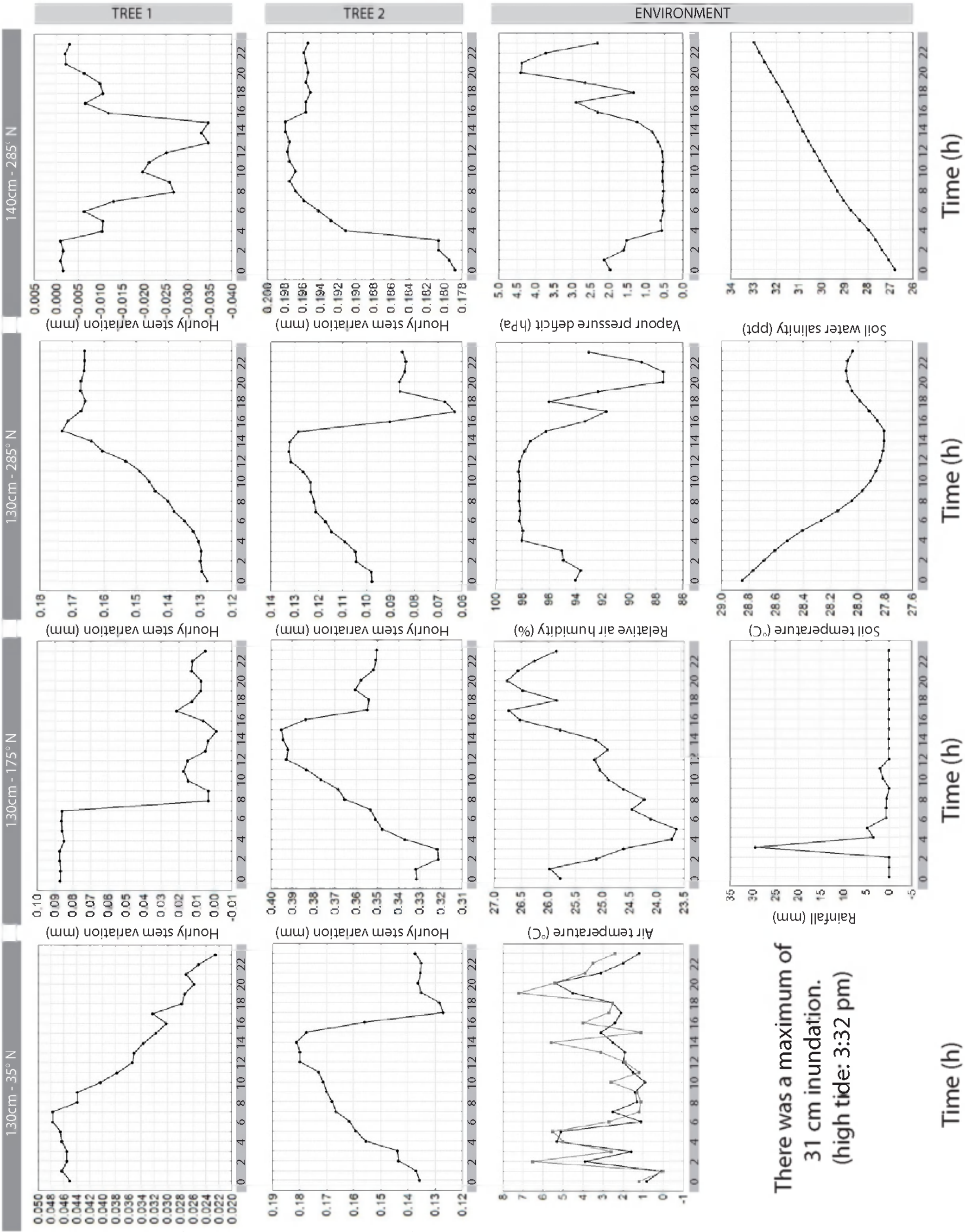
There was no rain during this period.



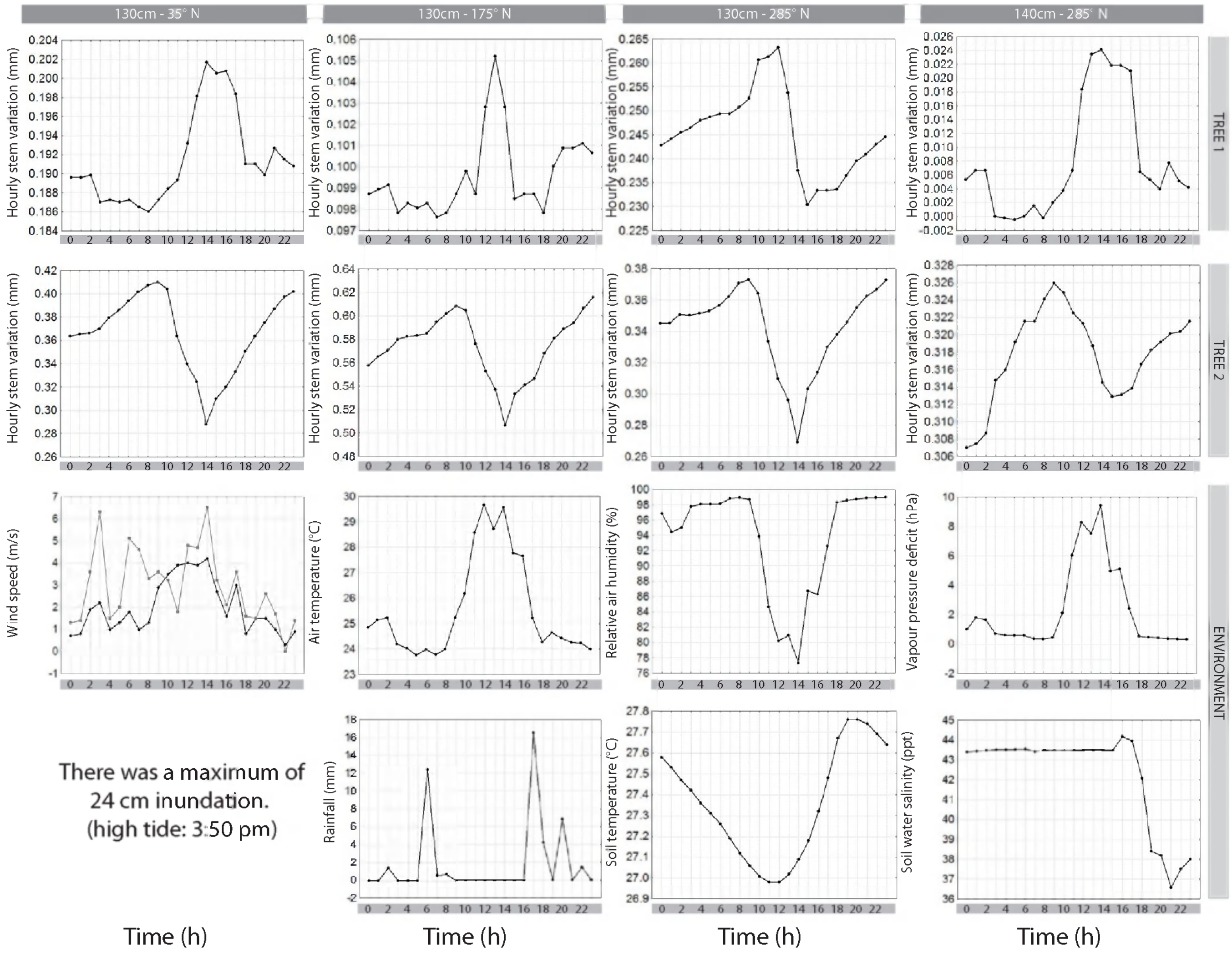
There was no inundation during this period.



There was no inundation during this period.



There was a maximum of 31 cm inundation. (high tide: 3:32 pm)



There was a maximum of 24 cm inundation. (high tide: 3:50 pm)

Discussion

Patchiness at different levels

The hypothesized patchy growth in *Avicennia* (Schmitz *et al.*, 2008b) is supported by the results of the pinning analysis. Radial increment was not always observed at all positions around and along the tree stems and small to large variation existed between the different positions (Figure 4, Table 1). It was not always at the same side that radial increment was absent, which would indicate a permanently inactive zone at least for the observed period. A clear example is the *ca.* 0.9 and *ca.* 2 mm increment between August 2006 and February 2007 in an *Avicennia marina* tree at southern and eastern direction of the stem respectively, in contrast to the absence of increment at the north-western side at the same stem height. The north-western side of this tree does not systematically show lower increment or absence of increment, further corroborating patchiness in growth. The differences in total increment after one year between the different positions around the stem circumference of two *Avicennia* trees (Table 2) also support a patchy growth mechanism, or at least do not contradict it.

Differences in the amount of increment around the stem circumference are well known in for example trees that form reaction wood (Schweingruber *et al.*, 2006) or tropical trees with buttresses (Woodcock *et al.*, 2000). However, little is known about the variation in increment around the stem circumference in trees with concentric stems or normally growing concentrically. Radial stem growth is mainly studied in a forest ecology or forestry context with the general practice of manual or automatic diameter measurements, integrating the diameter increment over the whole stem (Clark *et al.*, 2000). Usually, studies using point dendrometers examine only one side of the tree's stem (*e.g.* Zweifel & Häsler, 2001; Wimmer *et al.*, 2002; Bouriaud *et al.*, 2005; Turcotte *et al.*, 2009; Biondi & Hartsough, 2010; Hölttä *et al.*, 2010, Volland-Voigt *et al.*, 2011). However, significant differences in radial increment between different compass directions have already been found in *Picea abies* and *Pinus sylvestris* (Mäkinen *et al.*, 2003; Mäkinen *et al.*, 2008). Furthermore, differences in duration patterns and amplitude of daily stem variations with tree height have been recorded in *Picea abies* (Zweifel & Häsler, 2001; Bouriaud *et al.*, 2005; Anfodillo *et al.*, 2012). To better interpret our results, it would be desirable to have more data on the small-scale variation in radial increment around the circumference of temperate and tropical tree species allowing a reference to our high spatial and temporal resolution data.

Patchiness does not only occur in the internal structure (Robert *et al.*, 2011) and in the radial growth of *Avicennia*, it can also be observed in the swelling and shrinking patterns of *Avicennia* trees. Different positions around the stem circumference often show different swelling and shrinking behaviour (Figure 11 and 12). Especially after rainfall, the differences between different stem parts are prominent. This strengthens our hypothesis that patchiness can optimize the interaction of *Avicennia* trees with their environment. A patchy reaction of the vascular tissues to the environment could allow *Avicennia* trees to partition the available water over the tree stem. In this way, water, once accessible, could be used in a more directive way, investing all the attainable resources in only some locations of the tree stem so that at least at these locations there is enough water to, for example, overcome vessel embolisms or create new cells. As these locations change with time, the overall functioning of the tree can be maintained.

Patchy growth and a patchy reaction of vascular tissues to the environmental conditions of the mangrove forest is a potential rather than a constant feature in *Avicennia* trees. Different locations around the stem circumference can react in the same way, but they can also react differently depending on the prevailing environmental conditions. Seemingly the location of patch growth emerges in a random way. The similarity or dissimilarity occurs at different levels: between trees, between different locations around the stem circumference of one tree, in the overall growth pattern after one year and in daily or weekly stem diameter patterns. The variation in stem diameter on days without rain is much smaller than on rainy days. However, even on dry days without inundation, still not all positions around the stem circumference have the common daily pattern in swelling and shrinking. Further study should therefore be done on the alternation of patches reactive to the environment in order to prove if this process is random or not.

Increment in rainy conditions

Gradual stem increase, if present in the growth patch, mainly occurred between April and July, in the long rainy season (Figure 6). This period can be divided into two distinguishable phases: a first one, from April to June, with extensive rainfall, decreasing air temperature, soil water salinity and soil temperature and increasing relative humidity and a second phase, in July, with less rainfall but with minimum air temperature, soil water salinity and soil temperature. Hence, favourable conditions for radial growth are periods of precipitation like for many tropical tree species, where onset and interruption of cambial activity relates to changes in water availability (Bräuning *et al.*, 2008; Biondi & Hartsough, 2010; Krepkowski *et al.*, 2011; Voland-Voigt *et al.*, 2011). However, also the combination of a smaller amount of precipitation, low air temperature, low soil water salinity and low soil temperature apparently allow for radial increment in *Avicennia* trees. This could be explained by the combined effect of water availability and less demanding conditions for the water transport. Decreased tension on the water column at the atmosphere side (lower air temperatures) as well as at the soil side (lower soil water salinity), makes water transport less vulnerable to cavitation and thus enables higher water supply for cambial activity.

The short rainy season in the year of our study started in the last days of October and lasted until the end of November. The start of this short rainy season induced stem increment in four of the eight studied positions around the stem circumference of the two trees (Figure 6). The period of stem increment, however, lasted to the end of February at most of these positions, with an extensive increment phase at one position of tree B (130 cm - 35°N) in December. Trees appear to have a stop in radial increment in August to October, between the long and short rainy season, rather than in the dry season, despite a considerable amount of precipitation during this period. A possible explanation for this growth stop could be the combined strong increase of air temperature, soil temperature, soil water salinity and vapour pressure deficit. All these variables are at the highest respective levels in the dry season but without overall increase in this period. Possibly, the constantly high stress which the trees experience in the dry season is less limiting for the tree functioning than the steeply increasing stress level in between the long and the short raint season. The stop in radial increment also coincides with the peak in litter fall. Peak leaf fall in *Avicennia* trees occurs after peak leaf emergence (Duke, 1988; Ochieng & Erftemeijer, 2002; Wang'ondu *et al.*, 2010). However, August and September have been reported as months with high leaf production (Wang'ondu *et al.*, 2010) so that in this period, *Avicennia* trees rather invest available energy and water in leaf production than in water storage or wood formation.

A large difference, both in total increment after one year and in increment patterns, could be observed between both studied trees (Figure 6, Table 1). This difference could not be explained by any of the measured tree characteristics (general appearance, tree phenology data, pen root height, wood anatomy) and it is unclear whether the observed difference is due to differences at tree level or at time level (annual differences of growth in a seemingly homogeneous stand). Longer observation of the stem diameter changes of more trees could show whether one of the two growth patterns is the more general type or whether there is much variability. It could also contribute to establishing whether the two patterns occur in all trees with yearly variation. However, differences up to several millimetres in yearly increment are not exceptional between trees of a same stand (*e.g.* Deslauriers, 2003; Mäkinen *et al.*, 2003; Volland-Voigt *et al.*, 2011).

In tree A, the increment in one year happened for 33 to 100 % through sudden increases in the stem diameter (bursts), depending on the position around the stem circumference. These bursts happened at three different moments in time at one or two sides of the stem circumference and corresponded to environmental changes linked to precipitation events. Since these sudden increases of the stem do not reverse within the period studied and are not larger in width or shorter in time than observed in other species (Deslauriers, 2003; Čufar *et al.*, 2008a; Čufar *et al.*, 2008b; Čufar *et al.*, 2011), short periods of cell formation are not excluded. However, the methods used in this study cannot distinguish between radial increment due to water uptake by the vascular tissue or to formation of new cells adding to the circumference. The change of location with time of these sudden increases in stem size could allow for an overall concentric growth of the stem diameter as proposed by Schmitz *et al.* (2008b).

Daily stem diameter fluctuations

Unexpectedly, inundation by seawater had no direct effect on the daily stem variation patterns in *Avicennia* trees on days without rain (Figure 12). This is in contrast to the effect of fresh water from rain events, from which instant effects could be noticed in the stem diameter changes, at least at one position around the stem circumference of both studied trees. The larger and instant effect of fresh water could indicate that *Avicennia* trees cannot instantly extract fresh water when inundated with seawater. However, more daily patterns have to be observed to evaluate the effects of inundation on different hours of the day and the possible delayed effect of tidal inundation. Moreover, rain events are associated with changes in air temperature and relative air humidity, while inundation only has an effect on soil temperature. Consequently, the large and instant effect of rain events is more than a reaction from the tree on fresh water availability alone. Processes such as stomatal conductance, known to be instantly influenced by temperature and relative air humidity and of high importance for the water fluxes in trees, most probably play an equally crucial role in stem diameter variations during rain events.

The instant effect of rain events was also reflected in the patterns of maximum daily shrinkage of both trees, with generally lower but more varying maximum daily shrinkage in the rainy season. Also, the occurrence of more than one daily shrinkage phase is more frequent during the rainy season, indicating the division of the diurnal cycle by several shrinking and swelling phases and thus the short-term reaction of the tree stems on changing environmental conditions. Hence, we can conclude that fresh water input has a strong and direct effect on the water flow between the vascular tissues in *Avicennia* both accounting for replenishment of the stem with water and stem increment.

Conclusion

We can conclude that patchiness is a prominent characteristic of the hydraulic system of *Avicennia* trees. Patchiness occurs in the structure of their hydraulic tissues (Robert *et al.*, 2011), in their radial growth and in the shrinking and swelling patterns of their stems. Patchiness is however a potentially rather than systematically present feature. By this potential patchiness, *Avicennia* trees seem to have an extra tool to optimally react on the prevailing environmental conditions, leading to ecological success in the highly dynamic mangrove environment.

Radial growth increment is strongly linked to rainfall and rain events had an instant effect on the daily shrinking and swelling patterns of *Avicennia* tree stems. This shows the extreme importance of fresh water input for the functioning and water household of mangrove trees.

Further study should firstly consist of a second analysis of the obtained dataset addressing systematic analysis of the dataset on day-per-day level, in-depth study of the characteristics of the daily stem diameter cycles and a search for other potential patterns. So far, we only addressed clear patterns between the hourly stem variation data and the environmental data, with their differences and effects, in relation to the tested hypothesis. Deeper insight into the patchy functioning of the hydraulic system of *Avicennia* trees as well as in the water household within this patchy system could for example be obtained by dendrometer measurements over longer periods on more trees and more positions of the trees stem or by coupling dendrometer analysis to magnetic resonance imaging (De Schepper *et al.*, 2012).

The observations bring up two questions, which cannot be answered readily through the actual approach. Our actual approach was intended to test the hypothesis of patchiness in the water conducting tissues and to establish its timing and the mechanistic process, research steps which have all been taken. A first question addresses the evolutionary background of the patchiness. Though the transition from a regular concentric growth to a patchy growth does not seem to require major and fundamental changes in the growth process, but rather its location and timing, it must be investigated whether the generally observed meristematic flexibility in stems of *Avicennia* spp. and its successive cambia predisposed the species of this genus for evolutionary selection into the most demanding niches in the mangrove ecotone. This could easily be understood as a regulatory sloppiness in stem and tissue development in an ecologically graded environment. The second question addresses the wide ecology of *Avicennia* spp., both at a local and at a biogeographical level (latitudinal range). Though conditions which are adverse to tree growth (such as in the rarely flooded landward stands as well as at high latitudes in arid climates) explain the stunted and crooked dwarf growth of *Avicennia*, looking healthy in all other aspects, flowering and fruiting profusely, it must be confirmed that this is the result of efficient short and rare growth bursts at many moments of suitable growth conditions, even when unpredictable. Taken together this can be seen as a succesful growth strategy emerged from a fairly simple anatomical feature: successive cambia and patchy growth.

Acknowledgements

We are grateful to Hamisi Ali Kirauni, Hilde Robert and Tom Van der Stocken for their help during the field work, to Achim Bräuning, Julia Krepkowski and Franziska Volland-Voigt for their advice regarding the dendrometer installation and the dendrometer data anlysis and to Johan De Mey and Maarten De Munter for executing the CT-scans. The study was financially supported by the Agency for Innovation by Science and Technology (IWT, Flanders, Belgium), the Research Foundation - Flanders (FWO, Flanders, Belgium), the King Leopold III Fund for Nature Exploration and Conservation (Belgium), the 'Stichting ter bevordering van wetenschappelijk onderzoek in Afrika' (SBWOA, Belgium) and a BET/UTB budget of the Royal Museum for Central Africa (RMCA, Tervuren, Belgium). We thank the Department of Radiology of the Universitair Ziekenhuis Brussel for offering free CT-scanning.

Visualisation of the stem water content of two species with internal phloem through Magnetic Resonance Imaging (MRI)

Abstract

Shrubs and trees with internal phloem tissue mainly occur in habitats characterized by periodical or continuous lack of water availibility. Furthermore, the amount of internal phloem tissue in stems of *Avicennia* trees raises with increasing soil water salinity and decreasing inundation frequency. Hence, increased water storage in internal phloem tissue was put forward to be advantageous in harsh environmental conditions. The question however remains whether the internal phloem cells over the entire stem of woody species showing this wood anatomical feature, are water-filled. In this preliminary and pioneering study, we use magnetic resonance imaging (MRI) to visualize the stem water content of three species with internal phloem tissue, the mangroves *Avicennia marina* and *A. officinalis* and the non-mangrove *Bougainvillea spectabilis*. Measurements were conducted in living plants. We tested the hypothesis that not only the outermost phloem tissue has high water content but also the internal phloem tissues over the entire stem from the bark inward to the pith, herewith serving as water storage sites. We can conclude that the internal phloem tissue of both *Bougainvillea* and *Avicennia* has high water contents. This aligns with the contribution of internal phloem tissue to ecological success in conditions of physiological drought. Further study should however be done to understand the mechanisms through which internal phloem tissue contributes to the water household of plants in conditions of water shortage.

Introduction

We established that the internal phloem tissue in stems of *Avicennia* trees is more abundant with increasing soil water salinity and decreasing inundation frequency (Robert *et al.*, 2011) Hence, we hypothesize that internal phloem tissue allows for increased water storage. Phloem water storage may be advantageous in environmental conditions with variable water availability and frequent drought. Through estimation of the effective water content of internal phloem tissue in *Avicennia* trees, and the variation therein across the tree, we may gain insight into the role of internal phloem on the water balance of *Avicennia* trees.

Imaging methods based on nuclear magnetic resonance (NMR), called magnetic resonance imaging (MRI), are among the techniques that allow for direct *in vivo* observation of water content and water movement in plants. Other methods are heat tracing methods (heat balance, heat pulse or heat dissipation) and radioisotope or stable isotope tracing methods (Calder *et al.*, 1986; Lambs & Saenger *et al.*, 2011). However, the exceptional aspect of MRI is the possibility for spatially resolved results, allowing for sap flow and water content data on every location of the plant's stem. In the last decades, NMR flow imaging has become a growing discipline in the study of xylem and phloem sap transport over long and short distances in plants (*e.g.* Kuchenbrod *et al.*, 1996; Rokitta *et al.*, 1999; Olt *et al.*, 2000; Holbrook *et al.*, 2001; Scheenen *et al.*, 2002; Scheenen *et al.*, 2007; Windt, 2007; Kaufmann *et al.*, 2009; Mullendore *et al.*, 2010) with few studies on trees (Kuroda *et al.*, 2006; Windt *et al.*, 2006; Homan *et al.*, 2007; Merela *et al.*, 2009; Umebayashi *et al.*, 2011; De Schepper *et al.*, 2012).

All NMR experiments are based on the interaction between the magnetic moment of certain nuclei, especially ^{1}H, and an external magnetic field (Köckenberger, 2001). A strong permanent magnetic field is used to create sample magnetization that is then manipulated by radio frequency (RF) pulses and detected through induction of a weak voltage in a coil placed around the sample (Köckenberger, 2001). The magnetization is measured in the presence of a magnetic gradient, allowing for one-dimensional projection in the direction of the gradient after Fourier transformation of the detected signal as a function of time (Köckenberger, 2001).

It has already been proven that the bark tissues of *Quercus robur* (De Schepper *et al.*, 2012) and *Populus* (Windt *et al.*, 2006) trees, 1-2 cm in stem diameter and 1.1-1.5 m in height, have the highest water content when compared to the other tissues within the stems of these trees. Also, the non-conductive bark cells in the same *Quercus* trees were put forward as contributing most to the water storage capacity of the tree stem (De Schepper *et al.*, 2012). If the phloem tissue of the bark has an important role in the water household of trees, it is probable that also other living tissues within a tree's stems, especially internal phloem if present, significantly contribute to the tree's water reserves. It is generally accepted that phloem in plants with a single vascular cambium function for one year only (Raven *et al.*, 1999; Rosner *et al.*, 2001; Carlquist, 2007a). For plants with successive cambia, a greater area of conducting phloem area has been proposed, based on the continued production of internal phloem by the internal vascular cambia (Carlquist, 2007a). To our knowledge, physiological experiments providing evidence that internal phloem tissue in plants with successive cambia is still active are lacking, with the exception of one study by Fahn & Shchori (1967). Water conduction can take place in more than the outermost stem part of *Avicennia* trees (Robert & Schmitz, unpublished data). The question then remains: what about the actual water content of internal phloem tissue, if present, in woody species? Are the internal phloem cells water-filled over the entire stem? Are they active in long and/or short water transport in tree stems? A positive answer would confirm the ecological advantage that we suggested for species with internal phloem tissue in conditions of physiological drought due to high soil water salinity or water shortage (Robert *et al.*, 2011).

In this study, we used MRI to visualize the stem water content of three species with internal phloem tissue, the mangroves *Avicennia marina* and *A. officinalis* and the non-mangrove *Bougainvillea spectabilis*. We tested the hypothesis that not only the outermost phloem tissue has high water content but also the internal phloem tissues over the entire stem from the bark inward to the pith, herewith serving as water storage sites. While the xylem and phloem tissue in *Avicennia* trees has a reticulate structure (Robert *et al.*, 2011), numerous strands of vascular increments, composed of both xylem vessels and phloem tissue, are scattered over the stem of *Bougainvillea* (Figure 1). One tree of each species has been selected for the study. The *Bougainvillea* tree, cultivated in a temperate climate and directly subjected to MRI, was used as a positive control. The *Avicennia* trees were taken from the mangrove forest, transported and measured, after an acclimatisation period in conditions different from its natural environment. The general approach is viewed as a preliminary and pioneering step to assess an extra line of evidence for the ecological significance of internal phloem tissue. The outcome of the approach is evaluated in the framework of the insights gained and hypotheses developed in the previous chapters.

Materials and Methods

Plant material

A *Bougainvillea spectabilis* Willd. plant with a stem of 1.20 cm in diameter and *ca.* 80 cm in height, trimmed when cultivated as a single stemmed small tree, has been obtained from a local plant dealer in Belgium. Two small *Avicennia* trees have been transported from the mangrove forest of Gazi Bay (Kenya) (*Avicennia marina* (Forssk.) Vierh. - tree 1) and Leyte island (The Philippines) (*Avicennia cf. officinalis* Linn. - tree 2) to the Wageningen NMR Centre (Wageningen University, The Netherlands) where MRI measurements were conducted. Tree 1 was *ca.* 150 cm high and had a stem diameter of 1.25 cm while tree 2 was

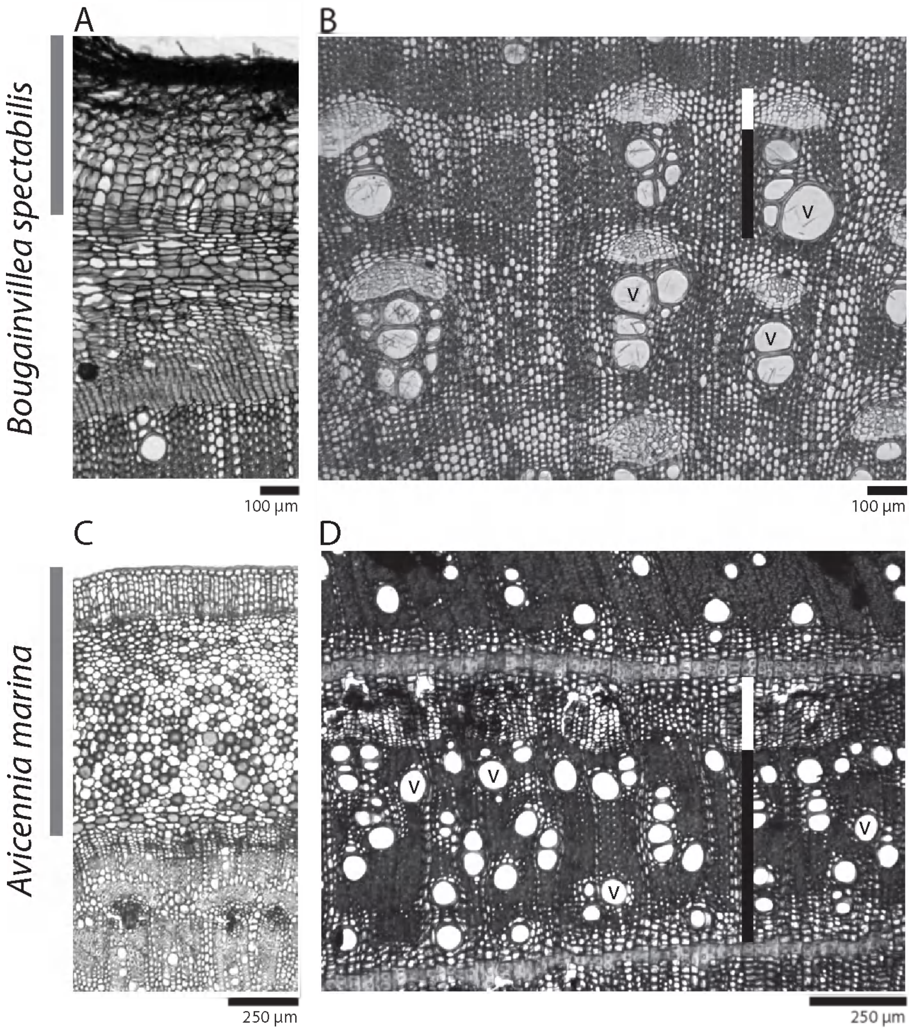


***Figure 1** - Internal stem anatomy of* Bougainvillea spectabilis (A, B) *and* Avicennia marina (C, D), *showing the bark tissue (grey bars) (A, C), the internal phloem tissue (white bars) and the xylem tissue (black bars) with water conducting vessels (v) (B, D). Stems were sectionned using a sliding microtome and double stained with safranin-alcian blue after which a picture was taken via a camera connected to a microscope.*

ca. 65 cm high with a stem diameter of 0.8 cm. During transport, the *Avicennia* trees were covered with insulation material and between arrival and actual MRI measurements there was an acclimatisation period of 2.5 months in the mangrove greenhouse of Burger's Zoo in Arnhem (The Netherlands) during which the tree was watered with fresh water (tree 1), and of one week in a climate chamber (air temperature: 22°C; relative air humidity: 70%) during which the tree was watered with saline water (20-30 g/l) (tree 2).

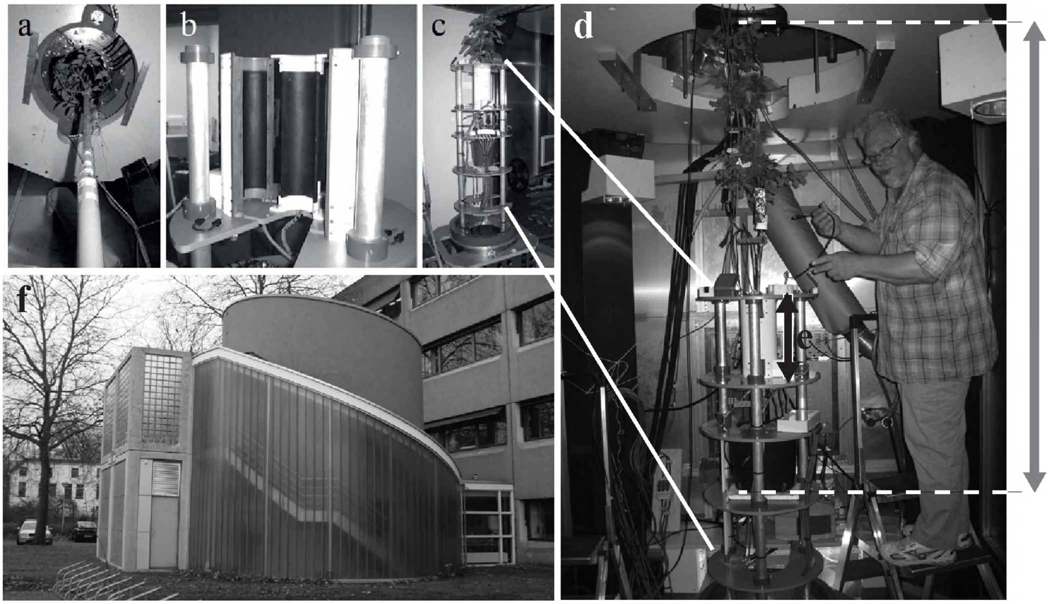


__Figure 2__ - 3T MRI system at Wageningen University (The Netherlands) showing (a) the lifting of a tree into the superconducting magnet, (b, c, d, e) the gradient coil, (b) open and (c, d, e) placed around a tree, (d) Edo Gerkema preparing an oak tree for MRI analysis in the space under the magnet and (f) the 3T MRI building from outside. The grey arrow indicates the length of a 1.80 m high tree, from roots to crown top. Figures a, b and c are adapted from Homan, 2007. Picture d is taken by Paul Copini.

Magnetic Resonance Imaging (MRI)

Measurements were done in a 3 T (*Bougainvillea* tree and *Avicennia* tree 1) and a 0.7 T MRI system (*Avicennia* tree 2) at the Wageningen NMR Centre (Wageningen University, The Netherlands).

The **3T MRI system** (Figure 2) consists of an Avance console (Bruker, Karlsruhe, Germany) and a superconducting magnet with a 50 cm vertical bore (Magnex, Oxford, UK), generating a magnetic field of 3 T (128 MHz proton frequency). Measurements were performed in the centre of a gradient coil that was mounted around the stem (Figure 2b-d) and that generates maximum gradient strengths of 1 T/m. For induction and detection of the signal, an radio frequency (RF) coil, consisting of two half cylinders, was placed around the tree stem at *ca.* 40 cm height above the pot surface.
The **0.7 T MRI system** consists of an Avance console and electromagnet (Bruker, Karlsruhe, Germany) with a 10 cm air gap, generating a magnetic field of 0.7 T (30 MHz proton frequency). Maximum gradient strength that can be generated is 1 T/m. For induction and detection of the NMR signal a solenoid RF coil was constructed by wrapping copper wire around a mould slightly wider than the tree stem. The coil was connected to a tuning circuit

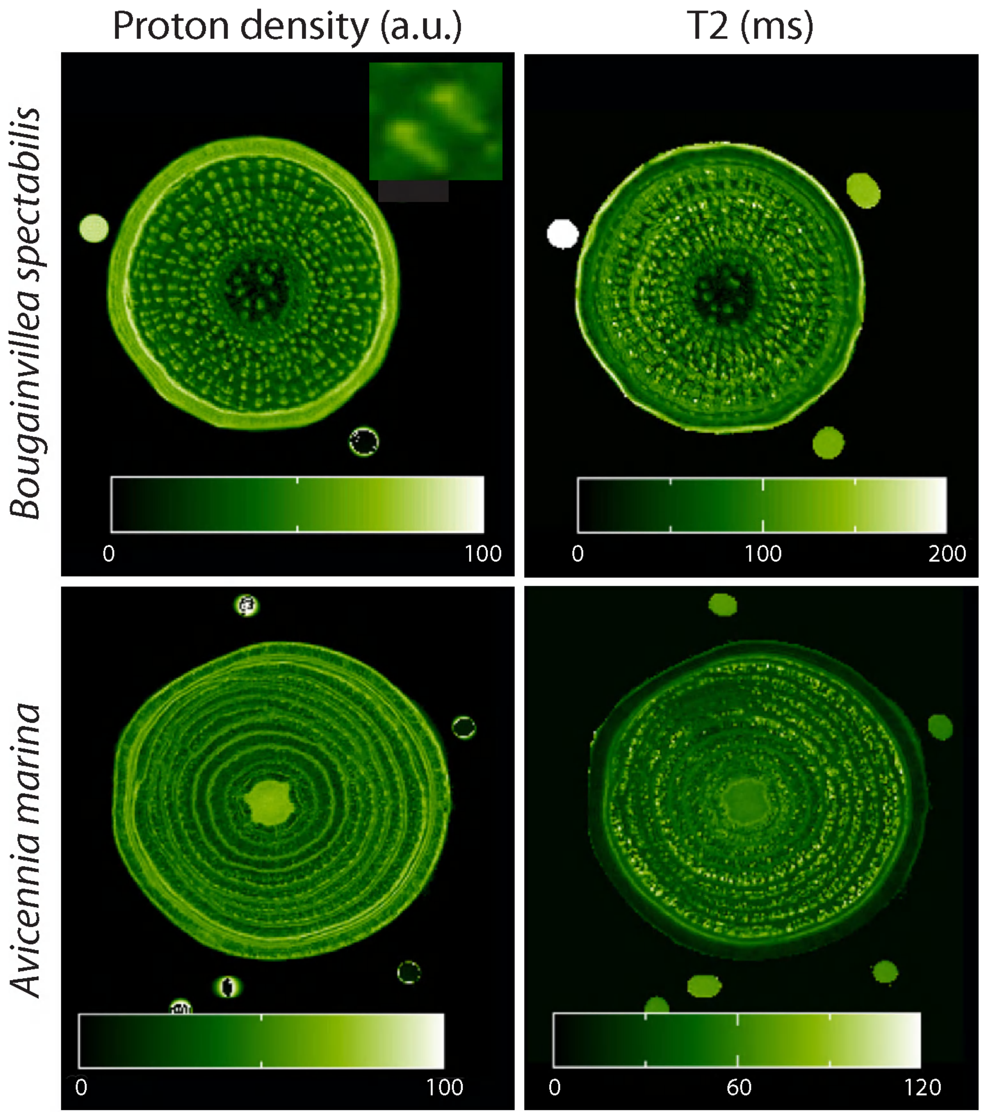


Figure 3 - Proton density (water content) on a gradient from 0 (black - no water) to 100 (white - purety water) and T2 images of intact Bougainvillea spectabilis *and* Avicennia marina *stems obtained through magnetic resonance imaging (MRI). The stem diameters are 1.20 cm and 1.25 cm for the* Bougainvillea *and* Avicennia *plant respectively. The inset in the upper left panel is a zoom of two vascular strands. Proton density is in arbitrary units (a.u.).*

and electromagnetically shielded with aluminium foil and copper tape.
For a more detailed description of the MRI systems: see Homan et al. *(2007).*

During the measurement periods air temperature ranged from 22°C to 26°C. Relative air humidity was 28-40% (3 T MRI) and 50-65% (0.7 T MRI). Trees were subjected to a day-night cycle of 12:12 hours and regularly watered with fresh water (*Bougainvillea* and *Avicennia* tree 1) and saline water (20-30g/l) (*Avicennia* tree 2). These conditions were chosen to maximally allow normal functionning of all three plants. The conditions imposed on the *Avicennia* trees were intended to represent natural conditions with high demand on the water transport system from the atmospheric side (low relative humidity and fresh water) or from the soil side (high relative humidity and saline water).

Measurement routines were selected by Edo Gerkema, with input of Dr. Henk Van As, Ir. Frank Vergeldt and Dr. Carel Windt of the Wageningen NMR Centre (Wageningen University, The Netherlands). For all quantitative T2 measurements, a multiple spin echo imaging sequence has been used (Edzes *et al.*, 1998). The following settings were applied: field of view: 20 x 20 mm (*Bougainvillea*), 18.5 x 18.5 mm (*Avicennia* 1) and 11.4 x 11.4 (*Avicennia* 1); matrix: 256 x 256; slice thickness: 3 mm; number of averages: 8 (*Bougainvillea*) and 16 (*Avicennia*); echo time: 7.19 ms (*Bougainvillea* and *Avicennia* 1) and 6.88 ms (*Avicennia* 2); number of echos: 96 (*Bougainvillea* and *Avicennia* 2) and 64 (*Avicennia* 1); repetition time: 2500 ms. Differences of values between the trees were to optimise the spatial (field of view) and the signal-to-noise and time resolution (number of averages). They furthermore depend on the longest relevant T2 (number of echos).

Several quantitative T2 measurements were made, both during day and night after which, measured proton density and T2 values were visualized over the stem disc through a colour scale from the smallest (black) to the largest (white) value. Proton density is a measure of the water content, while T2 is a time constant related to transverse relaxation after magnetisation and radio frequency pulsing of a sample (Köckenberger, 2001). T2 values change with the size of the measured compartment (van der Weerdt *et al.*, 2001) and are as such an indication of the tissue type in plants. No differences could be observed in water content and T2 patterns between the different measurements, neither in *Avicennia*, nor in *Bougainvillea*.

Results and discussion

Amplitude images showed the water content of the different tissues over the entire stem of both *Bougainvillea* and *Avicennia* trees (Figure 3). The bark and the vascular bundles of *Bougainvillea* had a high water content but the highest amount of water could be observed in the zone of the secondary cambium (up to the first lignified xylem cells). This is in agreement with observations in *Quercus* (Kuroda *et al.*, 2006; De Schepper *et al.*, 2012), *Populus* (Windt *et al.*, 2006) and *Pinus* (Umebayashi *et al.*, 2011) tree stems. High water content is expected in the cambial zone since water is crucial in cell formation and especially cell enlargement (Woodward, 2004; Arend *et al.*, 2007). When looking in more detail to the water content of the vascular bundles of *Bougainvillea*, we see that in the outermost zone of many bundles, the water content was higher than in the innermost zone, visible by the lighter yellow-green and the slightly darker green colour respectively (Figure 3 - inset in above left panel). Since each vascular bundle in this species is composed of phloem on the outside and xylem on the inside (Figure 1), the higher water content was situated in the phloem tissue. The phloem cells in *Bougainvillea* stems are thus full of water, whether or not living and/or active in vertical sap transport.

In *Avicennia*, amplitude images showed higher water content in the pith and in the outermost zone of the studied stems (Figure 3; tree 2: data not shown), although this was less pronounced than in the studied *Bougainvillea* tree. The phloem bands in both studied *Avicennia* trees had relatively high water content throughout the entire stem, despite the different treatments they got (tree 1: fresh water and low relative humidity *versus* tree 2: saline water and higher relative humidity). The considerable amount of water in the internal phloem tissue of both *Avicennia* and *Bougainvillea* throughout the entire stem strengthens the importance attributed to the internal phloem tissue in the water household of trees with successive cambia and other cambial variants. The presence of water storage tissues evenly distributed over the tree stem could indeed be responsible for their ecological advantage in conditions of water shortage as has been proposed earlier (Robert *et al.*, 2011).

T2 values are not radially homogeneous in the studied *B. spectabilis* and *A. marina* stems, which is indicated by the presence or absence of light green dots on the T2 images (Figure 3 - right panels) often in concentric zones. Since T2 values scale with compartment size (van der Weerdt *et al.*, 2001), narrower vessels in some zones of the stems could possibly be at the basis of lower T2 values in these zones. However, to obtain further understanding of the physiology in terms of hydraulic function behind the observed patterns, sap flow measurements should be conducted.

Though the constraints on the experimental approach limit maximum tree size, reduce naturalness of growth conditions during measurements and restrict the number of measurements and trees, the results obtained get their significance within the hypothetical framework we put forward: they align with the role of internal phloem as a water storage tissue. It is however appropriate to scrutinize the possible effects of these constraints. Actually we cannot establish whether our observations would also apply to tall or old trees, but the fact that *Bougainvillea* is not usually a thick stemmed tree respectively that the young *Avicennia* used show a general anatomy that is similar to an adult tree's anatomy gives us confidence. As for the naturalness of growing conditions: after the procedures none of the trees showed damage such as leaf abscission or blackening (common in *Bougainvillea* respectively *Avicennia*). As for the actually unavoidable limited data set, the fact that the results align strengthen the preliminary conclusions.

Conclusion

We can conclude that the internal phloem tissue of both *Bougainvillea* and *Avicennia* has high water content. This strengthens the contribution of internal phloem tissue to ecological success in conditions of physiological drought caused by water shortage or high soil water salinity which we proposed earlier (Robert *et al.*, 2011).

Our MRI results on *Bougainvillea* and *Avicennia* are preliminary and only a very few trees have been measured, yet we consider them significant. The non-destructive and realtime study of phloem transport and water exchange between the xylem and the phloem tissue in mature trees in the field is difficult (Sevanto *et al.*, 2011). The study of internal (not bark) phloem tissue in trees present additional complications. However, further studies of the functioning of internal phloem tissue and its role in the water household of plants could offer important insight in understanding the survival and thriving of plants under physiological drought and the functioning of trees in general. Combining techniques and new approaches may overcome the current difficulties and should therefore be continued. Studies on mangrove trees combining MRI and dendrometer measurements (De Schepper *et al.*, 2012), using portable NMR devices (Windt *et al.*, 2011) or specifically addressing the coupling between xylem and phloem through dendrometer analysis (Sevanto *et al.*, 2011) could be a first possible continuation.

Acknowledgements

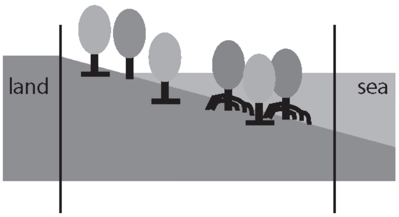
We are grateful to Hamisi Ali Kirauni and James Gitundu Kairo of the Kenya Marine and Fisheries Research Institute (KMFRI, Mombasa, Kenya), to Mohamed Omar Said Mohamed of Kenya Wildlife Service and to Hilde Robert and Mia Van Mellaert for their help and support in the transport of the *Avicennia marina* tree; to Joep Wensing and Ernst Kamphuis of Burger's Zoo (Arnhem, The Netherlands) for housing the *Avicennia* plants in the mangrove greenhouse of Burger's Zoo; to Paul Copini for his help during the set up of the research; to Edo Gerkema, Frank Vergeldt, Ari van Hoek, John Philippi, Carel Windt and Henk Van As of the Wageningen NMR Centre (Wageningen Univerity, The Netherlands) for the use of the MRI system and their assistance during scanning, scanning preparation and data processing and to Hilde Robert for her assistance in making thin-sections. The research was financial supported by the Agency for Innovation by Science and Technology (IWT, Flanders, Belgium), the Research Foundation - Flanders (FWO, Flanders, Belgium), the Company of Biologists (COB) through the Society for Experimental Biology (SEB), the European Cooperation in Science and Technology (COST) on the Terrestrial Biosphere in the Earth System (TERRABITES) via a Short Term Scientific Mission and the "Stichting ter bevordering van wetenschappelijk onderzoek in Afrika" (SBWOA, Belgium).

General summary

Mangroves make a virtue of necessity

Necessity

Mangrove environment = tropical intertidal zone



▷ SALT

▷ INUNDATION

highly dynamic & unpredictable

Physiological translation: increased tension on the water column of trees

▷ RISK FOR DROUGHT-INDUCED CAVITATION ↑

Mangrove trees need adapted survival strategies.

Vascular factor

Diverse phylogenetic origins — Mangrove signal in wood anatomy

- → NARROW VESSELS (small range)
- → NUMEROUS VESSELS (large range)

> Survival in the mangrove environment requires a wood anatomy consisting of numerous narrow vessels

Mangrove species 1 — Mangrove species 2 — Mangrove species 3 — Mangrove species 4

Location 1 — Location 2 — Location X

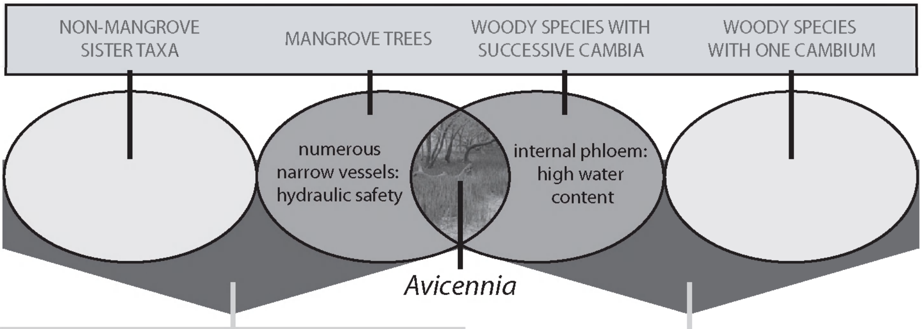
along a gradient in salinity and inundation stress

> With higher soil water salinity and lower inundation frequency, mangrove species and mangrove trees from different locations within the mangrove forest have more numerous and narrower vessels.

The level of physiological drought (*i.e.* cavitation risk) determines mangrove wood anatomy.

The ecological success of *Avicennia* trees

Broad local and global distribution



Wood anatomy

> *Avicennia* has the most pronounced mangrove wood anatomy (*i.e.* numerous narrow vessels).

Successive cambia

Internal phloem

> We found three lines of circumstancial evidence for the ecological advantage of successive cambia under harsh environmental conditions

- TREE AND SHRUB SPECIES WITH INTERNAL PHLOEM MAINLY OCCUR IN WATER LIMITED ENVIRONMENTS
- *AVICENNIA* TREES UNDER HIGH SOIL WATER SALINITY AND LOW INUNDATION FREQUENCY HAVE MORE INTERNAL PHLOEM TISSUE
- THE INTERNAL PHLOEM TISSUE OF *AVICENNIA* HAS HIGH WATER CONTENT OVER THE ENTIRE TREE STEM

Patchiness

> *Avicennia*'s internal structure consists of xylem and phloem patches arranged in a complex three-dimensional network extensively varying with tree height.

> This structure allows for patchiness in radial increment as well as in swelling and shrinking around the stem circumference, most probably for an optimal reaction on the prevailing environmental conditions at any moment.

The ecological success of *Avicennia* can, at least partially, be explained by its wood anatomical characteristics.

Conclusion & Perspectives

Mangroves make a virtue of necessity

The precarious conditions of the mangrove forest, *i.e.* the highly dynamic soil water salinity, inundation periods and evaporation intensity, demand a water transport system that is composed of numerous narrow vessels. This pattern has developed with similar features in different mangrove species, despite their diverse phylogenetic origin and it differs from features of their respective closest non-mangrove relatives. The fact that the environment plays an important role in determining the characteristics of the vascular system is not a new observation (*e.g.* Baas, 1983; Baas, 1986). However, the convergence in mangrove wood anatomy is astonishing if one considers the many possible strategies a tree's water transport system could develop to handle the same environmental conditions (Chave *et al.*, 2009). It would be highly interesting to complete the existing dataset by a comparison between mangrove species of non-eudicot lineages and their respective non-mangrove sister taxa. Such examples could be mangrove palms (*Nypa fruticans* and *Phoenix paludosa*) as monocots and mangrove ferns (*Acrostichum* spp.) as vascular spore plants, to be compared with respective non-mangrove relatives. If these comparisons generate data in the same direction, *i.e.* more numerous and narrower vessels in mangroves as compared to their respective non-mangrove relatives, then the additional evidence would render the requirements of the vascular system in conditions that are extremely demanding for a plant's water transport system strict.

Numerous narrow vessels seem to be a minimum requirement for trees to survive in the mangrove environment. But why? What could be a physiological explanation? The cohesion-tension theory explains the ascent of sap through tension gradients along a continuous water column (Dixon & Joly, 1894; Angeles *et al.*, 2004). In the mangrove forest, saline soil water and frequent soil drought generate extra pulling on the water column at the soil side while high air temperature and wind do the same at the atmosphere side. This obviously generates an increased risk of cavitation and subsequent vessel embolism in mangrove trees. With numerous narrow vessels, a larger amount of conducting area remains functional for the same amount of air-filled vessels, if compared to trees with fewer and larger vessels. However, investing in embolism resistance through numerous narrow vessels comes with costs, specifically carbon investment (numerous vessels demand more tissue formation) and reduced transport efficiency (conductivity efficiency scales with the fourth power of the vessel radius - Hagen-Poiseuille's law for laminar flow). This explains why plants only are as cavitation resistant as their environment requires them to be (Pittermann, 2010).

Regardless the mechanism for long distance water transport (Zimmermann *et al.*, 2002; Angeles *et al.*, 2004; Zimmermann *et al.*, 2004; Laschimke *et al.*, 2006; Westhoff *et al.*, 2009), a water transport system under increased tension is still vulnerable to vessel embolisms. If for example Laschimke *et al.* (2006) are right in their theory and a wall adherent bubble system generates the necessary energy for long distance water transport in trees, vessels under increased tension will completely fill with air, destructing the system of several small air bubbles inside the vessels necessary for water to be lifted. Apart from the reduced loss in conducting area for the same amount of vessel embolism, a system of numerous narrow vessels could in this case also be a way to distribute the many air bubbles over the vascular system in order to keep them small enough for long distance transport to take place. As such, more air can be stored inside the stem. Also under the theory of Westhoff *et al.* (2009), *i.e.* water lifting by short distance tension gradients in many steps and not by one step, numerous narrow vessels could bring advantage since they increase the short distance contact possibilities between the different hydraulic tissues.

Intervessel pit characteristics are considered more directly linked to cavitation vulnerability than vessel characteristics through the pit area hypothesis (Wheeler *et al.*, 2005; Sperry *et al.*, 2006; Choat *et al.*, 2008). Furthermore, pit membrane characteristics were suggested to be important in vulnerability to embolisms in plants (Choat *et al.*, 2008; Jansen *et al.*, 2009). Study of mangrove intervessel pits did, however, not reveal a clear link between pit (membrane) anatomy and the mangrove environment, unlike we found for mangrove vessels. While more narrow and more numerous vessels were found with increasing environmental stress at all levels (mangroves as a group, between species comparison and within species comparison), no clear trend in pit characteristics was reported. So far, we could only show a difference in the pit morphology of *Avicennia* and *Rhizophora* that supports their ecological distribution (Schmitz *et al.*, 2007a). A trend of more cavitation resistant pits with higher salinity or more demanding hydrostatic conditions within a tree could not be observed (Schmitz *et al.*, 2007a; Schmitz *et al.*, accepted). Is the ecological advantage of numerous narrow vessels with more stressful conditions unrelated to the intervessel pits? Further study should address the relation between the three-dimensional organisation of the vessel network and the vessel connections. In *Vitis vinifera* (Brodersen *et al.*, 2011) and *Fraxinus excelsior* (Burggraaf, 1972), it has been shown that vessel connections can be intermittent. Only by three-dimensional analysis (Brodersen *et al.*, 2011), it can be shown if the numerous narrow vessels in different mangrove species imply high or low vessel contact through intervessel pits. Also, a comparative study of the pit characteristics of mangroves and their non-mangrove sister taxa could be conducted in order to evaluate if there are minimum requirements in pit characteristics for trees to survive in the mangrove environment. It has already been shown that, despite the strong phylogenetic signal in intervessel pit characteristics, vestured pits (*i.e.* pits with extensions of the secondary wall in the pit chamber or the outer pit aperture - Wheeler *et al.*, 1989) and simple perforation plates are more present in plants growing in warm environments with periodical or continuous drought stress (Jansen *et al.*, 2004).

Vessel characteristics and related xylem hydraulics are important functional traits in the survival of trees in a certain environment (Westoby & Wright, 2006; Chave *et al.*, 2009). In this study we provide another line of evidence for the key role of vessel size and vessel density in the survival of trees under environmental conditions that are highly demanding for water transport. Evidently, it is the full set of ecologically significant plants traits that determines if species and individuals can succeed in a certain environment. However, despite alternative possible adaptations, evolution has equipped mangrove trees with decreased vessel number and vessel size, apparently crucial for thriving in their environment.

Avicennia in pole position

Vessel anatomy

Within the range of vessel characteristics found in diverse mangrove species, *Avicennia* show the most pronounced mangrove signature in our actual understanding, *i.e.* it has the most numerous and the narrowest vessels. This can be clearly observed in the seaward populations of *Avicennia*. Although soil water salinity is more constant close to the sea and soils are permanently wet, *Avicennia* trees growing in this zone of the mangrove forest have higher vessel density and narrower vessels than other mangrove species growing in the same environmental conditions. In addition, *Avicennia marina* trees have shorter vessel elements and higher vessel grouping when compared to *Rhizophora mucronata* trees. These two most widely occurring mangrove species have different ecological ranges which is reflected in their vessel characteristics. The features as observed in *Avicennia* are proper to numerous Acanthaceae and hence probably reflect the evolutionary and genetic background. Environmental pressure also leads to a gradient of values for these features within *Avicennia* trees with the highest vessel density and vessel grouping and the narrowest vessels occurring at high soil water salinity and low inundation frequency. Indeed, *Avicennia* is eurytopic in the mangrove forest ecotone, thriving in a wide range of inundation classes. Neither short vessel elements nor high vessel grouping are unequivocally linked to reduced cavitation vulnerability (Ellerby & Ennos, 1998; Schulte, 1999; Wheeler *et al.*, 2005; Hacke *et al.*, 2006; Choat *et al.*, 2008). However, short vessel elements can strengthen the reduced impact of cavitation coupled to numerous narrow vessels since they can also reduce the loss of conducting area as well as the spread of vessel embolisms throughout the vascular system (Baas *et al.*, 1983). Vessel grouping has been proposed to create alternative routes for the water to circumvent air-filled parts (Baas *et al.*, 1983; Zimmermann, 1983; Villagra & Roig-Juñent, 1997; Yáñez-Espinosa *et al.*, 2001; Cruiziat *et al.*, 2002; Kitin *et al.*, 2004; Lopez *et al.*, 2005), however further analysis must confirm whether high vessel grouping observed on stem cross-sections correlates to an increased number of effective vessel connections (Brodersen et al., 2011).

The pronounced safety strategy evolved in *Avicennia*'s vessel system, *i.e.* its protection against the effects of cavitation, contributes to the explanation of the wide distribution of *Avicennia* trees within the mangrove forest. The vessel characteristics of *Avicennia* trees allows for growth in extremely harsh conditions, with soil water salinity above 100 promille and long periods of soil drought and their adaptability also facilitates ecological success in a wide range of soil water salinity and drought intensity. Adaptation in *Avicennia* to the mangrove environment has evolved beyond what is known for other mangrove genera.

The safety signature of the intervessel pit characteristics of *Avicennia* can further explain the occurrence of *Avicennia* along the entire intertidal zone. Pits of *Avicennia* are vestured, unlike the intervessel pits of the other mangrove species. Vestured pits may be a survival strategy in continuous or periodical drought stress in warm (tropical) climates (Jansen *et al.*, 2003; Jansen *et al.*, 2004). Furthermore, *Avicennia* pit membranes are thick and pit apertures are minute (Schmitz *et al.*, 2007a). Both thick pit membranes and small pit apertures are associated to lower cavitation vulnerability (Hacke *et al.*, 2001; Ellmore *et al.*, 2006). The above results strongly indicate that in *Avicennia* wood characteristics reflect the evolution of a comprehensive set of adaptations for hydraulic safety. However, mangrove pit structure and function is not extensively studied so far, and between-species comparison, also over the different continents, should be further developed.

Successive cambia - presence of water

Being the only mangrove species with radial growth through successive cambia, *Avicennia* differs from the other mangroves in the composition of its internal conducting tissues. The xylem tissue of *Avicennia* trees is interspersed with internal phloem tissue. We found three lines of circumstantial evidence that support the ecological significance of internal phloem tissue and its advantage under conditions of water shortage. First, we noted that 84.9% of trees and shrubs with internal phloem tissue occur in saline or (periodically) dry habitats, *i.e.* habitats characterised by physiological drought. Second, the amount of internal phloem tissue inside *Avicennia* stems increases with increasing soil water salinity and soil drought. Third, magnetic resonance imaging (MRI) has shown the high water content of the internal phloem tissue throughout the entire stem in two woody species with successive cambia (the mangrove *Avicennia marina* and the non-mangrove *Bougainvillea spectabilis*).

Phloem and parenchyma have been suggested to play a major role in embolism repair, *i.e.* the refilling of embolized vessels with water (*e.g.* Hacke & Sperry, 2003; Salleo *et al.*, 2006; Salleo *et al.*, 2009; Brodersen *et al.*, 2010). The results of our study support the role of internal living tissue in the water household of a tree. Extensive internal phloem tissue seems to serve as water storage locations from which a tree can mostly benefit in conditions of water shortage. This indicates that a tree's water transport system is more than a collection of vertical pipes transporting water in a vertical direction only. Short distance forces such as the water exchange between xylem and phloem tissue are equally important in the overall functioning of the water transport system in trees, as has been suggested by for example Zimmermann *et al.* (2004), Brodersen *et al.* (2010) and Steppe *et al.* (2012).

Successive cambia - patchiness

In *Avicennia*, the connection and interaction of phloem and xylem tissue is even more important since both tissues are organized in a complex network of patches and thus strongly entangled. Xylem patches are at times almost entirely surrounded by phloem tissue. Moreover, the structure varies extensively with height, creating the possibility for a highly dynamic system of short-distance water exchanges between xylem and phloem tissue, both in space and time.

These results show the importance of a three-dimensional study of plant tissues and three-dimensional understanding, when plant functioning is concerned. Far too often, the third spatial dimension is not addressed and hence it is understudied in tree research (Kitin *et al.*, 2004; Brodersen et al., 2011). Especially in the case of woody species with cambial variants, this can lead to incomplete or wrong interpretations of data obtained. But also in other species, such as *Machilus thunbergii* and *Fraxinus lanuginosa*, vessels that were observed to be solitary at a certain height in the tree stem connected to other vessels some millimetres higher or lower in the tree, over the boundaries of growth rings (Fujii *et al.*, 2001; Kitin *et al.*, 2004). Furthermore, insight in intervessel connections that is important in the understanding of tree functioning and not accessible through two-dimensional analysis alone, was found through three-dimensional study of the stem anatomy of *Vitis vinifera (*Brodersen *et al., 2011).* The functioning of trees and the ecological significance of wood anatomical characteristics depend on the three-dimensional organisation of the wood tissues and cells. A complete understanding of water transport in trees and of tree ecology can only be obtained by integration of the three-dimensional variation into the hydraulic tissues of tree trunks. It is in this way that the study on the ecological significance of wood features must continue.

In depth studies of the changes of wood anatomical features with tree height and the ecological patterns herein, could be an important component in the ongoing testing of the mechanisms through which trees lift water. Westhoff *et al.* (2009) showed a height dependence of the amount of water in birch (*Betula pendula*) stems challenging the postulations of the cohesion-tension theory. Can this finding be linked to the wood anatomy of trees? Or more generally: do existing and newly obtained wood anatomy data support or contradict the newly proposed mechanisms for water transport in trees?

Strong evidence for patchiness in the swelling and shrinking and the radial increment of *Avicennia* tree stems has been obtained from a dendrometer study and pinning analysis. This introduces an exceptional interaction of *Avicennia* trees with their environment since they can react to it in a highly dynamic way, temporally as well as spatially. The results strengthen our hypothesis that ecological advantage is obtained through a patchwork of tissues that is, at any moment, adapted to the prevailing environmental conditions, at least at one part of the stem. It would be of great interest to see how water is running through this system of compartments and how the compartmentalisation in the hydraulic tissues is influencing the overall water transport. What are the triggers for growth and water uptake of tissues and how are they distributed over the stem? How does a tree divide its efforts over the tree trunk? A study over several years, with at least eight point dendrometers on minimum five different *Avicennia* trees could bring a first glimpse of deeper insight.

Difference in stem increment around the stem circumference of trees is well-known in the case of reaction wood formation (Schweingruber *et al.*, 2006). Also, the wedging of tree rings is a well-known phenomenon in tropical as well as in some temperate tree species (Worbes, 2002). These wedging sides can change around the tree circumference with time, indicating differences in local supply of water, carbohydrates, mineral elements and phytohormones around the stem circumference (Dünisch *et al.*, 2002; Worbes, 2002). Imagine that trees without successive cambia have a similar but less extensive interannual variation in swelling and shrinking patterns around the stem circumference. It would influence the current conclusions on tree growth as well as it would contain a treasure of information on tree functioning.

The vascular factor

Overviewing our results, we think it can be stated firmly that the 'vascular factor' is important in the species distribution within the mangrove forest as well as at a global scale. In *Avicennia* trees a wood anatomy evolve that favours their occurrence and thriving under extreme soil water salinity and long periods of soil drought, without excluding its presence under less harsh environmental conditions. Both the characteristics of *Avicennia*'s water transport system - the addition of highly grouped, numerous narrow vessels composed of short vessel elements, intervessel pits reducing proliferation of cavitation, internal phloem tissue and a complex vascular network that operates in a patchy way - and the flexibility in these characteristics, add to this distribution pattern. This contrasts to the vascular system of other mangrove genera that either do not show these characteristics known to be advantageous in lowering cavitation vulnerability or are less flexible in them, explaining their more stenotopic distribution within the mangrove forest and their smaller latitudinal extent.

Future research on mangrove wood anatomy can deepen the understanding in the ecological significance of the internal structures of trees. Through comparison of the strict requirements in the vascular system of mangrove trees to those of other phylogenetic diverse tree groups under extremely demanding environmental conditions, insight could be generated into the absolutely critical structural and functional features for trees to thrive in different biomes on Earth. In the light of climate change and the projected velocity of temperature change and climate resistance time of the biomes characterized by (physiological) drought or inundation (Loarie et al., 2009), this would be a crucial understanding on trees. Furthermore, a meta-analysis of the wood anatomical differences at root, stem and crown level of mangrove trees along latitudinal and intertidal gradients in the different continents could expose the total variation in mangrove wood anatomy, and generate insight into this variation with changing environmental conditions. It could furthermore test the coherence of these findings with newly proposed water transport theories. Combined with experiments that link sap flow under contrasting environmental conditions to wood anatomy, by techniques such as magnetic resonance imaging (sap flow) and micro-CT-scanning (anatomy), it could ultimately contribute to the still largely unsolved question: how does water flow through a tree under different environmental conditions?

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Zweifel R, Zimmermann L, Zeugin F & Newbery DM (2006) Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. *Journal of Experimental Botany* **57**: 1445-1459.

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2 - Oscar Wilde (1894) Phrases And Philosophies For The Use Of The Young. In: The Chameleon. Oxford student magazine (December 1894 - only issue), Oxford, United Kingdom.

3 - Hugh Tredennick & Cyril G. Armstrong (1935) Aristotle Metaphysics. Harvard University Press, Cambridge – Massachusetts, United States of America.

4 - Peter A. Bucky & Allen G. Weakland (1992) The Private Albert Einstein. Andrews McMeel, Kansas City, United States of America.

5 - Bernardus van Clairvaux (exact date unknown) Letter 106 to master Hendrik Murbach. In: André Louf (1991) De vrijheid bevrijd. Abdij der Trappisten, Westmalle, Belgium

Acknowledgements

I would like to thank my promotors, Nico Koedam, Hans Beeckman and Nele Schmitz for their continuous support and teaching during the years of my PhD research. Through their love for tree research, their input and their encouragement this research was enriching and inspiring for me. I thank James Gitundu Kairo, all my field work assistants, especially Hamisi Ali Kirauni and Abudhabi Hamisi Jambia, the people of the Kenya Marine and Fisheries Research Institute and all the inhabitats of Gazi for their help in conducting research in the mangrove forest. I am thankful for the joy they brought me during my field work missions.

I am grateful to all my colleagues from both the Laboratory of Plant Biology and Nature Management of the Vrije Universiteit Brussel and the Wood Biology Team of the Royal Museum for Central Africa in Tervuren. My special thanks goes to Mohamed Omar Said Mohamed, Tom van der Stocken and Ilse Boeren for the inspiring scientific discussions we had, to Ronny Merken for his permanent friendship and support and to Sandrine Godefroid for helping me in finding my way as a researcher. I thank my friends and colleague wood researchers, Flurin Babst, Achim Bräuning, Filipe Campelo, Paul Copini, Jožica Gričar, Polona Hafner, Julia Krepkowski, Ute Sass-Klaassen, Dario Martin Benito, Joana Vieira and Franziska Volland-Voigt for their enthousiasm and encouragement and their input to my research. Without the help of Maarten De Munter and Johan De Mey of the UZ Brussel, Elke Van den Casteele of SkyScan and Edo Gerkema, Frank Vergeldt and Henk Van As of the Wageningen NMR Centre this research would not have been possible. I owe my gratitude to them.

This thesis could be realized with the financial support of the Agency for Innovation by Science and Technology (IWT, Flanders, Belgium). I thank the IWT-Vlaanderen and all other instances that supported my work financially.

I am deeply grateful to my parents, Mia Van Mellaert and Jos Robert, my brothers Karel and Frans and my sister Hilde for their love, for their permanent support during the nice and the hard moments and for all they did to help me. I thank Leen Haagdorens, Claire De Decker and Nibedita Mukherjee, especially for their help in the last days before submitting. My gratitude also goes to Anne Marie Fournier and Walter Chaltin for giving me the encouragement and the support I needed.

And last but not least, I am thankful for the time I spent in nature. After all, it are the trees I owe most.

Curriculum Vitae

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Website: www.africamuseum.be/research

Academic achievements

Education

1) M. Sc. in Art (Ecole Nationale Supérieure des Arts Visuels de la Cambre, Brussels, Belgium - 1998-2003) - Graduated *cum laude*

2) M. Sc. in Biology (Vrije Universiteit Brussel, Brussels, Belgium - 2003-2007) - Graduated *magna cum laude*

> nomination for the Jacques Kets Biology Award of the Royal Zoological Society of Antwerp (Antwerp, Belgium) in 2007
> VLIZ Thesis Award for Marine Sciences of Flanders Marine Institute (VLIZ - Ostend, Belgium) in 2009

Professional experiences and scholarships

M. Sc.
Research at the Laboratory of Plant Biology and Nature Management (APNA, Vrije Universiteit Brussel, Brussels, Belgium): June 2006-Sept 2007

Ph. D
1) Transitional benefit from the Vrije Universiteit Brussel (Brussels, Belgium): 1 Oct 2007-31 Dec 2007
2) Grant of the Agency for Innovation by Science and Technology (IWT, Flanders, Belgium): 1 Jan 2008-31 December 2011
3) Grant for PhD accomplishment - Vrije Universiteit Brussel (Brussels, Belgium): 1 Jan 2012-30 June 2012

Fieldwork expeditions
Fieldwork missions to Kenya in 2007, 2009, 2010 and 2011
Funded by the Research Foundation - Flanders (FWO, Flanders, Belgium), The Royal Netherlands Academy of Arts and Sciences (KNAW, The Netherlands), the Flemish Interuniversity Council (VLIR, Flanders, Belgium), the King Leopold III Fund for Nature Exploration and Conservation (Belgium) and the "Stichting ter bevordering van wetenschappelijk onderzoek in Afrika" (SBWOA, Belgium).

Missions abroad

1) *Universität Erlangen-Nürnberg - Institut für Geographie*: January 2009. Financed by the Agency for Innovation by Science and Technology (IWT, Flanders, Belgium).

2) *Wageningen University - Wageningen NMR Centre / Forest Ecology and Forest Management Group*: November 2010. Financed by the European Cooperation in Science and Technology (COST – Action ES0805-TERRABITES) and the Society of Experimental Biology (SEB) - Company of Biologists (COB).

3) *Wageningen University - Wageningen NMR Centre / Forest Ecology and Forest Management Group*: April 2011. Financed by the Research Foundation - Flanders (FWO, Flanders, Belgium) and the Agency for Innovation by Science and Technology (IWT, Flanders, Belgium).

4) *University of Lisbon - Climate Change Impacts Adaptation and Mitigation Research Group*: August 2011. Financed by the Agency for Innovation by Science and Technology (IWT, Flanders, Belgium).

Research equipment

Micro-CT-scanner (SkyScan 1172 high-resolution-micro-CT) - Hercules Medium Sized Research Equipment - 350 000 euro

Publications

- Articles as first author in peer reviewed international journals: 6
- Articles as co-author in peer reviewed international journals: 5
- Oral presentations at international meetings: 9
- Oral presentations at national meetings: 1
- Posters presented at international meetings: 6
- Posters presented at national meetings: 5
- Reviews performed for international scientific journals: 4

Peer reviewed papers

1) Schmitz N., Robert E.M.R., Verheyden A., Kairo J.G., Beeckman H. & Koedam N. 2008. A patchy growth via successive and simultaneous cambia: key to success of the most widespread mangrove species *Avicennia marina*? Annals of Botany 101: 49 - 58. **IF5 (2010): 3.884**

2) Robert E.M.R., Koedam N., Beeckman H. & Schmitz N. 2009. A safe hydraulic architecture as wood anatomical explanation for the difference in distribution of the mangroves *Avicennia* and *Rhizophora*. Functional Ecology 23: 649-657. **IF 5 (2010): 4.742**

3) Robert E.M.R., Schmitz N., Kirauni H.A. & Koedam N. 2009. Salinity fluctuations in the

mangrove forest of Gazi Bay, Kenya: lessons to take for future research. Nature and Faune 24: 89-95.

4) Robert E.M.R., Schmitz N., Okello J.A., Boeren I., Beeckman H., Koedam N. 2011. Mangrove growth rings: fact or fiction? Trees - Structure and Function 25: 49-58. **IF 5 (2010): 1.900**

5) Robert E.M.R., Schmitz N., Boeren I., Driessens T., Herremans K., De Mey J., Van de Casteele E., Beeckman H., Koedam N. 2011. Successive cambia: a developmental oddity or an adaptive structure? PLoS One 6(1): e16558. **IF 5 (2010): 4.610**

6) Schmitz N., Koch G., Schmitt U., Robert E., Beeckman H. & Koedam N. 2011. Temporal changes of intervessel pit membrane thickness and lignin content: food for thought. IAWA Journal, accepted. **IF 5 (2010): 1.309**

7) De Ryck D., Robert E.M.R., Di Nitto D., Schmitz N., Kairo J.G., Dahdouh-Guebas F. & Koedam N. 2010. The chance of long-distance-dispersal for viviparous mangrove propagules: influence of size, density and predation. Aquatic Botany, accepted. **IF 5 (2010): 2.080**

8) Quisthoudt K., Schmitz N., Randin C.F., Dahdouh-Guebas F., Robert E.M.R., Koedam N. 2012 Temperature variation among mangrove latitudinal range limits worldwide. Trees - Structure and Function, under review.

9) Robert E.M.R., De Weerdt J., Huyghe F., Arquero Cabral C., Kairo J.G., Dahdouh-Guebas F., Beeckman H., Schmitz N. & Koedam N. 2012. Spare tubes for survival. Convergent evolution in the water transport system of mangroves. Submitted.

10) Oste J., Robert E.M.R., De Ryck D., Van der Stocken T., Koedam N., Schmitz N. 2012. Viviparous propagules of *Ceriops tagal* and *Rhizophora mucronata* show different survival strategies. In preparation.

11) Robert E.M.R., Jambia A.H., Schmitz N., De Ryck D., De Mey J., Kairo J.G., Dahdouh-Guebas F., Beeckman H. & Koedam N. 2012. How to catch the patch? A dendrometer study on the radial increment through successive cambia in the mangrove *Avicennia*. In preparation.

Oral presentations at scientific meetings

1) Robert E.M.R., Schmitz N., Beeckman H. & Koedam N. 2008. The safer, the better? A safe hydraulic architecture as wood anatomical explanation for the difference in distribution of the mangroves *Avicennia* and *Rhizophora*. TRACE 2008 - Tree Rings in Archaeology, Climatology and Ecology, 27 - 30 April, Zakopane (Poland).

2) Robert E.M.R. 2009. Hydraulische architectuur van *Avicennia marina* (Forssk.) Vierh. langsheen een ecologische gradiënt in Gazi Bay (Kenia). VLIZ Young Scientists' Day , 6 March, Bruges (Belgium).

> **VLIZ Thesis Award for Marine Sciences in 2009**

3) Robert E.M.R., Schmitz N. , Beeckman H. & Koedam N. 2009. Successive cambia in three dimensions: the mangrove *Avicennia* inside. TRACE 2009 - Tree Rings in Archaeology, Climatology and Ecology, 16 - 19 April, Otočec (Slovenia).

> ATR-Award: best presentation at the conference TRACE 2009

4) Robert E.M.R., Schmitz N., Driessens T., Herremans K., Beeckman H., Koedam N. 2010. Successive cambia: useful to survive in water stressed environments? TRACE 2010 - Tree Rings in Archaeology, Climatology and Ecology, 22 - 25 April, Freiburg (Germany).

5) De Weerdt J., Robert E.M.R. , Schmitz N. , Beeckman H. & Koedam N. 2010. Why do only mangroves survive in coastal environments? – A wood anatomical comparison with terrestrial relatives. TRACE 2010 - Tree Rings in Archaeology, Climatology and Ecology, 22 - 25 April, Freiburg (Germany).

6) Robert E.M.R., Schmitz N., Driessens T., Boeren I., Beeckman H., Koedam N. 2010. The ecological success of the mangrove *Avicennia*: the perfect combination of well-adapted wood anatomical characteristics and special radial growth? Worlddendro 2010 - 8th International Conference on Dendrochronology, 13 - 18 June, Rovaniemi (Finland).

7) Kitin P., Robert E.M.R., Ntamwira N., Van den Bulcke J., Takata K., Beeckman H., Lachenbruch B. 2011. Three-Dimensional Structure of Stems formed by Successive Cambia. Botany 2011, 9 – 13 July, St. Louis (USA).

8) Robert E.M.R., Schmitz N., Kairo J.G., Beeckman H., Koedam N. 2011. The ecological success of the mangrove *Avicennia*: the perfect combination of well-adapted wood anatomical characteristics and special radial growth? First Annual Mini-Symposium of the Plant Environmental Physiology Group, 20 December, London (UK).

9) Robert E.M.R., Schmitz N., Kairo J.G., Beeckman H., Koedam N. 2012. Exception among exceptions: the mangrove *Avicennia*. Annual Conference of the Society for Tropical Ecology – Islands in land- and seascape: the challenges of fragmentation, 22 – 25 February, Erlangen (Germany).

10) Robert E.M.R., Schmitz N., Kairo J.G., Beeckman H., Koedam N. 2012. The ecological success of the mangrove *Avicennia*: the perfect combination of well-adapted wood anatomical characteristics and special radial growth? MMM3 Conference (Meeting on Mangrove ecology, functioning and Management), 2 – 6 July, Galle (Sri Lanka).

Poster presentations at scientific meetings

1) Robert E.M.R., Schmitz N., Kairo J. G. , Beeckman H. & Koedam N. 2008. The safer, the better? A safe hydraulic architecture as wood anatomical explanation for the difference in distribution of the mangroves *Avicennia* and *Rhizophora*. Eurodendro 2008 The long history of wood utilization, 28 May - 1 June, Hallstatt (Austria).

2) Robert E.M.R., Schmitz N., Beeckman H. & Koedam N. 2008. The safer, the better? A safe hydraulic architecture as wood anatomical explanation for the difference in distribution of the mangroves *Avicennia* and *Rhizophora*. Wood matters: a celebration of the work

of John Barnett, 29 – 30 May, London (United Kingdom).

3) Robert E.M.R., Schmitz N., Beeckman H. & Koedam N. 2009. Successive cambia in three dimensions: the mangrove *Avicennia* inside. Starters in het Bosonderzoek, 19 March, Brussels (Belgium).

4) Robert E.M.R., Schmitz N., Beeckman H. & Koedam N. 2009. Successive cambia in three dimensions: the mangrove *Avicennia* inside. African Botany in Brussels, 27 March, Brussels (Belgium).

5) Robert E.M.R., Schmitz N., Beeckman H. & Koedam N. 2009. Successive cambia in three dimensions: the mangrove *Avicennia* inside. Impacts of Global Change on Tropical Ecosystems - Cross-cutting the Abiotic, Biotic and Human Spheres, 27-30 July, Marburg (Germany).

6) Driessens T., Robert E.M.R., Schmitz N. & Koedam N. 2009. Successive cambia in woody plants: an indicator of water stress? Impacts of Global Change on Tropical Ecosystems - Cross-cutting the Abiotic, Biotic and Human Spheres, 27-30 July, Marburg (Germany).

7) Robert E.M.R., Driessens T., Schmitz N. , Beeckman H. & Koedam N. 2009. Successive cambia as strategy in ecological stressful conditions: evidence from the Inside Wood Database and a three-dimensional study of *Avicennia*. Eurodendro 2009 - Developments, Advances, Challenges, 26-30 October, Cala Millor (Mallorca - Spain).

8) Robert E.M.R., Driessens T., Schmitz N. , Beeckman H. & Koedam N. 2009. Successive cambia as strategy in ecological stressful conditions: evidence from the Inside Wood Database and a three-dimensional study of *Avicennia*. VLIZ Young Marine Scientists' Day, 27 November, Ostend (Belgium).

9) Kodikara K.A.S., Robert E.M.R., Jayatissa L.P., Dahdouh-Guebas F., Beeckman H., Schmitz N. & Koedam N. 2011. Successive cambia in the mangrove *Avicennia*: a study on the three-dimensional structure of the cambia and the functioning of the internal phloem tissue. VLIZ Young Marine Scientists' Day, 25 February, Bruges (Belgium).

10) Oste J., Robert E.M.R., Schmitz N. & Koedam N. 2012. Growth and structural changes of viviparous mangrove propagules: the effect of environment on dispersal and establishment. VLIZ Young Marine Scientists' Day, 24 February, Bruges (Belgium).

11) Robert E.M.R., Schmitz N., Jambia A.H., De Ryck D., Kairo J.G., Dahdouh-Guebas F., Beeckman H. & Koedam N. Survival in the precarious conditions of the mangrove environment: the contribution of successive cambia to the ecological success of *Avicennia*. TRACE 2012 - Tree Rings in Archaeology, Climatology and Ecology, 9 - 12 May, Potsdam (Germany).

Reviews performed for scientific journals
Oecologia (1) / Trees - Structure and Function (1) / Environmental and Experimental Botany (1) / Plos One (1)

Other professional activities

Teaching

- Biogeography and Ecology, Vrije Universiteit Brussel, Prof. Dr. Nico Koedam - assistance for course documents and field expeditions to Andalusia (Spain) in 2008, to Catalonia (France and Spain) in 2009 and 2010, to Crete (Greece) in 2011 and to Tenerife (Spain) in 2012

- Functional Plant Science, Vrije Universiteit Brussel, Prof. Dr. Nico Koedam - assistance for practical work in 2007, 2008, 2009, 2010 and 2012

- Introduction to mangrove ecosystems, Kenya Marine and Fisheries Research Institute, Dr. James G. Kairo - assistance for mangrove introduction days in 2010 and 2011

Guidance of students

Master thesis

- Dennis De Ryck - *Moving and settling: experiments on the dispersal and establishment of hydrochorous propagules* - Academic year 2008/09 - Master student in Biology, Vrije Universiteit Brussel, Belgium

- Elisha M'Rabu Jenoh - *The impact of indirect effects of climate change on mangrove associated biodiversity* - Academic year 2008/09 - Master student in the international program Ecological Marine Management, Vrije Universiteit Brussel, Belgium

- Joëlle De Weerdt - *Is the wood hydraulic structure contributing to mangrove survival? A wood anatomical comparison with upland relatives* - Academic year 2009/10 - Master student in Biology, Vrije Universiteit Brussel, Belgium

- Charlene Cotillas - *What halts mangrove growing beyond the subtropics? A wood anatomical survey in the borders of Mauritania* - Academic year 2009/10 - Master student in the international program Ecological Marine Management, Vrije Universiteit Brussel, Belgium

- Jorien Oste - *Growth and structural changes of viviparous mangrove propagules: the effect of environment on dispersal and establishment* - Academic year 2010/11- Master student in Biology, Vrije Universiteit Brussel, Belgium

- Sunanda Kodikara Arachchi - *Successive cambia in the mangrove* Avicennia*: a study on the three-dimensional structure of the cambia and the functioning of the internal phloem* *tissue* - Academic year 2010/11- Master student in the international program Marine and Lacustrine Science and Management, Vrije Universiteit Brussel, Belgium

Bachelor assignment

- Ann Vanden Wyngaert - *Invloed van de kruidlaag in bossen op de soortenrijkdom van loopkevers* - Academic year 2008/09 - Bachelor student in Biology, Vrije Universiteit Brussel, Belgium

- Filip Huyghe - *Worth his salt? Comparative study of the wood anatomy of a greenhouse* Rhizophora *with that of mangroves grown under natural conditions* – Academic year 2009/10 - Bachelor student in Biology, Vrije Universiteit Brussel, Belgium

- Bob Ceuppens - *Zoet versus zout: fenotypische plasticiteit in mangrovespecies* Avicennia marina, Heritiera littoralis *en* Aegiceras corniculatum - Academic year 2010/11 - Bachelor student in Biology, Vrije Universiteit Brussel, Belgium

- Sarah Hublou - *Uitbreiding van het areaal van de mangrove* Xylocarpus moluccensis *naar de Afrikaanse oostkust* – Academic year 2010/11 - Bachelor student in Biology, Vrije Universiteit Brussel, Belgium

- Kristoffel Jacobs - *Effects of temperature and water availability on the wood anatomy of* Avicennia marina – Academic year 2010/11 - Bachelor student in Biology, Vrije Universiteit Brussel, Belgium

Internship

- Tess Driessens - *Successive cambia in woody plants: an indicator of water stress?* – Academic year 2008/09 – Bachelor student in Biology, Vrije Universiteit Brussel, Belgium

- César Arquero Cabral - *Is the wood hydraulic structure contributing to mangrove survival? A wood anatomical comparison between the mangrove* Xylocarpus *and its most related relatives* - Academic year 2009/10 – Erasmus student at the Université libre de Bruxelles, Belgium

- Lisa Rijsenbrij - *De mangrove* Xylocarpus *aan de Afrikaanse oostkust: één of twee soorten?* – Academic year 2010/11 – Student "Algemeen Secundair Onderwijs" in Purmerend, the Netherlands

- Filip Huyghe - *On the origin of mangroves. Which adaptations of the wood hydraulic structure enabled mangroves to survive in tropical intertidal zones?* – Academic year 2010/11 – Bachelor student in Biology, Vrije Universiteit Brussel, Belgium

- Hilde Robert - *Houtanatomie en dendrochronologie: een introductie aan de hand van twee case studies* - Academic year 2010/11 – Master student in Art Science and Archaeology, Vrije Universiteit Brussel, Belgium

Memberships

- International Association of Wood Anatomists (IAWA) – since 2008
- Association of Tree-Ring Research (ATR) - since 2008
- Association for Tropical Biology & Conservation (ATBC) - 2010
- The Society for Experimental Biology (SEB) – since 2010
- Society for Tropical Ecology (Gesellschaft für Tropenökologie - gtö) – 2012

Popularizing initiatives

1) Knock on Wood - Forest and Wood in Africa. Exposition at the Royal Museum of Central Africa in Tervuren (Belgium) in 2007-2008.

2) Wetenschapsfeest. Exhibition about Science for Secondary School Students in Mechelen (Belgium) in 2008.

3) Wetenschapskaravaan. Presentation together with Nele Schmitz and Nico Koedam in the Royal Museum for Central Africa, Tervuren, in 2008.

4) Quadra Medicinale. Contribution to the Art Project of Jef Geys for the Biennale of Venice in 2009.

5) Annual meeting of the "Forestry Network" (organized by the Agronomy Dept of Condorcet High School) - presentation about *Avicennia* in the Royal Museum for Central Africa, Tervuren, 26 May 2010.

6) Contribution to the mangrove section in the Victoria Greenhouse of the National Botanical Garden of Belgium in Meise, in 2010.

7) Nacht van de Onderzoeker: "Word jij de nieuwe Darwin?" Interactive Happening on Science for a General Public in the BoZar in Brussels, 24 September 2010.

8) Contribution to the mangrove greenhouse of Burger's Zoo in Arnhem, the Netherlands, in 2011.

Other relevant information

Knowledge of languages

- Dutch: mother tongue
- French: very good
- English: very good
- Spanish: basic
- German: passive
- Portuguese: passive
- Kiswahili: notion

mangrove sister taxa
sucessive cambia wood anatomy
mangrove micro-CT-scanning
dendrometers tree growth
Rhizophora salt stress
water transport ecophysiology
physiological drought Kenya *Avicennia*
internal phloem tissue inundation stress
water-conducting vessels
magnetic resonance imaging

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