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CANOPY WATER UPTAKE:
AN IMPORTANT SURVIVAL MECHANISM OF MANGROVES

Promoter: Prof. dr. ir. Kathy Steppe

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Shihab Uddin
Ghent, August 2014
SUMMARY

Mangroves are unique ecosystems dominating the coastlines and estuaries of both tropical and subtropical areas all over the world. They supply an indispensable habitat for a variety of flora and fauna. They prevent erosion and protect coral reefs and sea grass from sedimentation. Humans are also benefitted from mangrove forests by timber, charcoal and food. Their presence has a positive effect on fishery and protects the coastlines from storms, hurricanes and tsunamis. Mangroves are growing in intertidal zones, which imply frequent inundation with saline sea water and high salt concentrations in the soil. They have to struggle for thriving under such extreme environments of higher salinity and limited fresh water availability. Mangroves are therefore characterized by adaptation to these harsh environments. Aerial roots, specialized wood structures, viviparous germination, salt glands and exclusion of salts at the root level are some notable adaptive features of mangroves. The ecological importance of mangroves combined with the fact that they are designed to survive in exceptional environmental conditions makes them an interesting object for plant water relations study. Foliar absorption of water by mangroves also keep forwarded as an additional mechanism to survive under such saline conditions. Some recent studies reported growth peaks in widely distributed mangrove species Avicennia marina (Forsk.) Vierh during rainfall. This aforementioned finding led to the hypothesis of foliar uptake of rain water by A. marina. In order to prove this hypothesis an experiment was conducted under controlled environment in the greenhouse facilities of the Laboratory of Plant Ecology, Faculty of Bioscience Engineering, Ghent University, Belgium, starting from November 2013 to the end of April 2014. Several ecophysiological measurements such as sap flow dynamics (SFD) and stem diameter variations (SDV) were conducted on the seedling of A. marina for two selected study periods of three consecutive days. At the same time meteorological parameters such as photosynthetically active radiation (PAR) and vapour pressure deficit (VPD) of the greenhouse were also recorded. All the findings of A. marina were compared with the temperate species Populus tremula L. to distinguish their response during rainfall.

The results of ecophysiological measurements were found to be highly correlated with the environmental variables. Linking these measurements with artificial rain simulation resulted in an intriguing dataset. Starting with the SDV, apart from the daily pattern of shrinking and swelling, which was linked with the ecophysiological measurements induced by environmental conditions like PAR and VPD, an irregular growth pattern was found in A. marina. This daily pattern was alternated by steep peaks in SDV (much higher than nighttime values), which was found in perfect agreement with the onset of artificial rain simulation. The SFD of A. marina also showed a similar trend but completely opposite to SDV. Also reversed SFD were observed corresponding to the steep peaks in SDV during the rain simulation time
resulting in hydraulic redistribution. Since rain water was prevented from reaching the rhizosphere there was no possibility of altering soil water potential as well as faster water uptake by roots. Foliar uptake of rain water is the most plausible mechanism resulting in the observed diameter increases and reversed SFD.

Further verification of the proposed hypothesis spraying with dye solution also confirmed the foliar uptake of rain water by *A. marina*. Presence of dye in the transverse sections of petiole indicated the transport of water from the leaf to the stem. Stable isotopes analysis also confirmed the occurrence of foliar uptake of rain water by *A. marina*. Significant proportion of deuterium ($\delta^2$H) was present in both petioles and stem of *A. marina*. Since only leaves were submerged under enriched water, the presence of $\delta^2$H in the petioles and finally in the stem surely proved that, water absorbed by the leaves were transported towards the stem via petioles. In all treatments *P. tremula* showed negative response towards foliar uptake of rain water. From the above findings it can be summarized that foliar uptake of rain water was occurred in *A. marina* but not in *P. tremula*.

Rain water has an important role in suppressing transpirational water loss from leaves by dropping VPD, thereby ameliorating the overall plant-water balance. Further foliar absorption of intercepted rain water is an important water acquisition mechanism that can mitigate the deleterious effects of plant available soil water deficits for *A. marina* and other mangrove species. Both dew and rain are the two most frequent forms of available fresh water for the mangrove vegetation and playing important role for their thriving under such harsh environment.
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<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
<th>Unit</th>
</tr>
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<tbody>
<tr>
<td>$C_{\text{Leaf}}$</td>
<td>Leaf specific capacitance</td>
<td>$[\text{m}^3 \text{ m}^{-2} \text{s}^{-1} \text{ MPa}^{-1}]$</td>
</tr>
<tr>
<td>CRDS</td>
<td>Cavity ring down spectrometry</td>
<td>[-]</td>
</tr>
<tr>
<td>$C_{\text{Root}}$</td>
<td>Root specific capacitance</td>
<td>$[\text{m}^3 \text{ m}^{-2} \text{s}^{-1} \text{ MPa}^{-1}]$</td>
</tr>
<tr>
<td>$C_{\text{Stem}}$</td>
<td>Stem specific capacitance</td>
<td>$[\text{m}^3 \text{ m}^{-2} \text{s}^{-1} \text{ MPa}^{-1}]$</td>
</tr>
<tr>
<td>DOY</td>
<td>Days of the year</td>
<td>[-]</td>
</tr>
<tr>
<td>$E_N$</td>
<td>Nighttime transpiration</td>
<td>$[\text{m}^3 \text{ m}^{-2} \text{s}^{-1}]$</td>
</tr>
<tr>
<td>FWU</td>
<td>Foliar water uptake</td>
<td>[-]</td>
</tr>
<tr>
<td>g</td>
<td>Gravitational acceleration</td>
<td>$[\text{m s}^{-2}]$</td>
</tr>
<tr>
<td>h</td>
<td>Vertical height</td>
<td>[m]</td>
</tr>
<tr>
<td>HAS</td>
<td>Hydraulic activation of stomata</td>
<td>[-]</td>
</tr>
<tr>
<td>HFD</td>
<td>Heat field deformation method</td>
<td>[-]</td>
</tr>
<tr>
<td>HRM</td>
<td>Heat ratio method</td>
<td>[-]</td>
</tr>
<tr>
<td>i.e.</td>
<td>That is</td>
<td>[-]</td>
</tr>
<tr>
<td>$k_{\text{Atm-Leaf}}$</td>
<td>Hydraulic conductance of foliar uptake</td>
<td>$[\text{m s}^{-1} \text{ MPa}^{-1}]$</td>
</tr>
<tr>
<td>$k_{\text{Soil-Root}}$</td>
<td>Soil-to-root hydraulic conductance</td>
<td>$[\text{m s}^{-1} \text{ MPa}^{-1}]$</td>
</tr>
<tr>
<td>LVDT</td>
<td>Linear variables displacement transducers</td>
<td>[-]</td>
</tr>
<tr>
<td>LWE</td>
<td>Leaf wetting event</td>
<td>[-]</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetically active radiation</td>
<td>$[\mu \text{mol m}^{-2} \text{s}^{-1}]$</td>
</tr>
<tr>
<td>ppt</td>
<td>Parts per thousand</td>
<td>[-]</td>
</tr>
<tr>
<td>r</td>
<td>Radius</td>
<td>[m]</td>
</tr>
<tr>
<td>R</td>
<td>Ratio of the heavy to light isotope</td>
<td>[-]</td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
<td>[%]</td>
</tr>
<tr>
<td>SDV</td>
<td>Stem diameter variations</td>
<td>[$\mu \text{m}$]</td>
</tr>
<tr>
<td>SFD</td>
<td>Sap flow dynamics</td>
<td>[-]</td>
</tr>
<tr>
<td>SLAP</td>
<td>Standard Light Antarctic Precipitation</td>
<td>[-]</td>
</tr>
<tr>
<td>SPAC</td>
<td>Soil plant atmosphere continuum</td>
<td>[-]</td>
</tr>
<tr>
<td>TDS</td>
<td>Total dissolved solids</td>
<td>[g L$^{-1}$]</td>
</tr>
<tr>
<td>TMCF</td>
<td>Tropical montane cloud forests</td>
<td>[-]</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Unit</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>TS</td>
<td>Transverse sections</td>
<td>[-]</td>
</tr>
<tr>
<td>VPD</td>
<td>Vapour pressure deficit</td>
<td>[k Pa]</td>
</tr>
<tr>
<td>V-SMOW</td>
<td>Vienna Standard Mean Ocean Water</td>
<td>[-]</td>
</tr>
<tr>
<td>$\Delta \Psi_{\text{Atm-Leaf}}$</td>
<td>Water potential gradient between intercepted crown water and leaf</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\Delta \Psi_{\text{Soil-Root}}$</td>
<td>Water potential difference from soil to root</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\mu_w$</td>
<td>Chemical potential</td>
<td>[J mol$^{-1}$]</td>
</tr>
<tr>
<td>$\rho_w$</td>
<td>Density of water</td>
<td>[kg m$^{-3}$]</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Surface tension at the air-liquid interface</td>
<td>[N m$^{-1}$]</td>
</tr>
<tr>
<td>$\Psi$</td>
<td>Water potential</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\Psi_{\text{atm}}$</td>
<td>Atmospheric water potential</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\Psi_g$</td>
<td>Gravitational potential</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\Psi_{\text{leaf}}$</td>
<td>Leaf water potential</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\Psi_p$</td>
<td>Pressure potential</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\Psi_{\text{rain}}$</td>
<td>Rain water potential</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\Psi_{\text{root}}$</td>
<td>Root water potential</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\Psi_{\text{soil}}$</td>
<td>Soil water potential</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\Psi_{\text{stem}}$</td>
<td>Stem water potential</td>
<td>[MPa]</td>
</tr>
<tr>
<td>$\Psi_{\pi}$</td>
<td>Osmotic potential</td>
<td>[MPa]</td>
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</table>
 CHAPTER 1: INTRODUCTION

Mangrove forests are important vegetation, which generally grow in the intertidal coastal zone of tropical and subtropical regions. These forests are regularly inundated by saline sea water with high total dissolved solids (TDS) and need to cope with relatively humid conditions and high temperatures for their survival, growth and reproduction (Robert et al., 2009). The halophytic nature of mangrove vegetations keep them alive under such pervasive environmental conditions (Krauss & Ball, 2013). Presence of aerial root, viviparous germination, salt secretion and salt excretion are the typical characteristics of mangrove trees that explain their persistence under saline conditions (Shi et al., 2005; Robert et al., 2009). These aerial root systems enable oxygen diffusion which is 10,000 times more efficient than diffusion of oxygen in water when exposed to the atmosphere during the low tidal inundation periods (Ball, 1988a). Among various mangrove species, *Avicennia marina* (Forssk.) Vierh. is found to be most dominating characteristic genus throughout the mangrove ecosystems (Robert et al., 2009). In *A. marina* About 70% of the overall root volume is composed of aerenchyma, which helps to form a highly competent manner of root aeration (Curran, 1985).

The most important limiting factor for trees growing under saline condition is the threat for cavitation-entrance of air bubbles in the xylem sap (Cochard, 2006), which ultimately block the transport of water within the xylem system. Since cavaition highly affects the functionality of hydraulic systems of plant even under mesophilic conditions (Zimmermann & Brown, 1971), so the survival of mangroves under such saline environment is more fascinating (Robert et al., 2009). As a result, there is a trade-off between water conducting capacity and protection of transport against cavitation (Mauseth & Stevenson, 2004; Hacke et al., 2006). Higher vessel density and grouping, smaller vessel member and shorter vessels are the adaptive mechanisms to escape the decline in hydraulic conductivity following cavitation and subsequent filling of vessels by air (Baas et al., 1983).

Although the mangroves are growing in saline conditions, which need to struggle for water uptake from soil through root hair due to physiological drought conditions, but *Avicennia* species are well-known to be grown successfully along a salinity gradient. They can also tolerate higher evaporation which was characterized by higher salinity in the pore-water (Lambs et al., 2008). *Avicennia* trees can astonishingly develop both on the seashore and a number of kilometers inland, thus leading to queries on the source of the water used by these trees (Lambs et al., 2007). Frequent tidal inundation with saline sea water increases salt concentration in soil solution, which results in a lower soil water potential (Sobrado, 2002).
Under such conditions, the adjustment of osmotic potential helps them to take up water from the soil (Suàrez et al., 1998). Alternatively, salt secretion by leaf glands of *A. marina*, as well as other mangrove trees, helps to avoid the extreme ion accumulation in the leaf tissues (Ball, 1988a; Waisel et al., 1986).

Water relations of mangroves are of particular interest since it is not possible for trees to survive and grow without water but they are growing under condition of continuous physiological drought. Rainfall and dew are the most important forms of fresh water in mangrove ecosystems. A significant proportion (about 17%) of the annual rainfall is intercepted by the plant canopy (Holwerda et al., 2010). Absorption of this intercepted rainwater through the plant canopy can meet up the water demand of the plants to some extent. Eller et al. (2013) described water movement from the atmosphere, into the plant and towards the soil and demonstrated that foliar water uptake (FWU) strongly correlated with wetness of the leaf surface, which occurred as a result of cloud immersion in a tropical montane cloud forest during the dry season. Further research revealed that the possible entry of water occurs through the hydrophilic cuticle, and the organ that facilitates the storage of water is the hydrophilic tissues of the leaf surface (Eller et al., 2013). Different possible entry pathways have been proposed such as hydathodes, cuticle, trichomes (Limm et al., 2009) and stomata (Burkhardt et al., 2012).

The transport of water from leaves to the stem necessitates the presence of a driving force, resulting in a higher water potential in the leaves compared to the stem (Goldsmith, 2013). FWU increases leaf water potential which causes flow of water away from the leaves and moves towards the stem. This reverse water flow has been noticed and an increase in water potential gradient observed in the stem due to rehydradation by foliar uptake of water (Simonin et al., 2009; Goldsmith et al., 2013). FWU in the form of fog has been reported by Eller et al. (2013) and demonstrated occurrence of frequent fog events in cloud forest tree species. Deposition of fog on the leaves causes water droplets to enter the vascular system and moves to the soil which is considered as one of the most important hydrological input in tropical montane cloud forests (TMCF) under terrestrial ecosystems (Bruijnzeel et al., 2011).

Mangroves thrive in harsh conditions dominated by frequent flooding and nutrient poor soils. Several research efforts have been directed towards foliar uptake of fog water but no information is available yet on rainwater absorption by the foliage of mangrove trees. Until now the phenomenon of rainwater absorption by the leaves has been little investigated. A hypothesis regarding FWU has been put forwarded as the means of an extra mechanism for meeting up the water demand by *A. marina*. Therefore this thesis will further extend the research work towards leaf water uptake of this mangrove species. In this study, the impact of rain water uptake by *A. marina* will be examined since this is one of the most popular forms of fresh water in mangrove environments.
This thesis commence with a LITERATURE REVIEW, giving a generalized background regarding water in the soil plant atmosphere continuum and FWU mechanisms along with a brief description of mangrove ecosystems with special emphasis on *A. marina*. In the following chapter, MATERIALS AND METHODS, a detailed description of the experimental set up and equipment used are illustrated. Data obtained during the experiments are subsequently presented in chapter, RESULTS along with some preliminary connections between the different results. The DISCUSSION chapter interprets the results along with several links with the previous observations. This chapter forwarded to some recommendations for future research potentials. Finally, the CONCLUSIONS chapter summarizes the important findings of my proposed research.
CHAPTER 2: LITERATURE REVIEW

Mangrove forests consist of evergreen salt tolerant tree species found in the tropical and subtropical coastlines which provide an important habitat for a wide range of ecosystem services. Foliar water uptake (FWU) under such a harsh environment may contribute to the survival and growth of mangrove. Many researchers investigated various aspects of plant water relations under different ecosystems. Some related research findings focusing on FWU and mangrove ecosystems have been reviewed in this chapter.

2.1 Water in the soil plant atmosphere continuum

2.1.1 Role of water in plant functioning

Water is the most abundant constituent of living plant cells. Herbaceous plants may contain around 95% of water, whereas woody plants have lower water content. Water plays a crucial role in plant physiological processes. It also acts as most important medium for transporting metabolites through the cell. The major portion (95% or more) of the water that plants absorb from the soil by roots is not retained inside the tree, but evaporates through the stomatal pores; a process known as transpiration (Ridge, 2002). This inefficient use of water by plants is an unavoidable consequence of photosynthesis, the process of converting solar energy into sugars, the basic building materials of plants. On the other hand this flow of water is of crucial importance because of transporting essential mineral nutrients from the soil to the leaves. Transpiration also acts as a protective mechanism against high solar radiation by cooling down the leaves. Moreover, water helps the cell constituents to exert positive pressure on their walls (referred to as turgor pressure) that result in plant growth and structural support. Cell expansion and photosynthesis are retarded when plants lose this turgor pressure (Lambers et al., 2008). In order to maintain these aforementioned physiological processes plants continuously uptake water from the soil.

2.1.2 Water transport in plants

Plants continuously absorb and lose water. This absorption usually occurs from the soil through the roots due to a pressure gradient, which induced by the loss of water from the leaves through transpiration. The general pathway of water movement throughout the plant is illustrated in Fig. 2.1.
Figure 2.1: A schematic representation of water transport through the SPAC. Removed from the leaves by transpiration, water is sucked up from the roots, following the gradient in water potential, created according to the cohesion-tension theory (adapted from Campell & Reece, 2008).

Soil water entering into the roots through fine root hairs moves radially via the root cortex to the central cylinder (or stele) which consists of the vascular system. Water is conducted by the non-living, heavily thickened and lignified xylem vessels. They act as a network of tubes connected one to another, through which water can transport axially and radially from the roots up to the small veins in the leaves. Xylem components have numerous simple pits in their wall through which lateral transport of water between adjoining cells is possible. Water is released from the xylem at the end of the veins and moves towards the mesophyll cells and evaporates from the surface of the cell walls into the substomatal cavity.
It then diffuses as water vapour through the stomatal opening, which open due to rising of sun (stomatal transpiration). This process allows the entrance of CO$_2$ in the substomatal cavities, which is the raw material of photosynthesis. Here water potential gradient acts as the main driving force for the upward movement of water against the pull of gravity. The water potential of the atmosphere is highly negative, hence water vapour from the substomatal cavities and intercellular air spaces will be drawn away through the opened stomata.

2.1.3 Water potential as driving force

The driving force, which governs the water movement between two locations in a system, is originated from the difference in their water potential ($\Psi$) i.e. water potential gradients. Water flows spontaneously from a region of higher $\Psi$ (less negative) to one of a lower $\Psi$ (more negative) (Steppe, 2004; Taiz & Zeiger, 2006). Considering all the different forces acting upon water molecules, the total $\Psi$ can be written as (Nobel, 2009):

$$\Psi = \Psi_p + \Psi_\pi + \Psi_g$$  \hspace{1cm} (2.1)

where, $\Psi_p (= P)$ is called the hydrostatic potential or pressure potential (MPa), $\Psi_\pi (= \Pi)$ is the osmotic potential (MPa) and $\Psi_g (= \rho_w g h)$ is the gravitational potential (MPa). This value of water potential is expressed in comparison to a reference value of pure, free water at atmospheric pressure and at a temperature of 298 K by definition set equal to zero (Lambers et al., 2008).

Uptake of water from the soil by plant roots occurs when there is a driving force, develop by the transpiration loss of water due to the greater difference in $\Psi$ between the leaves and the surrounding dry atmosphere. This difference creates a $\Psi$ gradient, which force water to move most likely toward areas with relatively less water. The drier the air surrounding plant, the greater the $\Psi$ gradient i.e. the driving force for moving water through the plant, hence the faster the rate of transpiration. As a result, unidirectional water movement occurs from the soil, towards the plant system and finally out into the atmosphere (Goldsmith, 2013) which is called the soil plant atmosphere continuum (SPAC). This upward movement of water can be described by the unidirectional SPAC mass balance model:

$$k_{\text{Soil-Root}} \Delta \Psi_{\text{Soil-Root}} = C_{\text{Root}} \left( \frac{d\Psi_{\text{Root}}}{dt} \right) + C_{\text{Stem}} \left( \frac{d\Psi_{\text{Stem}}}{dt} \right) + C_{\text{Leaf}} \left( \frac{d\Psi_{\text{Leaf}}}{dt} \right) + E_N$$  \hspace{1cm} (2.2)
where, $\Delta \Psi_{\text{Soil-Root}}$ is the $\Psi$ difference between soil and root; $C_{\text{Root}}$, $C_{\text{Stem}}$ and $C_{\text{Leaf}}$ are root, stem and leaf specific capacitances defined as the change in tissue water content due to per unit change in $\Psi$; $\Psi_{\text{Root}}$, $\Psi_{\text{Stem}}$ and $\Psi_{\text{Leaf}}$ are root, stem and leaf water potential, respectively; and $E_N$ is nighttime transpiration (Dawson et al., 2007).

This SPAC framework presumes a mass balance between uptake and loss of water by plant root and leaf, respectively. Several researches suggest that atmospheric water condensing on aboveground plant parts can be utilized via direct uptake by the foliage (Rundel, 1982; Yates & Hutley, 1995; Burgess & Dawson, 2004; Breshears et al., 2008; Goldsmith et al., 2013). This form of leaf and crown water uptake can increase $\Psi_{\text{Leaf}}$ hence contribute to the transport of water from leaf to root, thus in the opposite direction than that normally considered by the SPAC model, and consequently, $\Psi_{\text{Leaf}}$ and water content of leaf are no longer controlled by $\Psi_{\text{Soil}}$ and root water uptake. Therefore, Simonin et al. (2009) proposed a bidirectional SPAC mass balance framework to evaluate the contribution of crown interception and subsequent foliar uptake to plant water status:

$$k_{\text{Atm-Leaf}} \Delta \Psi_{\text{Atm-Leaf}} = C_{\text{Leaf}} \frac{d\Psi_{\text{Leaf}}}{dt} + C_{\text{Stem}} \frac{d\Psi_{\text{Stem}}}{dt} + C_{\text{Root}} \frac{d\Psi_{\text{Root}}}{dt} - k_{\text{Root-Soil}} \Delta \Psi_{\text{Root-Soil}}$$ \hspace{1cm} (2.3)

where, $k_{\text{Atm-Leaf}}$ is the efficiency of foliar uptake and $\Delta \Psi_{\text{Atm-Leaf}}$ is the water $\Psi$ gradient between the intercepted crown water and the leaf.

### 2.1.4 The cohesion-tension theory

Albeit the concept of water potentials explicate the transport of water from a point of higher (less negative) to a lower (more negative) water potential, it does not indicate how these water potentials are created within the tree. In order to explain this, the cohesion-tension theory was proposed at the end of the nineteenth century by Dixon and Joly (1894). With the introduction of the Ohm's law analogue of sap flow some facets of this theory were set on a quantitative basis by van den Honert (1948) in the SPAC. According to the cohesion-tension theory, water ascends through the xylem of the stem in a metastable state under tension, i.e. with a pressure potential more negative than that of the vapour pressure of water (Tyree, 1997). This driving force is generated by the transpiration of water from the leaves resulting in tension in the xylary sap. The cohesive forces between water molecules caused by the hydrogen bonds forms a continuous 'string threads' inside the apoplast of trees with the cell wall continuum of a plant containing the dead conductive xylem cells. When water is attracted at any one of the string ends because of a lower water potential, the water molecules will resist to be pulled apart and a tension will be promulgated within the water string (Vandegehuchte, 2013).
The cohesive forces of water molecules along with the adhesive forces between water molecules and the walls of xylem elements, suck 'string threads' upward in the lumen of xylem to the regions of lower (more negative) pressure potential (Steppe, 2004). The process of transpiration is ultimately driven by the solar energy, which is needed to overcome the latent heat of evaporation of water and for breaking down the hydrogen bonds at the air-water interfaces (Tyree, 1997). Evaporation occurs predominantly from the mesophyll cell walls of the substomatal chambers under the influence of much lower water potential of the water vapour in the air. A meshwork of small interstices exists within the leaves, which are build up by the cellulose microfibrils in the cell walls. In the air water interface these interstices act as a fine capillary network. The strong adhesive forces of water molecules with these cell walls result in many curvatures or menisci. Surface tension forces lower the pressure potential ($\Psi_p$) of the liquid directly behind the menisci, which ultimately lower the $\Psi$ of the adjacent region including the cell walls and cell protoplasts of the surroundings. The pressure potential at the air water interfaces is negative and given by (Nobel, 1999)

$$\Psi_p = -\frac{2\sigma}{r} \quad (2.4)$$

where, $\sigma$ is the surface tension at the air-liquid interface (0.0728 N m$^{-1}$ at 20°C) and $r$ is the radius (m) of the meniscus, which is by convention positive for a concave surface. The curvature determines the negative pressure of the xylary sap, directly behind the menisci. Loss of water during transpiration has the tendency to decrease the radii of the menisci, and thus causing the pressure potential to be more negative (see. Eq. 2.4). This negative pressure potential ultimately lowers the total $\Psi$ of the xylary sap (see. Eq. 2.1). This negative pressure creates a tension and pulls the water string upward. Under this strong negative pressure, the water column in the xylem will be in a highly metastable state. Air bubbles act as ‘catalyzing stimuli’ to change this stage within the system (Wei et al., 1999). In conditions of sufficient water availability the intercohesion of water molecules and the adhesion of water molecules to the xylem cell walls maintain this metastable state. In fact the entrance of air bubble into the xylem vessels is prevented by the tiny diameters of the pits in the cell walls (Pockman et al., 1995; Sperry, 1995) and, consequently, by the strong capillary forces produced by surface tension within these pits (see Eq. 2.4). However under stress condition when the tension of the xylary sap become very negative even lower than the critical level, air bubbles will enter into the xylem element through the largest pores in the cell walls of the transport vessels. These air bubbles interrupt the continuity of the water in the in the xylem element and cease its function, i.e. it cavitates (Cruiziat & Tyree, 1990).
2.1.5 Hydraulic redistribution

Among the dynamic processes of plant water uptake and releases, hydraulic redistribution plays an important role in plant water relations. The phenomenon of relocating xylary sap within the xylem is known as hydraulic redistribution (Nadezhdina et al., 2010), which is a passive movement of water, regulated by challenging soil and plant $\Psi$ gradients and resistance of corresponding pathways (Richards & Caldwell, 1987; Burgess et al., 1998; Nadezhdina et al., 2009). This passive flow of water within the different soil horizons via plant roots have been studied and demonstrated by many researchers (Richards & Caldwell, 1987; Caldwell & Richards, 1989; Dawson, 1993; Burgess et al., 1998; Smith et al., 1999; Scholz et al., 2002; Meinzer et al., 2004; Oliveira et al., 2005; Brooks et al., 2006; Howard et al., 2009; Domec et al., 2010; Prieto et al., 2011). This hydraulic redistribution can be distinguished in different types depending on the direction of water flow into the soil (Fig. 2.2). The upward passive movement of water in the soil via plant roots from deeper wet layers to upper dry layers is referred as hydraulic lift (Fig. 2.2a) (Caldwell & Richards, 1989; Caldwell et al., 1998; Horton & Hart, 1998). The reverse downward movement of water from shallow to deeper layer is called ‘downward hydraulic redistribution’ (Fig. 2.2c) (Schulze et al., 1998; Smith et al., 1999). Besides to these vertical flows, water may also be transported horizontally (lateral redistribution, Fig. 2.2b) (Brooks et al., 2002; Sternberg et al., 2002, 2004; Smart et al., 2005). This process considerably improves plant water and nutrient status (McCulley et al., 2004), also provides benefits to other surrounding plants (Caldwell & Richards, 1989; Dawson, 1993), as well as soil biota (Duncan & Elmorshedy, 1996). This improvement of soil water status in an area prevents the dropping of soil water potential from the critical threshold that would cause root hydraulic failure due to cavitation (Domec et al., 2004; Warren et al., 2007; Siqueira et al., 2009).

Moreover this process accounts for more than 20% of soil water that is taken up by plant during transpiration, which can affects the land-surface climatology (Brooks et al., 2002; Lee et al., 2005). In broad scales, hydraulic redistribution can play crucial role in ecosystem water, and nutrient cycling (Jackson et al., 2000), and can also effects the energy balance (Lee et al., 2005). The importance of hydraulic redistribution is increasing globally with the abundance of deep-rooted woody plants through afforestation and other processes (Van Auken, 2000; Engel et al., 2005; Jackson et al., 2009).
Plants having large, extensive woody root systems are most effective redistributors of soil water. Because these large and deep root systems are capable of connecting the multiple soil compartments, which allow water to move virtually in any direction governed by soil water potential gradients (Bleby et al., 2010). Deep rooted plants are able to access deep water sources many tens of meters underground (Schenk & Jackson, 2002), and they can play a vital role in facilitating both hydraulic lift (Penuelas & Filella, 2003) and downward hydraulic redistribution (Hultine et al., 2003) in arid and semi-arid environments.

The measurement of soil water potential or content surroundings the plant roots accomplished by the use of stable isotopes has been used to characterize hydraulic redistribution (Dawson & Pate, 1996; Meinzer et al., 2004; Smart et al., 2005; Schoonmaker et al., 2007). These techniques can indicate dynamic water transport, but are costly, timely and labour intensive. The use of sap flow sensors, which allow directional flux measurements, can be an alternative approach. At present, the heat ratio (HR) method (Marshall, 1958; Burgess et al., 1998), the heat field deformation (HFD) method (Nadezhdina et al., 1998, 2008, 2009; Saveyn et al., 2008) and the modified heat dissipation technique (Brooks et al., 2002) are used for such measurements.
2.1.6 Foliar water uptake

The uptake of intercepted water from leaf surfaces into the leaves is a phenomenon referred to as foliar water uptake (FWU), which has previously demonstrated in many plants under dew and cloud-affected ecosystems (Stone, 1957; Louw & Seely, 1982; Kramer, 1983; Nobel, 1983; Boucher et al., 1995; Yates & Hutley, 1995; Munné-Bosch et al., 1999; Martin & von Willert, 2000; Gouvrà & Grammatikopoulos, 2003; Burgess & Dawson, 2004; Limm et al., 2009; Simonin et al., 2009; Goldsmith et al., 2013). Furthermore FWU can also occur in ecosystems, where intercepted rainfall by plant canopies does not always increase soil water availability (Breshears et al., 2008).

FWU has been recognized in about 70 species representing 34 plant families in several diverse ecosystems (Goldsmith et al., 2013), which provide the evidence of bidirectional water movement through plants (Goldsmith, 2013). Furthermore water can moves in more than one direction simultaneously (Fig. 2.3). Conceptually and mathematically this bidirectional movement of water is already described in the SPAC (see Eq. 2.3), which can occurs as a function of Ψ gradients of all possible water sources (Simonin et al., 2009).

![Figure 2.3: Different potential scenarios of water movement through the plants depending on gradients in water potential (Ψ in MPa). In scenario (a), water moves from higher Ψ\text{soil} to lower Ψ\text{atm} by transpiration. In scenario (b), water moves from higher Ψ\text{atm} (during a leaf wetting event) to lower Ψ\text{stem} by foliar water uptake, while also simultaneously moving from higher Ψ\text{soil} to lower Ψ\text{stem}, thus refilling the plant from two directions. In scenario (c), water moves from higher Ψ\text{atm} (during a leaf wetting event) to lower Ψ\text{soil} by foliar water uptake. Note that additional scenarios, such as the hydraulic redistribution of water from one soil layer to another by roots, are not included. Hypothetical values of Ψ based on Nobel (2009) (adapted from Goldsmith, 2013).](image-url)
The movement of water into leaves requires the existence of a driving force, which may result from the lower $\Psi_{\text{leaf}}$ than the adjacent atmospheric boundary layer (Rundel, 1982). Presence of dissolved salts in the mesophyll cell sap may trigger FWU by lowering $\Psi_{\text{leaf}}$. Eller et al. (2013) demonstrated that FWU after leaf wetting events (LWE) resulted from cloud absorption during the dry season in a TMCF. FWU is likely restricted to LWE (Goldsmith, 2013). Water potential at a given temperature rapidly become very low when RH is less than 100% (e.g. -1.36 MPa for 99% RH at 20°C; Nobel, 2009). Hence when water has intercepted on the leaf surface under the condition of internal water deficit then the leaf tissues are likely to have lower $\Psi$ than the surrounding boundary layer (Goldsmith, 2013). The strongest evidence of FWU has found in the seasonally dry fog and dew affected ecosystems, where LWE occur in a condition of limited soil water availability (Munné-Bosch et al., 1999; Limm et al., 2009; Goldsmith et al., 2013). However, LWE resulting from sporadic dry season rainfall are predominant in various ecosystems and there is no reason to think that these events do not also lead to FWU (Breshears et al., 2008).

The movement of water from leaves towards the stem requires the presence of a driving gradient, where FWU results in a higher $\Psi_{\text{leaf}}$ than that of the $\Psi_{\text{stem}}$. This increase in $\Psi_{\text{leaf}}$ as well as the movement of water from leaf to stem as a result of FWU was demonstrated by Eller et al. (2013). Reverse flow of water in stems has been observed (Burgess & Dawson, 2004; Nadezhdina et al., 2010; Goldsmith et al., 2013), but the $\Psi_{\text{stem}}$ gradients related with rehydration by FWU are not well known (Simonin et al., 2009). If the plant stem contains water less than its full storage capacity, it has a water potential such that, after beginning of FWU, water moves spontaneously from both the roots and the leaves towards the stem (Fig. 2.3b). Filling of the stem from both directions continues until no driving gradient exists. Under drier soil condition, this movement of water can continue from plants to the soil along a driving gradient, which has previously been observed only during hydraulic redistribution (see section 2.1.5) (Neumann & Cardon, 2012). Eller et al. (2013) demonstrated the redistribution of water derived from FWU into the roots, if not the soil itself (Fig. 2.4c).

2.2 Mangrove forests

2.2.1 Distribution of mangroves

The ecological term mangrove refers to the diverse association of woody perennial trees and shrubs that grow successfully in the tropical and sub-tropical coastline and saline wetlands between land and sea (Ball, 1988a). One of the remarkable features of mangrove forests is that the species distribution follows a noticeable zonation pattern normally close to the sea shore (Semeniuk & Warm, 1987).
Frequency and duration of tidal inundation determine the zonation patterns of mangrove vegetation (Macnae, 1968). As a result, their dominance is normally found in the regions of 25°N to 25°S (Fig. 2.4). But it is a matter of regret that mangrove forest area declines very sharply each year, occupying globally 13 million ha of coastline sea shore (Wilkie & Fortuna, 2003). Mangrove usually formed dense forest consisting of mono-specific patches in an intertidal muddy shore (Sternberg et al., 2007; Hogarth, 2007). Mangrove communities are sometimes quantified by the term 'mangal' which constitute several halophytic plant species (Tomlinson, 1986). The halophytic nature of mangrove causes salt tolerance upto 90 ppt (gL⁻¹) which does not mean that salt requirement for their growth but still beneficial for them (Komiyama et al., 2008; Krauss & Ball, 2013).

2.2.2 Importance of mangrove vegetation

Mangrove vegetation is found mostly in flooded and water-saturated environments in intertidal tropical and sub-tropical areas. In such an oxygen deficient environment, mangrove roots form a habitat for many terrestrial as well as marine plants, algae, invertebrates and vertebrates (Ellison & Farnsworth, 1992). A variety of microflora and microfauna presents in these coastal areas are vulnerable to both natural and human induced threats.

Figure 2.4: Worldwide distribution of mangroves showing their abundance between 25°N to 25°S (National geographic magazine, 2007).
Mangrove also provides a variety of profit for human beings. Many studies have reported the direct link between the presence of mangrove and fisheries or prawn cultivation (Manson et al., 2005; Meynecke et al., 2007; Nagelkerken et al., 2008). Mangrove provides food and shelter for predators (Manson et al., 2005). Mangroves also have significant contribution in the reduction of soil erosion, water purification and protection against natural calamities such as Tsunami (Mazda et al., 1997; Kathiresan & Rajendran, 2005; Thampanya et al., 2006). Despite of having a great importance mangroves are diminishing due to human activities and natural calamities as well. Therefore, mangroves are always struggling and adapting some mechanisms to cope with such harsh environmental conditions.

2.2.3 Adaptation of mangroves to harsh environmental conditions

Mangroves are trees growing in a challenging environment. They have some special adaptive mechanisms which enables them to continue to exist under such harsh conditions. It must tolerate a wide range of salinity, water stress, temperature and many others environmental factors. Mangroves have had to adapt physiological, morphological and reproductive methods in the form of special breathing roots under flooding condition, salt secretion through the leaves and vivipary germination (Kathiresan & Bingham, 2001). Survival under saline condition occurs due to the halophytic nature of mangrove, implying that they can flourish successfully in the presence of some sort of salt in the soil solution while these are not necessary for their growth (Krauss & Ball, 2013). Presence of pneumatophores, knee roots, still roots and buttress roots (Fig. 2.5) enable them to survive under water logged conditions (Tomlinson, 1986). During low tidal inundation, these roots are exposed to the atmosphere and oxygen possibly diffuses through the pores (Ball, 1988a).

![Different types of aerial roots in mangrove trees (Kathiresan & Bingham 2001).](image)

**Figure 2.5:** Different types of aerial roots in mangrove trees (Kathiresan & Bingham 2001).
Viviparous germination is another important survival mechanism in mangrove trees. In this method, when the propagules become mature, they detach from the parent trees and drop down into water. The fundamental difference between vivipary and crypto-vivipary is that viviparous propagules germinate out through the pericarp (e.g. *Rhizophora* spp.), while crypto-viviparous propagules germinate inside the intact fruit wall (e.g. *Avicennia* spp.) (Fig. 2.6) (Kathiresan & Bingham, 2001).

Figure 2.6: Crypto-viviparous *A. marina* propagule, germinating inside the intact fruit wall (a) and viviparous *Rhizophora mangle* propagule, germinating out through the fruit wall (b) (adapted from De Groote, 2013).

### 2.2.4 Specific adaptations and ecophysiology of *A. marina*

Mangrove vegetation comprises nearly 110 species from 30 families of both dicotyledonous and monocotyledonous groups (Gibson, 2010). Among various genera, one of the most well recognized is the *Avicennia* genus which belongs to the family Acanthaceae. *Avicennia marina* is the most popular one among the ten species of *Avicennia* throughout the world and considered as white or grey mangrove. This species has a wide distribution both latitudinally and longitudinally (Tomlinson, 1986; Ball, 1988a). The most identifying feature of *A. marina* is the presence of thicker, ovate shaped leaves with waxy and glossy upper surface and whitish at the below surface and also bearing small yellowing color flowers. Bark is grayish in color, lenticellate and flaky (Tomlinson, 1986). The height of *A. marina* is approximately 30 m which is considered as either shrub or tree based on the branching pattern. This flexible growth pattern is also associated with environmental gradients (Naidoo, 2010). The growth rate of *A. marina* is strongly governed by water availability, high evapo-transpiration and nutrient uptake, and is frequently hampered by the tidal inundation and causes dwarf sizes tree (Naidoo, 1987). Addition of nutrients like phosphor and nitrogen can enhance the growth rate of *A. marina* to a certain extent (Lovelock et al., 2004).
Mangrove vegetation needs to struggle with oxygen availability due to the presence of water logging conditions. Generally *A. marina* has a shallow root system with extensive arenchymatous tissues and numerous lenticels, allowing the tree roots to get oxygen under oxygen deficient conditions (Tomlinson, 1986). Pneumatophores are produced by *A. marina* (Fig. 2.7b). These pneumatophores are exposed to the atmosphere during low tidal inundation and oxygen diffuses 1000 times more rapidly in air relative to water. It arises from horizontally lateral roots called cable roots supported by anchoring roots (Fig. 2.8) (Tomlinson, 1986).

**Figure 2.7:** Survival in water logging condition (a) and development of pneumatophores (b) of *A. marina* with cable roots system (modified from Robert et al., 2009 and Hoste, 2011).

**Figure 2.8:** Root system of *A. marina*. Specialized root structures are referred to as cable roots (Ca), pneumatophores (Pn) and anchoring roots (An). The dotted line is the substrate level (adapted from Tomlinson, 1986).
Salt secretion and exclusion mechanisms present in *A. marina* make them able to survive under high saline condition. Root ultrafiltration can exclude around 90% of the salt (Ball, 1988b) resulting in an increased salinity in the rhizosphere root zone (Passioura *et al.*, 1992). Frequent flooding by tidal water removing excess salinity is one of the processes for existence in high saline water (Saenger, 2002). *A. marina* can secrete up to 40% of the remaining salt at the leaf level through the specialized salt gland present on the surfaces of the leaf (Waisel *et al.*, 1986). These glands can secrete mainly Na⁺ and Cl⁻ ions (Boon & Allaway, 1982). These solutes travel through the xylem and reach to the leaves by the transpiration stream. Salt solution moves apoplastically via cell walls and also through the leaves it passes to the atmosphere by the stomata as pure water. The remaining part becomes denser and it enters the inner side of the cell membrane named symplast and goes out through the gland (Fahn, 1988). In *A. marina*, salt gland are scatterly arranged and fewer to the upper surface but more densely found on the abaxial leaf surface where they are not sunken but still surrounded by palisade cell which are covered by 3-4 cells of non-glandular hairs (Tomlinson, 1986) (Fig. 2.9). These hairs prevent water loss and secrete the salt droplet far away from the leaves cuticle (Ye *et al.*, 2005; Parida & Jha, 2010).

Salinity also affects the nutrient availability and tolerance to salinity associated with hypoxia along with K⁺ and Na⁺ selectivity (Ball, 1988a). Several findings report that *A. marina* has the highest capacity of selectively uptake K⁺ when the salinity level is low (Downton, 1982; Ball & Farquhar, 1984a; Clough, 1984; Ball *et al.*, 1987). Salt regulation by the roots is the most important survival mechanism of mangroves. As the life span of evergreen mangrove leaves is around 14 months (Coupland *et al.*, 2005), sufficient salt secretion mechanisms must exist to continuously uptake salt along with water and to defend the tissues of being accumulated with excessive salt (Parida & Jha, 2010).

**Figure 2.9:** Schematic diagram of salt glands in *A. marina*. (a) abaxial epidermis, (b) adaxial epidermis (Tomlinson, 1986) and (c) salt glands in abaxial epidermis of *A. marina* seedlings (Uddin, 2014).
A. marina can tolerate a wide salinity range in a very flexible way. Plants decrease water uptake with increased soil salinity due to salt exclusion (Ball, 1988b) and want to keep the leaf area to plant mass ratio as low as possible to store water (Ritchie, 1974). Presence of a waxy cuticle and thicker epidermis along with sunken stomata of A. marina can reduce water loss. As a result the leaves of A. marina are called hypostomatous where the lower surface of the leaves possess stomata which are covered by tricellular hair for minimizing water loss (Ball & Critchley, 1982; Parida & Jha, 2010). Therefore, dwarf growth of A. marina is noticeable in strong salinity areas (Naidoo, 2010). Reduction of stomatal conductance is another mechanism of this species under stressful conditions.

Anatomical adaptations such as the presence of successive cambia and specialized wood vessel structures also contribute to the fact of enduring higher salinities. Several modifications in wood vessels of A. marina help in preventing cavitations and avoiding reduction of hydraulic conductivity. Robert et al. (2009) studied the water transport system of mangroves species A. marina and Rhizophora mucronata under saline condition and found that with higher salinity, both species have higher vessel density, whereas higher vessel grouping was found only in A. marina. They also reported a higher vessel density, vessel grouping with smaller vessel diameters and lengths in A. marina as compared to R. mucronata (Fig. 2.10). Both small vessel diameters and larger proportion of the tiniest vessels make A. marina less vulnerable to cavitation (Lo Gullo & Salleo, 1991; Hargrave et al., 1994; Lo Gullo et al., 1995; Choat et al., 2003) and result in a double safe water transport system. In addition Schmitz et al. (2007a) observed that A. marina has very small vestured pits with thick pit membranes which offer an extra safety mechanism against cavitation. The findings of Robert et al. (2009) in combination with Schmitz et al. (2007a) explain the fact that A. marina has a wider global distribution than R. mucronata and is found especially to be more successful in the higher latitudes (both north and south) were environmental conditions become less optimal for mangroves (Spalding et al., 1997).

In addition to the wood vessel structure, A. marina has some other particular wood anatomical features worth mentioning, such as secondary growth by successive cambia. This exceptional feature was previously observed by Studholme and Philipson (1966); Zamski (1979); Carlquist (2001) and Schmitz et al. (2007b). The ecological importance of successive cambia in relation to a safer hydraulic architecture under water stress condition was studied by Schmitz et al. (2008) and Robert et al. (2011).
Figure 2.10: Overview of the observed wood vessel characteristics of A. 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 characterized by a safer water transport system. On the other hand, trees growing on the seashore, with better environmental conditions seem to have a more efficient water transport system, rather lacking adaptations to avoid air in the water column (Robert et al., 2009).

Despite vascular cambia are usually aligned in a concentric way in woody plants a reticulate arrangement of the vascular cambia producing a three-dimensional network of secondary xylem patches braided with strings of secondary phloem (Robert et al., 2011) was observed (Fig. 2.11) in A. marina. This cambium structure results in a patchy growth pattern in A. marina. Secondary growth in A. marina via successive cambia provides ecological benefits of repairing embolised cells and water storage for growing under drought stress (Schmitz et al., 2007b; Schmitz et al., 2008; Robert et al., 2011). Combining all the adaptive mechanisms, it is not surprising that A. marina is the most widespread mangrove species in the world (Tomlinson, 1994).

Generally A. marina has higher water use efficiency (Ball, 1988a) but less photosynthetic activity under higher salinity conditions (Ball & Farquhar, 1984b). In the past there was contradiction about the transpiration rate of mangroves (Chapman, 1976; Tomlinson, 1994). Frequent inundation with water and mangrove environment’s high insolation suggest high rates, although availability of seawater might be relatively less for plant because of its low osmotic potential (Becker et al., 1997).
Figure 2.11: C T scan of (a) a transverse and (b) a longitudinal plane of a tree stem portion of 7 cm diameter, with circles to indicate the positions of branching phloem bands (Schmitz et al., 2008).

But survival and wide distribution of *A. marina* under such conditions lead to the question about source of freshwater. This directed many researches to identify available fresh water and the pathways involved in water uptake of *A. marina*. Even the well known water uptake mechanism through plant roots cannot explain reversed sap flow observed by De Wal (2011) and Hoste (2011) corresponding to the time of dew deposition in *A. marina*. Maximum stomatal conductance was found in *A. marina* during sunrise (De Deurwaerder, 2012) whereas Hoste (2011) and De Wal (2011) suggested reverse sap flow associated with foliar uptake of dew, gathered during early morning. This argument is reasonable as mangroves receive low fresh water input during the dry season and the possible entry of fresh water may play a major role for their survival and growth. Higher salinity in leaf sap increases the osmotic gradient, supporting the possibility of dew uptake.

The possibility of up taking rain water is higher than dew by mangroves. Since deposition of dew melts the salt glands present on the leaf surfaces resulting in the lowering of intercepted water potential. So a strong water potential gradient cannot be established with the saline sap of the leaf. On the other hand rain can flush away these salt glands and fresh intercepted rain water ($\Psi_{\text{rain}} = 0$) can establish a strong $\Psi$ gradient with $\Psi_{\text{leaf}}$. Reverse sap flow was observed during rainfall events and attributed to foliar water uptake (De Wal, 2011; Hoste, 2011; De Groote, 2013). However, the role of rain water on the growth of mangrove plants under saline conditions is not well understood. Therefore, experimental evidence on canopy water uptake during rain simulation by mangroves will be collected in this master thesis.
CHAPTER 3: MATERIALS AND METHODS

3.1 Experimental set-up

In order to focus on the dependency of rainwater as a fresh water source for mangrove growth and to prove the hypothesis of canopy water uptake an experiment was conducted in the greenhouse (2 x 2.5 x 4 m) facilities of the Laboratory of Plant Ecology, Faculty of Bioscience Engineering, Ghent University, Belgium from November 2013 to the end of April 2014. Seedlings of the mangrove species *Avicennia marina* (Forssk.) Vierh. were planted in pot containing a mixture of 80% sand and 20% mangrove mud substrate on 7th November 2013. Stem cuttings of the temperate tree species *Populus tremula* L. were planted on 17th December 2013 in a pot containing peat soil. A pumping system was built to inundate the seedlings of *A. marina* with saline water for a period of twenty minutes and twice a day at 0800h and 1500h. A multi-range conductivity meter (HI 9033, Hanna Instrument Inc., Woonsocket, RI, USA) was used to measure the salinity of the pumped water and was maintained at 24±0.5 mS cm⁻¹ (corresponding to ppt of 15) by adding sea salt and tap water. Photosynthetically active radiation (PAR), air temperature and relative humidity (RH) inside the greenhouse were measured with LI190 Q17529 (LI-COR Biosciences, Lincoln, NE, USA), Thermocouples (type T, Omega Engineering, UK) and humidity sensor (HIH-4000-001, Honeywell Sensing, USA), respectively. To maintain a higher RH of the air within the greenhouse, two air humidifier (type Boneco 7135, Plaston, Widnau, Switzerland) were continuously used. Vapour pressure deficit (VPD) was calculated from these measured temperature and RH (Abtew & Melesse, 2013). All meteorological data and sensors data were recorded at a 30 seconds interval and averaged every 5 minutes using a datalogger (CR1000, Campbell Scientific Inc., Logan, Utah, USA).

3.2 Sap flow dynamics

Measurement of sap flow is the crucial part in plant water relations studies. Many methods have been developed based on thermodynamic principles (Marshall, 1958; Pickard & Puccia, 1972; Pickard, 1973; Cohen et al., 1981; Swanson & Whitfield, 1981; Swanson, 1983; Granier, 1985; Nadezhdina et al., 1998; Nadezhdina, 1999; Green et al., 2009) to measure the sap flow in plant. Although these thermodynamic methods have solved the disadvantages of using dye into the sap stream where the plant has to be cut (Kramer, 1940; Canny, 1977), most of them still have the limitations of measuring low and reverse flows (Becker, 1998; Green et al., 2009; Steppe et al., 2010).
In order to solve the above mentioned problems the HFD method was developed (Nadezhdina et al., 1998). However the knowledge on sap flow of small seedlings having smaller diameter is still limited, since most of the currently available sap flow sensors are only suitable for stems or branches of larger dimension. The main constraint of these sensors to be used on small seedlings, branches or peduncle of fruits is that they consist of needles, which have to be inserted into the sapwood of the plant. Moreover, other externally installed sensors are not capable of detecting small sap flow values (Sakuratani et al., 1999; Smith et al., 1999) or need a period of zero flow condition for their calibration. Sap flow dynamics (SFD) of both species were measured by using the non-invasive mini HFD (Hanssens et al., 2013) sensor (Fig. 3.1), which is based on the the same principle of heat field deformation (HFD) method (Nadezhdina et al., 1998). Fig. 3.2 gives a conceptual representation of the sensor configuration.

![Figure 3.1: Mini HFD sap flow sensors wrapped with aluminum foil are mounted on the stem of A. marina (a) and P. tremula (b)](image)

Likewise Hanssens et al. (2013) the axial (T₁ & T₂) and tangential (T₃) thermocouples were positioned 10 and 3 mm, respectively apart from the center of the heater. A continuous voltage of 2V was applied to the heater to generate the heat field. The K value for calculating the SFD ((K + dTₛₐ) dTₛ⁻¹) in absence of zero flow condition was determined by the linear extrapolation of dTₛₐ versus dTₛ/dTₐ curve (Nadezhdina et al., 1998).
3.3 Stem diameter variations

The exchange of water between the internal storage pools and the transpiration stream causes small but detectable changes in the stem diameter of the plant, which results in a typical day-night pattern. Moreover, the irreversible growth of the plant causes a more linear increase in stem diameter over time during well-watered conditions. In recent years, research has been carried out to unravel and interpret stem diameter variations (SDV) in trees with respect to tree water and carbon status (e.g. Huguet et al., 1992; Goldhamer & Fereres, 2001; Sevanto et al., 2003; Daudet et al., 2005; De Schepper et al., 2010; De Schepper & Steppe, 2011). The effect of plant water status on SDV and the potential of plant water status indicators inferred from the SDV measurements have also acquired extensive interest as an approach in plant-based irrigation scheduling (e.g. Jones, 2004; Steppe et al., 2008; De Swaef et al., 2009, 2012; Fernandez & Cuevas, 2010; Ortuño et al., 2010). These small variations in stem diameter are relatively easy to record continuously by using point dendrometers or linear variable displacement transducers (LVDTs) (De Swaef et al., 2013). In our experiment SDV of both A. marina and P. tremula were recorded (Fig. 3.3) with LVDT sensors (Solartron Metrology, Bognor Regis, UK).
The LVDT sensor is an electro-mechanical transducer, which produces an electrical output proportional to the displacement of a separate movable core. In this sensor a pushrod of smaller diameter can move freely back and forth along with the long axis of a hollow metallic cylinder. The pushrod ends in a rod-shaped magnetic core which must be within the coil assembly when the device is operating. Variation in stem diameter i.e. swelling or shrinkage of stem moves the core inside the coil assembly which induces a voltage output. This output can then be correlated to the displacement of the core by calibrating the LVDT in a fixed set-up during which a stepwise displacement is enforced on the sensor.

![Figure 3.3: Solartron LVDT sensor installed on the stem of a young seedling of A. marina (a) and P. tremula (b), supported by a custom-made stainless holder.](image)

3.4 Dye experiment

To evaluate the pathways involved in foliar water uptake, we sprayed both A. marina and P. tremula with a dye solution at 1500h on 7\textsuperscript{th} and 8\textsuperscript{th} of April 2014, respectively. About 5-6 drops of toluidine blue (C\textsubscript{13}H\textsubscript{16}N\textsubscript{3}SCI) having a color index of 52040 was added to one liter of tap water. The prepared dye solution was sprayed on the plants for a period of 20 minutes. Before spraying the dye solution all the petioles and stems were covered with tissue paper underneath of aluminum foil (Fig. 3.4) to prevent direct contact with the dye solution and to avoid absorption of water through them. Just after completion of spraying the dye solution 3 leaves of each species were harvested and washed with distilled water and then dried with towel. Thin transverse sections (T S) of petioles from the harvested leaves were examined under light microscope (leitz laborlux-S microscope, Wetzlar, Germany) and captured photograph with a
digital camera (Nikon Coolpix 8400) mounted on the microscope using Debut video capture software (version pro. 1.2). To see the effect of toluidine blue on different tissues, T S of petioles from the control plants were submerged in 2.5% dye solution for a period of 5 minutes. Both of them were compared with the T S of petioles from the control plants.

![Image](image1.png)

**Figure 3.4:** Petioles and stems of *P. tremula* wrapped with tissue paper underneath aluminum foil to prevent their contact with dye solution.

3.5 Rain simulation

To evaluate the effect of rainfall on sap flow and stem growth, artificial rain simulation was performed. Tap water was sprayed by using a hand sprayer for a period of 20 minutes on the seedlings previously installed with mini HFD and LVDT-sensors. This experiment was conducted in two study periods. During the first period rain simulation was done on *P. tremula* during 28th to 30th March 2014 (87 to 89 DOY) at 1400h in first two days and at 1300h in the 3rd day. In the second study period rain simulation was done on *A. marina* during 14th to 16th April 2014 (104 to 106 DOY) at 1300h. In this period *P. tremula* was irrigated twice in a day corresponding to the time of pumping saline water to *A. marina*. Before spraying of water all the petioles and stems were covered with tissue paper and aluminum foil (Fig. 3.4) to prevent direct absorption of water by any plant parts other than the leaves. Soil at the base of the plant was covered with aluminum foil to prevent absorption of rain water through the plant roots. The rain simulation was repeated for three consecutive days and its impacts on SFD (-) and stem growth were assessed from the data of both mini HFD and LVDT-sensors.
3.6 Deuterium labeling experiments

Stable water isotope (e.g.: deuterium, $^2$H; oxygen, $^{18}$O) studies have become progressively more important in the field of ecology (Sidle, 1998; Dawson et al., 2002), hydrology (Driscoll et al., 2005; Jasechko et al., 2013), soil science (Förstel et al., 1991; Scrimgeour, 1995), plant physiology (Leaney et al., 1985; Yakir et al., 1990; Flanagan & Ehleringer, 1991; Ehleringer & Dawson, 1992; Brunel et al., 1997; Schwinning et al., 2002) and forensics (Koeniger et al., 2011). In most cases water has to be extracted from the sample such as soil or plant tissues in order to enable their analysis by mass spectrometry or cavity ring down spectrometry (CRDS). Over the past five decades various water extraction methods have been developed and used (Walker et al., 1994) including mechanical pressing (Wershaw et al., 1970; White et al., 1985), microdistillation with zinc (Turner & Gailitis, 1988), displacement through centrifugation with an immiscible liquid (Whelan & Barrow, 1980), azeotropic distillation with kerosene or toluene (Reves & Woods, 1990) and cryogenic vacuum distillation (Wershaw et al., 1970; Ehleringer & Osmond 1989; Dalton 1989; Ehleringer et al., 2000; West et al., 2006). Among these methods, we used the cryogenic vacuum distillation, which generates less fractionation (Araguás-Araguás et al., 1995). According to Araguás-Araguás et al. (1995) the sample should be rejected from the analysis if the water extraction is less than 98%. Isotopic analysis was carried out at the ISOFYS laboratory facility, University of Ghent, Belgium. Isotopes were measured by using cavity ring down spectrometry (PICARRO L2130-i-Analyzer, Santa Clara, California, USA). Hydrogen and oxygen isotope ratios ($^2$H/$^1$H or $^{18}$O/$^{16}$O, respectively) are both obtained from the analysis. The isotopic composition was expressed in delta notation ($\delta$ $^2$H ‰).

\[
\delta^N E = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]  

where, N is the heavy isotope of element E and R is the ratio of the heavy to light isotope ($^2$H/$^1$H or $^{18}$O/$^{16}$O). The $\delta$ values are reported relative to internal calibration standards standardized with international standards V-SMOW and SLAP. Long-term precision is 0.1% ($\delta$ $^2$H) and 0.03% ($\delta$ $^{18}$O).

3.6.1 Set-up to submerge leaves under labelled water

A set-up (Fig. 3.5) of small trays was built to evaluate water uptake by leaves of plant, by submerging leaves of the plant under enriched water while they were still attached to the plant. On the day of experimentation *A. marina* was inundated twice like previous days but no irrigation was provided to *P.*
tremula. Enriched water (3107.08‰ δ²H) used to submerge the leaves in this experiment was composed of a mixture of tap water (-44.48‰ δ²H) and water rich in deuterium oxides (99.85%, Cambridge Isotope Laboratory, Andover, MA, USA). Leaves of P. tremula and A. marina were submerged for a period of 20 minutes at 1400h on 4th (94 DOY) and 21st (110 DOY) April 2014, respectively. Proper care was taken to prevent any contact of petiole with enriched water. During this time all the trays were covered with transparent thin polythene to prevent evaporation, which would lead to fractionation of enriched water. Enriched water from each trays was sampled with a plastic syringe through the polythene at 0, 10 and 20 min of experimentation to see the pattern of fractionation. Detached dry leaves of P. tremula were also submerged under enriched water and that water was sampled as previous manner. Fractionations of water from these two sources were compared with the control trays, containing only enriched water. Afterwards, the shoots were harvested and sampled separately as leaf, petiole and stem to analyze their water hydrogen isotope composition. To remove residual water from the leaf surfaces during sampling, we thoroughly dried individual leaves with paper towels. To prevent any change of their isotopic composition, plant samples were kept in a freezer at -20°C until water was extracted by cryogenic vacuum distillation (see section 3.6.2). The isotopic enrichment of submerged leaves compared with control leaves (only P. tremula was selected as control plant but not A. marina due to lack of individuals) was used as an evidence of FWU, since both soils and roots were completely isolated from the enriched water to prevent their contamination and uptake by roots.

Figure 3.5: Set-up of leaves submergence under water (a) P. tremula leaves submerged under enriched water and covered with thin polythene to avoid evaporation (b) plastic trays placed beneath leaves of A. marina just before submerging leaves under enriched water.
3.6.2 Cryogenic vacuum distillation

The vacuum distillation method for water extraction has been increasingly achieving popularity for its relative simplicity and the lack of necessity for addition of a solvent as well as less fractionation during water extraction (Araguás-Araguás et al., 1995). During this process water is sublimated from the frozen plant samples and condensed in a collection tube. Each extraction unit consists of a connector glass tube along with a sample tube and collection tube (Fig. 3.6 A, B, C) (see West et al., 2006 for details). The entire vacuum line was connected to a vacuum pump (Edwards, Sint-Pieters-Leeuw, Belgium) (Fig. 3.6D) in order to remove the air, until 1 mBar was reached and maintained. Then the sample tubes were placed on a heating block (HI 839800 Code Reactor) (Fig. 3.6E) at 105°C which sublimes water from the samples. Water vapor from the samples passes through the glass arm and accumulates in the collection tube, which was immersed in a dewar containing liquid nitrogen (Fig. 3.6F). As a result, water vapor was condensed and become frozen. This water extraction was continued for a period of three hours (Araguás-Araguás et al., 1995). After completion of the distillation process, both the sample tube and the collection tube were removed from the heating block and the dewar, respectively. The collection tube was removed and kept for a while to allow the frozen water to melt. The melted water was pipetted out of the collection tube into a syringe with a 0.45µm filter attached to it. Then the water was filtered and stored in a 700 µL storage vial and retained for isotopic analysis.

![Figure 3.6: The picture on the left: (A) Connector glass tube along with sample (B) and collection tube (C), attached with the vacuum pump (D). On the right, the set-up of water extraction showing the heating block (E) and the Dewar with liquid nitrogen (F).]
CHAPTER 4: RESULTS

4.1 Environmental variables

Two selected growth periods were studied in detail during this experiment. Both periods consist of 72 hours (three days). The first study period started on March 28th (DOY 87) and ended on March 30th (89 DOY). During this period artificial rain simulation was performed on *Populus tremula* but no rain simulation on *Avicennia marina*. The second selected study period started on April 14th (DOY 104) and ended on April 16th (DOY 106). During the second study period artificial rain simulation was performed on *A. marina* and daily irrigation was given to *P. tremula*. Both environmental and ecophysiological parameters for the selected periods were studied in detail and are presented in Fig. 4.1, 4.2 and 4.3. Photosynthetically active radiation (PAR) and vapour pressure deficit (VPD) comprised the environmental parameters whereas the ecophysiological parameters consisted of sap flow dynamics (SFD) and stem diameter variations (SDV).

![Figure 4.1](image)

**Figure 4.1:** Meteorological variables during the two selected study periods. During the first study period rain simulation was done on *P. tremula* (a); whereas in second study period rain simulation was done on *A. marina* and regular irrigation in *P. tremula* (b). The grey line indicates the photosynthetically active radiation (PAR) and the black line indicates vapour pressure deficit (VPD). The vertical dashed lines represent rain simulation time.
Meteorological parameters of both study periods are presented in Fig. 4.1. PAR showed a versatile pattern of peaks and falls during the day time and a constant pattern at night time due to sunset as well as for switching off the light of the greenhouse (no artificial light from 2000h to 0800h). When the sunrises a steep increase in PAR takes place in the morning. But most of the peaked values were concentrated around 1500h to 1700h. The observed oscillations on PAR are due to the blockage of shortwave radiation by clouds.

VPD also showed a similar daily pattern. Rising during the day, reaching the highest values around 1600h to 1800h, and decreasing afterwards reaching a minimum value early in the morning, before sunrise. The higher VPD values in the afternoon caused a maximal transpiration, which resulted in a strong suction of water towards the foliage. A small time lag of up to one hour (Hoste, 2011) was observed between the peaks of PAR and VPD (Fig. 4.1). This time lag in VPD, can be explained by the time difference between sunrise and air warm up. When sunset approaches, PAR began to drop rapidly, whilst VPD diminished more moderately. This, certainly is also a consequence of the heat capacity of the humid air (De Wal, 2011). Since at night there was no more heating of the air, VPD continued a gradual decrease until the sunrise when the cycle started again.

When the environmental parameters of both study periods are compared, higher peaks of PAR in the second period (1330 µmol m\(^{-2}\) s\(^{-1}\)) (Fig. 4.1b) can be distinguished from the former period (885 µmol m\(^{-2}\) s\(^{-1}\)) (Fig. 4.1a). This might be due to the perpendicular incoming solar radiation, common during early summer season. As a result, the mean temperature was higher during the second study period, as well as the peaks in VPD (Fig. 4.1b). The highest and lowest values of VPD in the first study period were 3.67 and 0.05 (kPa), respectively, whereas during second study period these values were 7.62 and 1.05 (kPa). This is quite logical since the mean temperature of second study period was much higher (31.4°C) than the first period (26.8°C).

The effects of artificial rain simulation were also clearly noticeable in these two study periods (Fig. 4.1). Since rain simulation was performed by a hand sprayer so it did not affect incoming solar radiation. With rain simulation, the RH in the greenhouse increased and reached its maximum value (around 97-98%), plummeting VPD value lower than before and after the rain simulation events (Fig. 4.1). During the first study period, VPD dropped even below 1 kPa (0.34 kPa) (Fig. 4.1a) still higher than the nighttime lowest value (0.05 kPa). In the second period this dropped value during rain simulation was higher than 1 kPa (1.53 kPa) (Fig.4.1b) and the nighttime lowest value was recorded 1.05 kPa.
4.2 Sap flow dynamics

The course of the sap flow dynamics (SFD) patterns of the two selected periods are shown in Fig. 4.2a & b (A. marina) and Fig. 4.3a & b (P. tremula). A diurnal pattern of SFD with maximum value around the afternoon (around 1500h to 1800h) and the minimal during nighttime was observed. In comparison with Fig. 4.1 it is clear that SFD had a similar pattern to PAR and VPD, which is not surprising since these variables govern transpiration rates and consequently sap flow. Apart from this SFD also showed some unusual pattern of steep drop during day time at the onset of rain simulation due to the sudden decrease in VPD.

![Figure 4.2: Ecophysiological parameters of A. marina during the two selected study periods. Sap flow dynamics (-) of A. marina during the first study period i.e. without rain simulation (a); Sap flow dynamics (-) of A. marina during the second study period with rain simulation (b); stem diameter variations without rain (c) and with rain simulation (d). The horizontal dotted line indicates the zero line and the vertical dashed lines represent rain simulation time.](image-url)
Apart from some little drops, SFD of *A. marina* during the first period showed an usual daily pattern (Fig. 4.2a) with a value ranging from 0.131 to 0.49. At this period a small drop in SFD was noticeable corresponding with the time of rain simulation on *P. tremula*. The lowest value recorded in *A. marina*, during rain simulation on *P. tremula*, was even higher (0.25) than the nighttime lowest value (0.131) (Fig. 4.2a). So the decrease in SFD of *A. marina* during the day time was due to the decrease in VPD, which was observed even below 0.5 kPa within the greenhouse (Fig. 4.1a). During the second study period (with direct rain simulation on *A. marina*), SFD of *A. marina* showed some unusual sudden drops, corresponding to the time of rain simulation (Fig. 4.2b). The SFD of *A. marina* at this period showed steep drop, corresponding to the rain simulation time. This dropped value in SFD of *A. marina* was much lower (-0.07) than the nighttime lowest value (0.02) (Fig. 4.2b).

When SFD of *A. marina* is compared between the two study periods, the dropped value of the second period is much lower than the former period. During the second study period despite of higher VPD, a steep drop in SFD of *A. marina* was noticeable (Fig. 4.2b) at the rain simulation time. Although both VPD and PAR of this period are much higher than the former period, the SFD value is lower. Also the decreased value in SFD during the rain simulation time was much lower (-0.07) than the previous period (0.25), even crossed below the zero line (indicated by the horizontal dotted line) (Fig. 4.2b). This negative SFD might be not only for the decrease in VPD but also for the uptake of water by the foliage of *A. marina*. Since the petioles and stem of the *A. marina* seedling were covered with tissue paper underneath of aluminum foil to prevent their direct contact with rain water, the uptake of water might be taken only with leaves. This foliar water uptake (FWU) changed the direction of water flow within the plant resulting in reversed SFD as presented in Fig. 4.2b.

Figure 4.3a represents the SFD of *P. tremula* with rain simulation treatment, which resulted the lowering in VPD (Fig. 4.1a). This lower VPD also resulted a sudden decrease in SFD of *P. tremula* (0.08) (Fig. 4.3a). Although the SFD showed a steep drop at the time of rain simulation, but that dropped value was higher than the nighttime value (-0.02). During night SFD became negative and cross the zero line (indicated as dotted horizontal line in Fig. 4.3a). This might be due to the redistribution of water from stem to the root, even soil under dry conditions. SFD of *P. tremula* during the second study period is presented in Fig. 4.3b. During this period *P. tremula* was irrigated twice in a day corresponding to the pumping time of *A. marina* (at 0800h and 1500h). There was a little drop in SFD (Fig. 4.3b) during the rain simulation on *A. marina*, which resulted from the sudden decrease in VPD (Fig. 4.1b).
Figure 4.3: Ecophysiological parameters of *P. tremula* during the two selected study periods. Sap flow dynamics (-) of *P. tremula* during first study period i.e. with rain simulation (a); Sap flow dynamics (-) of *P. tremula* during second study period with daily irrigation (twice) (b); stem diameter variations with rain simulation (c) and with irrigation twice in a day (d). The horizontal dotted line indicates the zero line and the vertical dashed lines represents rain simulation time.

When SFD of *P. tremula* is compared between the two study periods the second period has more than 10 times higher value than the former period (Fig. 4.3a & b). This was due to the higher PAR and VPD values of the second period (Fig. 4.1b) as well as the availability of fresh soil water. The effect of rain simulation on SFD of *A. marina* (Fig. 4.2b) was more prominent than the *P. tremula* (Fig. 4.3a). During rain simulation wetting of the leaves reduced SFD of *P. tremula* by restricting the transpiration loss of water. As a result SFD decreased to the minimal rate but did not cross the zero line. Whereas reversed SFD in *A. marina* might be resulted from both reduced transpiration and uptake of rain water by the leaves.
4.3 Stem diameter variations

The measurement of stem diameter variations (SDV) of *A. marina* during first and second study periods are presented in Fig. 4.2c and Fig. 4.2d, respectively. Whereas the SDV results of *P. tremula* for the first and second study periods are presented in Fig. 4.3c and Fig. 4.3d, respectively. A clear daily pattern of swelling and shrinking was observed in both periods. The shrinking occurred during the daytime and it was accomplished by the loss of internal water from the plant. Shrinkage started in the morning with rising of the sun and reached its minimum diameter corresponding to the peaks of VPD (Fig. 4.1). Swelling of the stem started in the evening, continued to rise throughout the whole night and reached its maximum in the early morning when VPD became lowest. SDV seemed to be related with the environmental variables PAR and VPD, since it showed a reversed pattern of them as well as SFD.

In case of *A. marina* during the first study period (Fig. 4.2c) the SDV showed a regular daily pattern (ranging from 1.75 to 1.90 µm), apart from a very small peak during the time of rain simulation on *P. tremula*. This peak was resulted from the decrease in VPD (Fig. 4.1a), which diminishes the rate of transpiration. But during the second study period the SDV of *A. marina* showed very steep peak (2.41 µm) (Fig. 4.2d), which was even much higher than the night time SDV value (2.35 µm). It also showed an opposite pattern with SFD (Fig. 4.2b & d), which decreased drastically during rain simulation. Since rain simulation was done directly on *A. marina* it had a double effect: steep decrease in VPD (Fig. 4.1b) and uptake of water through the foliage. This FWU may results in the radial growth of *A. marina*, which is indicated by the steep peak in SDV. But shortly after rain simulation (around 1 to 2 hours later) stem diameter started to shrink and reached its lowest, corresponding to the peaks in SFD (Fig. 4.2b & d), PAR and VPD (Fig. 4.1b).

Stem diameter of *P. tremula* during the first study period showed a regular shrinkage with the sunrises in the morning. But with the rain simulation it started to swell and reached upto the night time diameter value (1.90 µm) (Fig. 4.3c). This is due to the decrease in VPD and the reduction of transpiration by wetting of leaves. *P. tremula* in the second study period (Fig. 4.3d) showed higher SDV (4.2 µm) than the former period (Fig. 4.3c). The extent of stem diameter shrinkage during this period was also lower (3.9 µm) than the previous period (Fig. 4.3c). Despite SFD in this period were10 fold higher than previous period still day to day radial growth is noticeable. This was due to the availability of fresh irrigated water. Stem diameter of *P. tremula* (Fig. 4.3d) increased more significantly than *A. marina* under irrigated condition. Whereas during the rain simulation, increase in SDV of *A. marina* was more significant than *P. tremula*.
4.4 Dye experiment

Transverse sections (T S) of petioles of both *A. marina* and *P. tremula* observed under light microscope are presented in Fig. 4.4. These photomicrographs are distinguishing the effect of spraying dye solution on both species. The dye was not noticeable in T S of petioles of control (without any spray treatment) plants (Fig. 4.4a & b). Both the vascular bundles and the surrounding parenchymatous tissues of *A. marina* became stained when thin sections of petioles were submerged in 2.5% dye solution for a period of 5 minutes (Fig. 4.4c), whereas in *P. tremula* the presence of dye is more clearly visible in the vascular bundles than the surrounding tissues (Fig. 4.4d).

Transverse sections of the petioles of *A. marina* of which the leaves were sprayed with dye solution is showing the presence of dye (Fig. 4.4e). In comparison to the control condition (Fig. 4.4a), spray treatment showed darker color (Fig. 4.4d), although lighter than the submerged condition. Since the petioles and stems were covered with tissue paper underneath of aluminium foil, only the leaves were in contact with dye solution during spray treatment. The presence of dye in the petiole of *A. marina* represents that water absorbed by the leaves during spray is transported through the petioles most likely towards the stem (not sure yet as stem section was not observed under microscope).

Negative value in SFD (Fig. 4.2b) and steep peaks in stem diameter (Fig. 4.2d) during the rain simulation events also suggested the phenomenon of water absorption by the foliages of *A. marina*. The decrease in VPD during rain simulation (Fig. 4.1) and the presence of saline soil (by lowering the water potential of the rhizosphere) might also trigger the water uptake by the foliages of *A. marina*. Spray of dye solution did not show any noticeable effects on *P. tremula* (Fig. 4.4f). There was no remarkable distinction between the control and spray treatment of *P. tremula*. Even T S of petioles (Fig. 4.4f) from the plant sprayed with dye solution did not show any similarities with submerged condition (Fig. 4.4d).

Both SFD (Fig. 4.3a) and SDV (Fig. 4.3c) in *P. tremula* did not show any proof of up taking water through the foliage. The decrease in SFD and the steep increase in SDV during artificial rain simulation events were merely due to the decrease in VPD (Fig. 4.1a).
Figure 4.4: Photomicrographs of transverse section (T S) of petioles showing the vascular systems of both A. marina and P. tremula under the light microscope. T S of petioles from a control plant of A. marina (a), P. tremula (b). T S of petioles submerged under dye solution for a period of 5 minutes A. marina (c), P. tremula (d). T S from the plant previously sprayed with dye solution for a period of 20 minutes A. marina (e) and P. tremula (f).

4.5 Stable isotope analysis

We performed stable isotope analysis as an ultimate proof of the hypothesis of FWU, where leaves of both species (A. marina and P. tremula) were submerged under deuterated water (3107.086‰ δ²H) for a period of 20 minutes. Results of isotopic analysis are presented in figure 4.5a, and the fractionation of
enriched water during the experiment with *P. tremula* (Fig. 4.5b) and *A. marina* (Fig. 4.5c). The observed value (600.63‰) of δ²H in *P. tremula* leaves was significantly higher than the control (1.56‰) plant (Fig. 4.5a). This positive value of δ²H in leaves of control plant is due to the fractionation of water during transpiration which is also reported by Ellsworth and Williams (2007). Both adsorption and absorption of enriched water by the surface of the leaves might result in such higher value of δ²H in *P. tremula*. Rough leaf surfaces with less waxy cuticle facilitate adsorption of enriched water by *P. tremula*. Absorption of water through the leaves may transport water to the stem through petioles. Although *P. tremula* showed a positive value (10.41‰) of δ²H in the petioles, it was not significantly different from the control plant (-41.37‰) (Fig. 4.5a). This positive value might be due to the transport of enriched water from the foliage or by the contamination of petioles with enriched water during submergence of leaves under water. Also no noticeable difference of δ²H was found in the stems (-39.59‰) of *P. tremula* when compared with its control plant (-42.00‰) (Fig. 4.5a). This negative value of δ²H in the *P. tremula* stem proved that no water was transported from the leaves to stem. Since FWU of *P. tremula* was not supported by the findings of SFD (Fig. 4.3a), SDV (Fig. 4.3c) and dye experiment findings (Fig. 4.4f) we could assume that the amount of δ²H present in the petioles is mainly due to contamination with enriched water.

Leaves of *A. marina* also showed a higher value (327.85‰) of δ²H but lower than *P. tremula*, which might be the result of both adsorption and absorption of enriched water (Fig. 4.5a). Further higher values of δ²H in petioles (254.32‰), which is significantly higher than the control plant (-41.37‰) (Fig. 4.5a) indicate enriched water was transported from the leaves to stem. This transportation of enriched water from leaves to stem is confirmed by the significantly higher value of δ²H (67.67‰) in the stem of *A. marina* than the control plant (-42.00‰) (Fig. 4.5a). Since only leaves were submerged under enriched water the presence of δ²H in petioles and finally stem suggested that water was up taken by the leaves and transported towards the stem. More negative water potential in the rhizosphere of *A. marina* may also trigger FWU. This uptake of water by the leaves of *A. marina* was also supported by the SFD (Fig. 4.2b), SDV (Fig. 4.2d) and dye experiments (Fig. 4.4e). This general trend demonstrates that water with a high δ²H was directly absorbed into internal leaf tissues and transported to the stem through petioles, which confirms that foliar water uptake occurred in *A. marina*. The observed value of δ²H was significantly higher in the leaves of *P. tremula* than *A. marina*. But both petioles and stem of *A. marina* showed significantly higher value of δ²H than *P. tremula*. These findings proved that δ²H after being absorbed by the leaves of *A. marina* was transported to the stem via petioles. Whereas in *P. tremula* δ²H was just adsorbed on the leaves but not absorbed. Hence, no transportation of δ²H from the leaves towards the stem.
Figure 4.5: Plant scale experiments assessing the foliar absorption in both *A. marina* and *P. tremula* by using a *P. tremula* control plant and submerging the leaves of both species under $\delta^2$H enriched water (a). Fractionation of enriched water ($\delta^2$H) during the 20-min lasting experiment with *P. tremula* (b) and *A. marina* (c).

The fractionation of enriched water during the 20-min lasting experiment are presented in Fig. 4.5b (*P. tremula*) and Fig. 4.5c (*A. marina*). Although both species showed a similar pattern, the fractionation in *A. marina* was higher than in *P. tremula*. *A. marina* being a salt tolerant species, therefore it has higher capability of fractionation than *P. tremula*. A significant positive correlation between the salinity tolerance and magnitude of fractionation for mangrove species was also supported by Ellsworth & Williams (2007) and Lin & Sternberg (1993).
CHAPTER 5: DISCUSSIONS

5.1 Reversed sap flow

Although the first evidence of reversed sap flow in a branch of an apple tree was already demonstrated about three centuries ago by Hales (1727), only few in situ measurements of such reversed sap flow have been made (e.g. Burgess & Dawson (2004); Burgess & Bleby (2006); Nadezhdina et al. (2009), Nadezhdina et al. (2010); De Wal (2011); Hoste (2011); Goldsmith et al. (2013)). This fewer availability of information about reverse flow can be explained by the necessity of sap flow sensors which can detect very low and reversed sap flow. Only two types of sap flow sensors the heat ratio method (HRM) (Burgess et al., 2001) and the heat field deformation method (HFD) (Nadezhdina et al., 1998), are capable of detecting such low and reversed flow (Nadezhdina et al., 2010). Most of the abovementioned studies on reversed sap flow by using these sensors linked their observations with a mechanism called hydraulic redistribution. However, another phenomenon called FWU can also result in reversed sap flow (Burgess & Dawson, 2004). Both of these phenomena will be discussed further in detail and linked with the reversed sap flow dynamics and stem diameter variations recorded in this thesis in the following sections.

5.2 Rain induced hydraulic redistribution

The phenomenon of hydraulic redistribution has already described earlier in the Literature Review chapter, where the concept of hydraulic redistribution was explained due to the variations in water content and thus water potential throughout the soil surrounding the plant roots. Of course there are some other factors that can influence the water potential. In the mangrove forest tidal inundation with saline sea water results in the accumulation of salts throughout the soil horizons. But more salt is accumulated in the upper horizon than the lowers. This higher salt level in the upper layer results in a lower (more negative) $\Psi_{\text{soil}}$. During nighttime due to the decrease in VPD, the transpirational demand diminishes which ultimately increases $\Psi_{\text{stem}}$. No water uptake is occurred when $\Psi_{\text{stem}}$ becomes equal to $\Psi_{\text{soil}}$ and the plant comes to equilibrium (Richter, 1997; Simonin et al., 2009). The lower soil horizons might be less influenced by the deposited salt from the inundated sea water, which results in a water potential gradient from the deeper to the surface soil layers. Therefore plant roots reaching the deeper soil layers experience a higher soil water potential than the shallow roots. At night this water potential gradient can cause uptake of water by the deep roots and release towards the shallow roots. This can also happen in a reverse way during rainfall.
When it rains, the fresh rainwater cannot easily penetrate upper NaCl enriched surface to dilute the lower horizons in the clayey soil, which results in the fact that the upper soil layers are diluted and less saline due to this fresh rain water. At the same time, due to the slow penetration of fresh water the deeper layers remain saline. As a result a water potential gradient is created, which can result in hydraulic redistribution from upper to the lower soil layers through the tree and thus reversed sap flow during nighttime.

As far known, Hao et al. (2009) studied first the influence of interstitial soil salinity gradients on hydraulic redistribution with the mangrove species Rhizophora mangle. Changing salinity throughout the different soil horizons can also cause a water potential gradient, which can result in water flow towards the more saline soil layers by the similar mechanism of hydraulic redistribution by plant roots. This hydraulic redistribution under saline condition can result in nighttime negative sap flux densities in mangrove species A. marina (De Wal, 2011; Hoste, 2011).

But other than roots, hydraulic redistribution can also take place in tree stems. Substantial information is available about the process of root mediated hydraulic redistribution, but much less information is available about hydraulic redistribution within above-ground tree parts (Nadezhdina et al., 2009). The first evidence of reverse flow and the possibility of hydraulic redistribution in tree stems was demonstrated by Hales (1727) (Fig. 5.1). Under normal natural conditions, such reverse flow in tree stems can be resulted from foliar uptake during foggy or drizzly weather (Burgess & Dawson, 2004).

![Figure 5.1](image-url)  
**Figure 5.1**: Schematic illustration of the experiment conducted by Hales (1727) representing the bidirectional passive movement of water in a large branch of an apple tree. The sap flow changed from roots (1)-leaves (2) direction during transpiration under natural conditions (a); to the leaves (2)-roots (1) direction after branch severing when its cut upper tip was put into water (b) (adapted from Nadezhdina et al., 2009).
The role of stems in the exchange of water between roots was first proved by Burgess & Dawson (2004). The redistribution of water by plant stems and roots is possible in any direction, but always according to the gradient in $\Psi$. Most of the findings reported the occurrence of hydraulic redistribution during the nighttime. During daytime, such redistribution of water does usually not takes place, since $\Psi_{\text{leaf}}$ is too low (due to the higher value of both PAR and VPD (Fig. 4.1), which cause transpiration through the foliage) and water is pulled upwards. Immediately after artificial rain simulation, the measurement of SFD in *A. marina* showed a rapid decrease even below the zero line (Fig. 4.2b). Instantly after stopping the rain simulation it started to rise again. Since a mini HFD sensor was installed in the main stem of the *A. marina* (Fig. 3.1a) seedling the negative SFD surely proved that water was transported downward through the stem. It is more surprising since the reversed SFD was found at around noon time when the transpiration demand by the plants is usually higher. Stem diameter variations recorded during that time also showed steep peaks (Fig. 4.2d).

Although the effect of rain simulation on SFD of *P. tremula* was not significant irrigation influenced the SFD significantly (Fig. 4.3b). The SFD of *P. tremula* under irrigated condition increased tenfold higher than the non-irrigated plant (Fig. 4.3a & b). The SFD of *A. marina* was always lower although it was irrigated twice in a day. From these findings it can be concluded that rain simulation on *A. marina* caused reversed sap flow, which ultimately results in downward hydraulic redistribution from leaf to stem (since petioles and stem were covered to prevent contact with rain water). Several findings under field conditions also suggested this reversed sap flow in *A. marina* during rainfall both day and nighttime (De Wal, 2011; Hoste, 2011; De Groote, 2013).

Since we carried out our experiments on a young seedling of *A. marina* (120 days old) grown in a small pot (Fig. 3.1a) there is no possibility of occurring reversed sap flow or hydraulic redistribution by the water potential gradient as a result of changing salinity throughout the different soil horizons as stated by Hao et al. (2009). A considerable time delay of about 3.5 hours between reversed flow at root level and at stem level was reported by Nadezhdina et al. (2009). However, during this experimentation, reversed SFD was found immediately (within 1 to 2 minutes) with the starting of the rain simulation. Hence another possible explanation for this reversed SFD during the rain simulation events will be discussed in the following section.
5.3 Foliar water uptake

In the normal process, water is up taken by the plant roots from the soil, which is subsequently transferred through the stem towards the leaves, where transpiration takes place. In combination with several strategies for uptake and use of water, plant growth and survival are influenced by the distribution and accessibility of soil water (Drake & Franks, 2003). However the ability of plants to thrive especially under harsh conditions puzzled many scientists since it appeared most unlikely that these plants could survive with only this known water transport system. This led to many research to clarify the pathway of water uptake under such adverse conditions. Based on the normal water uptake system via roots the reason of the instant reversed sap flow dynamics detected in our experiments remained unresolved and seems to indicate an additional water source. Yates and Hutley (1995) detected foliar water in *Sloanea woollsii* F. Muell., an Australian subtropical rainforest tree at a location 200 km south-west of Brisbane. It is already described in the Literature Review chapter that extensive research has been conducted towards FWU, especially under condensed fog and dew conditions (e.g. Stone, 1963; Louw & Seely, 1982; Kramer, 1983; Nobel, 1983; Yates & Hutley, 1995; Hutley *et al.*, 1997; Burgess & Dawson, 2004). But the foliar uptake of rain water has not been recorded that much, apart from Oliveira *et al.* (2005) and Breshears *et al.* (2008). Foliar uptake of intercepted rainfall could be particularly important in both dryland and saline wetland ecosystems and will be able to prevent the widespread tree mortality due to drought stress (Allen & Breshears, 1998; Breshears *et al.*, 2005).

Different possible pathways and structures have been suggested for FWU including cuticle (Vaadia & Waisel, 1963; Yates & Hutley, 1995; Limm *et al.*, 2009; Limm & Dawson, 2010), hydathodes (Martin & von Willert, 2000) and specialized trichomes (Franke, 1967; Rundel, 1982; Schimper, 1888) and narrow clefts between leaves (Lai *et al.*, 2007). The cuticle is not the impermeable layer as it was traditionally considered. It is capable of transmitting water to the epidermis and the leaf as a whole (Franke, 1967). Yates and Hutley (1995) also suggested that deposition of moisture from the saturated atmosphere onto the leaves was taken up by imbibitions through the cuticle in *Sloanea woollsii*. Cuticles are complex and dynamic in nature that can modify their permeability in response to varying environmental conditions (Schreiber *et al.*, 2001; Schönherr *et al.*, 2005; Rosado *et al.*, 2010). However the entry of water into leaves depends on several factors, including the wettability of the cuticle. The capability of water to wet a leaf surface is a function of its contact angle on that surface and hence the nature of a leaf surface as well as surface tension affects wettability.
Stomatal uptake of water, solutes and dispersed substances were also proved after the visualization of the stomatal uptake of nanoparticles via confocal laser scanning microscopy (Eichert & Goldbach, 2008). Recently Burkhardt et al. (2012) also confirmed stomata as another additional pathway for water entry especially in the presence of salts, which ended a 40 years old paradigm. These stomatal pathways play vital role in the transport of different ions/minerals and liquid water in the plant.

Hydraulic activation of stomata (HAS) for the transport of water is a recent idea whereby a continuous connection of liquid water along with the stomatal walls is established, as long as the threshold is exceeded (Burkhardt, 2010). HAS implies the formation of continuous thin films of liquid water on the stomatal walls connect the apoplast and the leaf surface, thereby enabling bidirectional transport of water and solutes between the leaf interior and the leaf surface (Burkhardt et al., 2012). Once the liquid water connection is established, it provides a continuous pathway for the transport of water and solutes as long as the stomata are not completely closed. Transport of water via this hydraulic connection through the stomata will be faster and efficient than across the higher cuticular resistance (Burkhardt, 2010).

Martin and von Willert (2000) observed FWU in Crassula species through specialized structures called hydathodes. These structures represent a gap in between the leaf cuticle and epidermis and they are ontogenetically derived from stomata. Their ability to exude water i.e. guttation (Dieffenbach et al., 1980) presumably correlates with their ability to absorb water. The findings of Martin and von Willert (2000) were particularly important, since the presence of hydathodes are observed in the leaves of some mangrove species (Tomlinson, 1986) as well, including Avicennia spp. (Negi, 1996). Furthermore, Tomlinson (1994) denied the involvement of hydathodes of mangroves in the process of guttation. Hence, this might be a sign of the possibility of FWU in A. marina, which would also explain the fact that this plant can survive in such harsh environments. In order to demonstrate the FWU, Burgess & Dawson (2004) and Eller et al. (2013) carried out isotopic analyses of the xylem sap as well as sap flow measurements. Direct foliar absorption was detected by the reversed direction of sap transport as well as by the presence of labelled isotope in the sap after fog event.

The combination of all these aforementioned factors leads to the hypothesis that reversed sap flow dynamics (SFD) recorded in A. marina (Fig. 4.2b) during the artificial rain simulation events are a result of FWU, which is also confirmed by the presence of significant proportion of δ²H in its petiole and stem (Fig. 4.5a). Furthermore the stem diameter variation also showed steepest peaks (Fig. 4.2d) immediately with the onset of rain simulation, which might also be linked to the FWU. While rainfall is known to stimulate stem diameter increase due to a sudden drop in xylem water potential, likewise during stomatal closure in the evening, here the growth peaks occurred at daytime when PAR was high (Fig. 4.1b), that
implies another mechanism. As the responses of diameter were immediate, and SFD was even negative (Fig. 4.2b) during these growth peaks, also rain water uptake from the soil can be excluded. Foliar uptake of rain water is the most plausible mechanism resulting in the observed diameter increases.

This FWU can be occurred by the following mechanisms. It was discussed in the Literature Review chapter that A. marina can partly exclude the dissolved salt in the water that is taken up by the roots. But a substantial portion, 10% or more according to Ball (1988b) of the salt is uptaken by the roots and transported through the tree. Upto 40% of this transported salt can be excreted by the salt glands on the leaves (Waisel et al., 1986), which is also shown in Fig. 2.9c. However, this indicates that water within the leaves are at least partly salty, which lowers the osmotic water potential. Therefore, moistening of leaves with fresh rain water ($\Psi_{\text{rain}} = 0$) develops a water potential gradient, which causes the fresh water to penetrate the leaves, with or without the help of hydathodes. In addition the soil was highly saline and $\Psi_{\text{root}}$ was not influenced by rain simulation (since covered with aluminum foil). Hence, the $\Psi_{\text{leaf}}$ is able to rise a value higher than the $\Psi_{\text{root}}$. Therefore this water potential gradient results in a reversed sap flux from leaves to roots, observed as negative SFD in the stem (Fig. 3.2b). Once within the plant, the water flows by diffusion from cell to cell and through the cell walls (short-distance transport) or by conduction through the xylem (long-distance transport) along a water potential gradient (Larcher, 1995).

5.4 Ecological significance of rain for A. marina

A significant proportion of annual rainfall is intercepted by the plant foliage under diverse ecosystems. Zinke (1967) reported this interception loss in conifers and hardwoods forests commonly 20% to 40% and 10% and 20%, respectively. Xiao et al. (2000) found a gross precipitation interception of 15% for the Pyrus calleryana tree and 27% for the Quercus suber tree. In the off-shore mangrove ecosystem dominated by Rhizophora mangle this canopy interception was recorded 16% (Wanek et al., 2007). This intercepted water is generally assumed to evaporate back into the atmosphere or leak to the soil without being directly absorbed (Breshears et al., 2008), a hypothesis held in different disciplines like in ecology (Waring & Running, 1998), hydrology (Brooks et al., 1991), and atmospheric science (Bonan, 2002). Although all precipitation events are intercepted by the plant foliage to a greater or lesser extent, only a few larger rainfall events considerably increase soil water content (Loik et al., 2004, Owens et al., 2006). Breshears et al. (2005) found Juniperus monosperma to survive under drought condition, where precipitation events were very limited and collectively insufficient to substantially increase soil moisture through the shallow depth to reach the active rooting zone (Breshears et al., 1997, 2005). Hence it was hypothesized that J. monosperma absorbed water through the plant foliage.
Precipitation in many tropical and subtropical regions occurs most frequently as medium to small events (Loik et al., 2004). Therefore, if substantial foliar absorption occurs from these medium to small rain events it could be more important for the survival of plant under such harsh condition than previously appreciated. Rainfall diminishes the VPD, which drives evaporation and transpiration from plant surfaces thus ultimately reduces plant water demand.

The ecological significance of FWU by vascular plants may be an issue of dispute in various groups (Vaadia & Waisel, 1963; Mooney et al., 1980), but it has long been recognized (Rodin, 1953) for many epiphytic Bromeliaceae. Despite the fact that wetting events of leaves are often considered to negatively affect the plant performance in regards of gas exchange and photosynthesis (Brewer & Smith, 1997) there is many evidence of improvements in plant water status resulting from FWU, leading to increased plant water potential (Grammatikopoulos & Manetas, 1994; Boucher et al., 1995; Yates & Hutley 1995; Gouvrà & Grammatikopoulos, 2003; Breshears et al., 2008; Simonin et al., 2009). Furthermore this water, uptaken by the plant canopy can play an important role in both plant stem and soil water redistribution, as well as the water balance of many ecosystems during drought events (Yates & Hutley, 1995; Burgess & Dawson, 2004; Breshears et al., 2008; Limm et al., 2009; Simonin et al., 2009). Additional physiological benefits of FWU are enhanced gas exchange after drying of the leaves (Grammatikopoulos & Manetas, 1994; Martin & von Willert, 2000; Simonin et al., 2009), improved survival ability (Stone et al., 1956; Vaadia & Waisel 1963), and even promoted plant growth (Boucher et al., 1995) by expansion of cells (Burgess & Dawson, 2004). A. marina in our experiment also showed a large diameter increase at the onset of rain simulation (Fig. 4.2d). But no such similar effect was found in the temperate species P. tremula. Irrespective to the mechanism of rainwater uptake, it is clear that the rain events are crucial for the survival of A. marina as without rain it clearly suffered from the stress conditions caused by the saline environment.

### 5.5 Future perspectives

Although several observations indicating foliar water uptake by the seedlings of A. marina were presented in this thesis, future research to further confirm this hypothesis by avoiding the limitations of our experiments would be very fascinating. Although we conducted our experiments under controlled environment in the greenhouse, to regulate the biotic and abiotic parameters, we failed to maintain higher light intensity similar to the tropical and subtropical region. The major limitation of our experiment was the lack of replication, since we had just a few seedlings of A. marina and only one of them was suitable for installing both LVDT and mini HFD sensors together.
Future studies with higher replicates will strengthen our findings. Further experiments with different duration and intensity of rain simulation and salinity levels should be conducted to see the effect of rain on growth and vitality of *A. marina*.

Further investigation to determine the critical amount of rainfall for the variation in stem diameter would also be interesting. The effect of rain on growth of *A. marina* can be compared with fog under controlled environments to evaluate their ecological and physiological significance on *A. marina*. Determination of sap flow to link the reversed flow with the foliar water uptake will be more logical than the sap flow dynamics. Measurement of water potential (which is missing in this thesis) in soil, stem, petiole and leaf level before and on the onset of rain simulation will be able to explain this foliar water uptake of *A. marina* more precisely. Foliar water uptake of *A. marina* can be further investigated by weighing the whole plant before and after submerging the leaves under water. Even though we used only *P. tremula* as control, the use of a reference plant from the same species is highly recommended during any experiment undertaken, especially with stable isotopes.
CHAPTER 6: CONCLUSIONS

The main goal of this thesis was to confirm the hypothesis of canopy water uptake, and its role to the growth of mangrove species *Avicennia marina*. Several ecophysiological parameters such as sap flow dynamics (SFD) and stem diameter variations (SDV) were measured together with the environmental variables like photosynthetically active radiation (PAR) and vapour pressure deficit (VPD) during the time span of the experiment. Canopy water uptake of rain water by *A. marina* was further verified by using toluidine dye and deuterium isotopes. All the results of *A. marina* were compared with a temperate species *Populus tremula*. Sap flow dynamics and stem diameter variations of both species were measured with mini HFD and LVDT sensors, respectively. The resulting data led to some fascinating conclusions regarding sap flow dynamics and stem diameter variation of *A. marina* during rain simulation. First of all, a strong correlation was observed between SFD and both VPD and PAR, which indicates that meteorological variables are one of the driving forces for sap flow in plant. Although there was a clear daily pattern in SFD with maximum value during the noon and minimum at night, rainfall had a great influence on it. The rain simulation events resulted in a sudden fall of SFD in both species, but the decrease in SFD was more prominent in *A. marina* than in *P. tremula*. Even during the day time under condition of higher PAR value, rain simulation on *A. marina* resulted in reversed flow.

SDV also showed a clear daily pattern of shrinkage and swelling and could be related to the internal turnover of water. Apart from this daily pattern of shrinkage and swelling some hydration peaks were noticeable during the rain simulation time. Similar to SFD those hydration peaks of SDV were also significant in *A. marina*. The steep expansion of stem diameter at the onset of rain simulation could be explained by the fact that rain simulation stopped transpiration but root water uptake continued for a while since a time lag between transpiration at leaf level and water uptake at root level exists. Although the refill of storage tissues can expand the stem diameter up to the night time diameter, but steepest peaks of stem diameter higher than night time were recorded during the rain simulation.

Since soil water potential corresponded well with inundated saline water and the rain water was prevented from reaching the root zone, the possibility of that the rain water reduced the soil water potential thereby facilitating root water uptake was rejected. Considering the hypothesis of foliar water uptake together with the observation of meteorological parameters and hydration peaks led to the hypothesis of foliar uptake of rain water. The water potential gradient originated between the salty xylem sap and the fresh rain water on the leaf surfaces is suggested as the main driving force for this FWU.
Further verification with dye also proved the foliar uptake of rainwater by *A. marina*, while no such indication was found in *P. tremula*. Moreover, the presence of deuterium in both petioles and stem of *A. marina* was considered as the ultimate proof of foliar uptake of rain water. From all the observations of this thesis it can be concluded that growth peaks in *A. marina* were resulted from the foliar uptake of rain water. Rainfall has significant contributions in ameliorating the overall plant-water balance and is also important for the survival of *A. marina* under physiological drought conditions. Since it is unlikely that rain will cause a substantial increase in the available soil water under saline condition, which is even inundated by saline sea water several times a day, so the foliar absorption of intercepted rain is the best mechanism for *A. marina* to survive such harsh environment.
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