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Coordinated Tree Responses to Drought -Vulnerability and Sustainable Production: Hypotheses on Arid Ecosystem Adjustments to Limitations in Water Resources

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Abbreviations and symbols

Symbol	Meaning	Units
Ψ	Water potential	MPa
Π	Osmotic potential	MPa
$\Delta\psi$	Change in water potential	MPa
ψ_L	Leaf water potential	MPa
Π^{100}	Osmotic potential at full turgor	MPa
$\delta^{13}C$	Carbon 13 isotope discrimination	MPa
ε	Modulus of elasticity	MPa
ε_{max}	maximum modulus of elasticity	MPa
Ψ_L	Leaf water potential	MPa
Ψmd	Midday leaf water potential	MPa
ψ_{pd}	Predawn leaf water potential	MPa
ψ_s	Soil water potential	MPa
ψtlp	Water potential at turgor loss point	MPa
E	Transpiration rate per leaf area	mmol m^{-2} s^{-1}
E_{max}	Maximum E	mmol m^{-2} s^{-1}
Gs	Stomatal conductance rate	mmol m^{-2} s^{-1}
Gs$_{max}$	Maximum gs	mmol m^{-2} s^{-1}
HPa	hecto Pascal	hPa
K_h	Hydraulic conductance (whole tree) –unit xylem area	mmol MPa^{-1} m^{-2} s^{-1}
K_{s-l}	Leaf specific hydraulic conductance- unit leaf area	mmol MPa^{-1} m^{-2} s^{-1}
LA	Leaf area	cm^2 or m^2
Ldwt	Leaf dry weight	g
MPa	Mega Pascals	MPa
MV	MilliVolt	mV
P	Turgor pressure	MPa
PAR	Photosynthetic active radiation	μmol m^{-2} s^{-1}
P-V	Pressure-Volume	
Q(tree)	Whole tree sap flux	kg h^{-1}
R*	Relative water content	%
r:s	Root:Shoot ratio	
R*tlp	Relative water content at turgor loss point	MPa
Rdwt.	Root dry weight	g

Symbol	Meaning	Units
SFD	Sap flux density	$Kg\ m^{-2}\ h^{-1}$
SWC	Soil water content	$m^3 m^{-3}$
T	Temperature	$^{\circ}C$
ΔT	Temperature difference	$^{\circ}C$
T_{min}	Minimum temperature	$^{\circ}C$
T_{max}	Maximum temperature	$^{\circ}C$
Tdwt.	Total dry weight	g
V	Volts	V
VPD	Vapor pressure deficit	hPa
VPD_{max}	Maximum VPD	hPa
VPD_{min}	Minimum VPD	hPa
WUE	Water use efficiency	

Acknowledgements

Abstract.

Field and controlled greenhouse experiments were carried out to investigate tree responses to declining soil water content. Field experiments were conducted on naturally growing trees of *Acacia tortilis* and *A. xanthophloea* in the savanna region of Kenya and *Quercus suber* in the Mediterranean region of Portugal. The selected field sites were regions that experience regular drought periods during the year. Greenhouse experiments constituted two watering regimes. Seedlings of *A. tortilis* and *A. xanthophloea* grown from seeds initially obtained from the Kenya field site were raised and arranged on a greenhouse bench into two groups per species. The first set of plants were watered every other day (controls) while the second set were watered every seven days (water stress treatments). Field measurements included weather parameters, soil and plant water status, growth, sap flux density, leaf transpiration and stomatal conductance, tissue water relations and isotope labeling. Similar measurements were conducted on plants growing in the greenhouse. Also examined in the greenhouse were root biomass, root structure as well as whole plant biomass accumulation. A second set of experiments was carried out in the greenhouse by subjecting plants initially stressed and non-stressed to severe water stress by withholding water until plants were wilted overnight. The wilted plants were then re-watered regularly and their recovery after stress alleviation was monitored.

Declining soil water content significantly affected plant water status in all the trees studied. Lowest Ψpd recorded during the study period occurred in the month of June and were -2.0 and -1.1 MPa for *A. xanthophloea* and *A. tortilis* respectively. The same species subjected to repeated water stress in the greenhouse attained mean minimum Ψpd of -2.4 and -1.2 MPa for *A. xanthophloea* and *A. tortilis* respectively at the end of the drying cycle. Mean minimum Ψpd recorded for *Q. suber* during summer was -1.8 MPa and occurred in September. There were however, significant differences among trees. Decline in Ψ associated with increasing soil drought led to decline in leaf initiation and leaf expansion and both processes ceased at higher water stress levels. For the *Acacia* species, even leaf shedding occurred at higher stress levels. There was also a decline in stomatal conductance (gs) during water stress, leading to decrease in transpiration rates (E). Maximum stomatal conductance of 340 mmol m^{-2} s^{-1} were observed during rainy seasons for the *Acacia trees* while mean maximum values of 300 mmol m^{-2} s^{-1} were recorded for *Q. suber* when soil moisture conditions were favorable. Stomatal conductance declined by 31%, 67% and 67% in *A. tortilis* and *A. xanthophloea* in the savanna and *Q. suber* in the

Mediterranean regions respectively. Daily tree water use ($Qtree$) as well as leaf transpiration reflected changes in Ψ and gs. Root to leaf hydraulic conductance equally declined with increasing soil drought. *Q. suber* trees adjusted osmotically by a magnitude of 0.7 MPa, while bulk modulus of elasticity (ε) increased by a magnitude of 17 MPa. Osmotic adjustment of 0.48 MPa was observed in greenhouse plants of *A. tortilis* while ε declined by a magnitude of 7 MPa in *A. xanthophloea*. *A. tortilis* plants in the greenhouse showed increased absolute root growth, root depth and root:shoot (r:s) ratio. The dimorphic rooting pattern in *Q. suber* resulted into hydraulic lift and this could as well occur in *A. tortilis* because of similarity in their rooting patterns.

Most plant responses were reactionary and were aimed at enhancing soil water uptake and reducing transpiration water loss when soil water content was declining. Similar responses were observed for both greenhouse and naturally growing field plants of the same species. Decline in leaf initiation and leaf expansion as well as leaf senescence reduced tree crown size hence potential tree transpiration. This however, had negative impact on plant productivity. Increased root growth as well as osmotic adjustment increased tree water uptake from the soil. The balance between root water uptake and leaf transpiration through growth and stomatal regulation was aimed at protecting xylem integrity. The overall results showed that soil characteristics, root activities and root distribution patterns are the main factors determining tree functioning and productivity in drylands, while the coordinated interaction between the aboveground shoot and belowground root activities ensures survival during drought. Maintained production and survival will ensure distribution and success in the arid environments. Repeated water stress imparted water stress resistance qualities on seedlings enabling them to survive longer during severe stress. The study emphasizes the role of soil resource base as well as species interactions in the functioning and balance of dryland ecosystems.

Zusamenfasung

Im Rahmen der vorliegenden wurden Experimente in Freiland und Gewächshaus durchgeführt, um die Reaktionen von Bäumen auf abnehmenden Bodenwassergehalt zu untersuchen. Freilanduntersuchungen wurden an natürlich wachsenden Bäumen der Arten *Acacia tortilis* und *A. xanthophloea* in der Savannenregion Kenias und *Quercus suber* in der mediterranen Region Portugals durchgeführt. In den ausgesuchten Regionen treten während des Jahres Dürreperioden auf. Die Experimente im Gewächshaus wurden unter zwei Bewässerungsbedingungen vorgenommen. Hierfür wurden Setzlinge von *A. tortilis* und *A. xanthophloea*, die aus im Freiland in Kenia gesammelten Samen gezogen wurden, im Gewächshaus in 2 Gruppen pro Art aufgeteilt. Die erste Gruppe wurde jeden zweiten Tag gegossen (Kontrolle), während die zweite Gruppe siebentägig gegossen wurde (Trockenstressbehandlung). Die Freilandmessungen beinhalteten Wetterparameter, Boden- und Pflanzenwasserzustand, Wachstum, Saftflussdichte, Blatttranspiration, stomatäre Leitfähigkeit und Gewebewasserzustand. Ähnliche Messungen wurden an den im Gewächshaus wachsenden Pflanzen vorgenommen. Hier wurden auch Wurzelbiomasse, Wurzelstruktur und Biomasseakkumulation der gesamten Pflanze untersucht. Im Gewächshaus wurde ein zweite Versuchsreihe durchgeführt, in der die ursprünglich gestressten und nicht gestressten Pflanzen erheblichem Trockenstress ausgesetzt wurden, bis die Pflanzen über Nacht welkten. Die welken Pflanzen wurden danach wieder regelmäßig gegossen und ihre Erholung nach der Stresserleichterung verfolgt.

Der abnehmende Bodenwassergehalt beeinflusste signifikant den Pflanzen-Wasserzustand in allen untersuchten Bäumen. Der niedrigste gemessene Wert für Ψpd während der Untersuchungsperioden wurde im Juni gefunden, und zwar -2.0 und -1.1 MPa für *A. xanthophloea* bzw. *A. tortilis*. Die selben Arten, die im Gewächshaus wiederholtem Trockenstress ausgesetzt waren, erreichten ein mittleres Minimum des Ψpd von -2.4 und -1.2 MPa für *A. xanthophloea* und *A. tortilis* am Ende der Austrocknungsphase. An *Q. suber* wurde ein mittleres minimales Ψpd von -1.8 MPa während des Sommers gemessen, und zwar im September. Es gab jedoch signifikante Unterschiede zwischen den Bäumen. Die Abnahme von Ψ in Zusammenhang mit steigender Bodentrockenheit führte zur Abnahme der Blattinitiation und Blattexpansion, und beide Prozesse hörten bei höherem Trockenstessniveau auf. Die *Acacia*-Arten warfen auch Blätter ab. Während des Trockenstresses verringerte sich auch die stomatäre Leitfähigkeit (gs), was zur Abnahme der Transpirationsraten (E) führte. Die maximale

stomatäre Leitfähigkeit von 340 mmol m^{-2} s^{-1} für die Akazienbäume wurde während der Regenzeit gemessen, die mittleren Maximalwerte von 300 mmol m^{-2} s^{-1} für die Korkeichen bei günstigen Bodenfeuchtigkeitsbedingungen. Die stomatäre Leitfähigkeit verringerte sich um 31 %, 67 %, und 67 % in *A. tortilis* und *A. xanthophloea* in der Savanne und *Q. suber* in der mediterranen Regionen. Der tägliche Wasserverbrauch der Bäume (*Qtree*) und auch die Blatttranspiration spiegelten Änderungen in Ψ und gs wider. Die hydraulische Leitfähigkeit zwischen Wurzel und Blatt verringerte sich gleichermaßen mit steigender Bodentrockenheit. Die Korkeichen-Bäume passten sich osmotisch in einer Größenordnung von 0.7 MPa an, während sich der Elastizitätsmodul (ε) in einer Größenordnung von 17 MPa erhöhte. Eine osmotische Anpassung von 0.48 MPa wurde bei den Gewächshauspflanzen von *A. tortilis* beobachtet, während sich bei *A. xanthophloea* ε um 7 MPa verringerte. Die *A. tortilis*-Pflanzen zeigten im Gewächshaus ebenfalls erhöhtes absolutes Wurzelwachstum, Wurzeltiefe und Wurzel-Spross-Verhältnis. Die dimorphe von *Q. suber* resultierte in diurnaler Wasserhebung („hydraulic lift"), und dies könnte aufgrund der ähnlichen Wurzelverteilung auch bei *A. tortilis* geschehen.

Die meisten Pflanzenreaktionen richteten sich auf verstärkte Bodenwasseraufnahme und reduzierten Transpirationswasserverlust wenn sich der Bodenwassergehalt verringerte. Ähnliche Reaktionen wurden für Pflanzen der gleichen Art sowohl im Gewächshaus als auch im Freiland beobachtet. Die Verringerung der Blattinitiation und -expansion sowie die Blattseneszenz reduzierten die Größe der Baumkronen und folglich der potentiellen Baumtranspiration. Dies jedoch hatte negativen Einfluss auf die Pflanzenproduktivität. Erhöhtes Wurzelwachstum ebenso wie osmotische Anpassung erhöhten die Wasseraufnahme der Bäume aus dem Boden. Die Ausgeglichenheit zwischen Wurzelwasseraufnahme und Blatttranspiration durch Wachstum und Stomataregulation zielte auf den Schutz der Xylemintegrität. Die Ergebnisse zeigten insgesamt, dass Bodencharakteristika, Wurzelaktivität und Wurzelverteilungsmuster die Hauptfaktoren für die Baumfunktion und Produktivität in Trockengebieten sind, während die koordinierten Interaktionen zwischen den oberirdischen Spross- und den unterirdischen Wurzelaktivitäten das Überleben während der Trockenzeiten sichern. Die Aufrechterhaltung der Produktion und das Überleben sichern Verbreitung und Erfolg in ariden Gebieten. Wiederholter Trockenstress verleiht der Keimlingen Trockenstressresistenz-Eigenschaften, die es ihnen ermöglichen, länger unter größerem Stress zu überleben. Diese Arbeit unterstreicht die

Rolle der Bodenressourcen ebenso wie die der Interaktion der Arten im Funktionieren und Gleichgewicht von ariden Ökosystemen.

Chapter One

General introduction and literature review

1.1. Introduction

About 40% of the total land surface of the globe are classified as arid and semi-arid lands (ASAL) (FAO 1998). These are areas where the ratio of precipitation to potential evaporation (PET) ranges from 0.05 to 0.65. Large areas of these drylands occur in Asia, Africa, the Mediterranean, Oceania and the Americas (UNEP 1997, Reynolds 2001) and are considered to experience varying degrees of heat and water stress (Mauat and McGinty 1998), limiting the establishment, growth and distribution of trees (Kramer 1980). In Africa, these conditions cover 13 million km^2, 43% of the continent's land area on which 270 million people or 40% of the continent's population lives (UNDP, 1997). Analysis of arid and semi-arid areas of Africa show potentially large losses of plant species diversity, with low landscape heterogeneity as a result of climate and land-use changes (Rutherford et al. 1999, Climate change 2001) and over-exploitation, leading to serious land degradation (Burley 1982). In Kenya, ASAL occupy about 38 million ha or 60% of the total land area, most of which is currently under great pressure from extended drought, sedentarisation of pastoralists and migration of people into these vulnerable areas (GOK 1986). The annual loss of the woody vegetation in the Kenyan drylands currently stands at approximately 19,000 ha. The loss of forest cover and other types of woody vegetation will lead to increasing scarcity of a wide range of forest products, environmental degradation and loss of biodiversity, unless urgent measures are taken to address the degradation process. Land degradation in ASAL is, however, difficult to repair and trees have a major role in their protection. This calls for new hypotheses that clearly define ecosystem interactions and can predict the extent and nature of ecosystem changes and plant species geographical shifts in the event of increased drought and changes in land-use systems. Such studies must be broad-based and aimed at designing adaptive and mitigating strategies with respect to ecosystem management, biodiversity conservation and vulnerability to stress. These are few or are lacking for the African ASAL.

Soil water availability is recognized as a key factor determining tree growth and activity, species composition and distribution as well as ecosystem functioning and long-term water, carbon and nutrient balances in the ASAL (Noy-Meir 1973, Walker and Noy-Meir 1982, Ehleringer 1994, Reynolds et al. 2004). Soil moisture recharge in the ASAL is mainly through rainfall, yet precipitation in these regions

are usually low and very erratic, leading to different patterns of soil moisture recharge (Ehleringer 1994, Reynolds et al. 2004). The characteristics of soil water uptake by the different plant species encountered in any given dryland ecosystem, therefore, are fundamental to understanding ecosystem functioning and the adaptations exhibited by different tree species, which enable their growth and success in the dryland. Generalized information related to root distributions and knowledge of the basic mechanisms of soil water extraction and transport by aridland species are a suitable starting point in addressing differences among trees with respect to their habitat preferences and ecological potentials. Extensive and deep rooting systems with a large active surface area over which water absorption takes place will facilitate soil water uptake by trees. For trees successful in arid habitats, a greater allocation of photosynthates to the roots as compared to shoots is found, and in some cases, absolute root growth is enhanced (Jones 1992, Scholz et al. 2002). This enables extraction of water from a large volume of soil or from a deep water table when the upper soil horizons are dry (Jones 1992, Jackson et al. 2000). As water becomes limiting, certain trees also decrease osmotic potential of the cell sap, thus increasing the gradient between soil water and root cells, maintaining water uptake, and promoting physiological activity despite a declining soil water content (Tyree and Jarvis 1982).

Patterns of uptake, use and redistribution of soil water by tree roots have considerable impact on the hydrological cycles of the terrestrial ecosystems (Jackson et al. 2000). Differences in rooting patterns exhibited by different plant species could have significant influence on ecosystem water use and balance (Jackson et al. 2000), a fact that may influence tree growth and distribution in the ASAL. Experimental evidence (Caldwell and Richards 1989, Dawson 1993, Ryel et al. 2002, Ludwig et al. 2003, Espeleta et al. 2004), show that root systems can transfer water from deep moist soils to the upper drier soil layers during the night, where it could be used at a later time by the same individual or other trees of the same or different species. This process has been termed as hydraulic lift (Caldwell and Richards 1989, or hydraulic redistribution: see Ryel et al. 2002). The volume of water transferred by hydraulic lift is quite substantial and could buffer species against the potential damaging effects of seasonal water deficits (Richard and Caldwell 1987, Caldwell and Richards 1989, Espeleta et al. 2004), improving their productivity as well as species diversity, ecosystem and water balance (Jackson et al. 2000). Hydraulic lift is therefore a beneficial mechanism that must have evolved with plant species successful in the ASAL in which plants can improve their own soil water status as well as ability to

meet daily water demands. Although the role of hydraulic redistribution in aridland stability and ecosystem functioning is recognized and already appreciated, little is known on how widespread it is in aridland species as well as the exact quantitative contribution of hydraulically lifted water to the transpiration stream, hence productivity (Jackson et al. 2000).

Overall tree performance in drylands, however, cannot be evaluated without considering constraints within the plant that will influence overall carbon gain (Ehleringer 1994). For example, trees with an effective water supply system may lack specific adaptations for controlling water loss, resulting in low tissue water status that will affect overall plant performance (Levitt 1980, Kramer 1980). Thus, as soil water supply becomes limited due to increasing drought, high transpiration rates will result in decreases in leaf water potential (Ψ_L), leading to large gradients in water potentials between root and leaf and may reach a point at which cavitation occurs (Tyree and Sperry 1989). Decreases in maximum stomatal conductance and increased sensitivity of stomata to changes in water status may be required to maintain leaf water potential above a critical threshold and to avoid cavitation in the xylem (Tyree and Sperry 1989, Tyree and Ewers 1991, Jones and Sutherland 1991). In certain tree species however, stomatal conductance has been observed to decline long before any noticeable change in soil water content is recorded, imposing an early restriction to CO_2 uptake (Sperry 2000). Stomatal regulation of water loss must, therefore, balance transpiration with efficiency of water supply to the leaves so that dangerous decrease in Ψ_L is avoided without unnecessary restriction of carbon gain (Meinzer 2002). There is growing evidence that transpirational water loss may be reduced via changes in hydraulic conductance of the water-conducting pathway (Hubbard et al. 1999, Sperry 2000, Schultz 2003). In this case, increased hydraulic resistance at the root surfaces will lead to steep gradients in Ψ in the soil-plant system during the day, even when soil water conditions are favorable, potentially initiating stomatal closure to avoid cavitation (Tyree and Sperry 1989), and limiting daily water use from the soil water store. This has the ecological advantage of reducing excessive tree water use when soil water is abundant and prolonging the period with favorable soil water status, but it reduces the potential maximum stomatal conductance, hence potential productivity (see Figure 1).

Controversy still surrounds the regulation of both liquid and vapor phase conductance (Jones and Sutherland 1991, Meinzer 1993, Slindra et al. 1995, Cochard et al 2002, Mainzer 2002). However,

there is a consensus that stomatal closure during water stress is associated with maintenance of xylem integrity through regulation of changes in Ψ_L (Tyree and Sperry 1988, 1989, Cochard et al 2002). Differences in the diurnal and seasonal fluctuations in Ψ_L among individual trees or species at a common site may, therefore, reveal differences in access to soil water reserves at varying soil depths and water conducting behavior between roots and shoots, which in turn influences stomatal responses (Jones and Sutherland 1991). Differences in stomatal behavior must, therefore, not only depend on differences in sensitivity to environmental factors associated with the development of water deficit, such as high evaporative demand, high temperatures, or low leaf or soil water potentials (Davies and Zhang 1991), but also on the development of the root system (Larcher 2003). Such differences in stomatal sensitivity between species expressed during the development of drought would serve to limit transpiration and compensate for differences in vulnerability of the xylem with respect to cavitation (Tyree and Sperry 1988, Jones and Sutherland 1991, Ehleringer 1994). Since gaseous exchange, primary productivity and plant fitness are linked with each other (Ehleringer 1994, Saliendra et al. 1995), continuous monitoring of transpiration through sap flux measurements on trees growing at a common location coupled with instantaneous measures of gas exchange activity at single points in time during the development of drought, must provide reliable information related to species performance and ecological potentials. In certain tree species, reduction of water loss is also achieved through reduction of total leaf area (LA) (Turner 1986, Munne-Bosch and Alegre 2004). Reduction in LA is achieved either through reduced leaf size, leaf rolling or leaf shedding as soil water becomes limiting, thus reducing the transpiring leaf surface but with significant negative impact on carbon gain and overall plant productivity (Jones 1992). Thus, monitoring of leaf phenology may equally provide valuable information regarding species fitness.

In summary, the overall adjustment mechanisms that occur during the development of drought must be the result of coordinated behavior responses between the aboveground shoot system and belowground root system (Bradford and Hsiao 1992) and must depend on species differences in root growth potentials, accessibility to water at varying soil depths, root activity and water transport efficiency. The capacity to access and maintain stable water supply to the shoots as well as to effectively maintain communication between roots and shoots, which in turn ensures a balance between water supply and transpirational water loss must determine success of individual tree species in arid habitats. The

perceived role of soil and root water relations with respect to shoot responses has implications for our understanding of adaptations to stress. In particular, the relative importance of the root as the determinant of plant water status and productivity during water deficits must depend on the adaptations of the roots as well as of the shoots to water deficits, but more so on their coordinated activity during the development of drought and throughout the drought period.
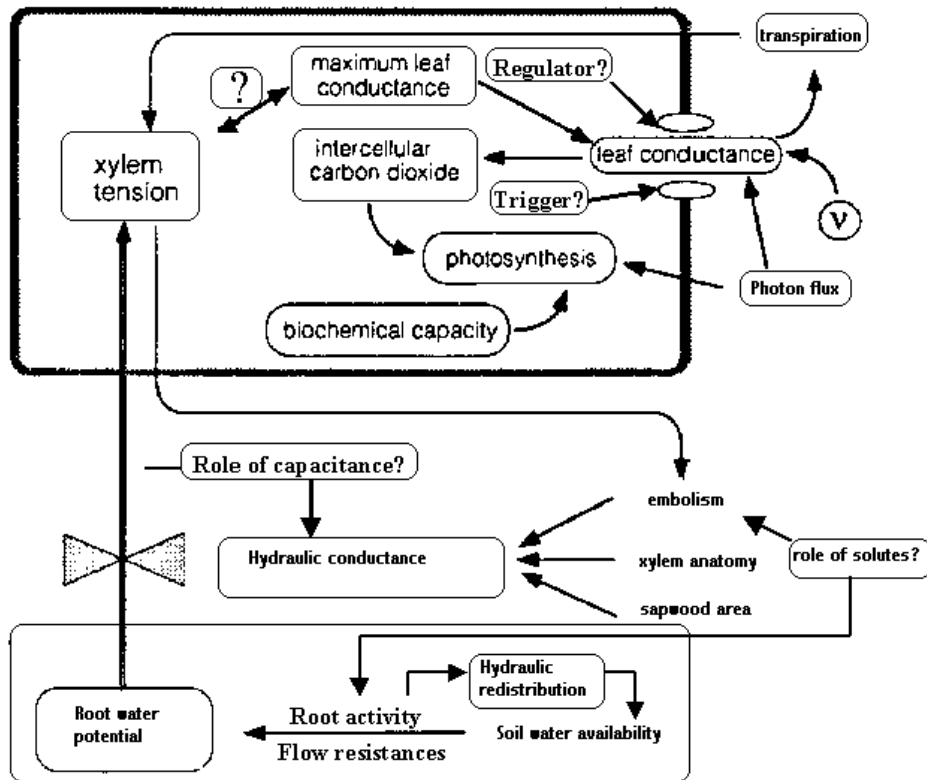
Figure 1. A generalized model of coordinated tree responses occurring during the development of soil water stress and how they could impact on the overall tree productivity. Processes that are not well understood are marked with question marks. Adapted from Ehleringer (1994)

In this study, intensive investigations were carried out to understand the role of root water uptake patterns in influencing shoot responses that determine plant water status of aridland tree species during drought and to determine how coordinated interactions between root and shoot contribute to tree productivity and, thus, allow establishment and survival of trees in aridland ecosystems. It was hypothesized that root distribution plays a major role in water uptake from soil, however, maintenance of favorable plant water status, productivity and survival of tree species during drought is the product of timely and coordinated regulation of water uptake, transport and loss. Implications of the perceived

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role of root distribution as well as coordinated responses for determining soil and ecosystem water balance (and therefore species distribution and survival) in arid ecosystems were explored.

The study was carried out in two phases. Phase one comprised of two field experiments conducted on naturally growing tree species in their natural environments. The two regions where the field experiments were conducted were those that experience extended dry periods during the year with erratic rainfall patterns. These were dry savanna ecosystem in Kenya (experiment 1) and a Mediterranean-type ecosystem (modified savanna-type) in Portugal (experiment 2). The information was used to develop common hypotheses regarding plant interactions and productivity in arid environments. Coordination of response to drought should be a general phenomenon. Thus, it was further hypothesized that a similar suite of response characteristics would be apparent at both sites, and that a similar shift in characteristics will occur with changes in root system access to soil water stores. As a result of difficulties encountered during the field studies in the Kenyan savanna due to lack of suitable equipment, some of the intended investigations were not carried out. However, extensive investigations were carried out during the field studies in Portugal and the results have been extrapolated to explain some of the observed field responses in the Kenyan savanna, when there were similarities in behavior patterns. Phase two of the study comprised controlled greenhouse experiments. Seedling of *Acacia* trees studied in the field in Kenya were raised and subjected to different watering regimes before specified measurements were carried out. It was hypothesized that even under controlled conditions, a similar suite of responses can be identified. The particular responses to drought should be of further value in considering use of these species in reforestation efforts and should guide in the development of methods appropriate for reaching such goals.

1.2. Literature review

1.2.1. Savanna ecosystems

Savannas are tropical or sub-tropical ecosystems characterized by the presence of a dominant continuous graminoid stratum and a discontinuous woody stratum that forms the upper canopy of the vegetation, often strongly affected by seasonal changes of rainfall (Walker and Gillison 1982, Lüttge 1997). They have alternating wet and dry seasons, receiving rainfall amounts between 150 – 500 mm per year, falling within relatively short time period (Tinley 1982). Tropical savannas occupy 65% of Africa, 60% of Australia and 45% of South America. The tropical savanna of Africa is one of the least researched and poorly understood terrestrial ecosystems of the world (Menaut 1983). However, their immense contribution in terms of food production as well as other socio-economic benefits, which are vital for the people inhabiting these areas have been recognized. The demand to develop them without compromising their sustainability as well as predicting their status in an event of climate change has called for the understanding of how they function.

Moisture conditions within the savanna are determined by precipitation, which is strongly seasonal (Tinley 1982, Walker 1987). Areas that experience high amounts of rainfall remain wet during most part of the year and are referred to as moist savanna. Areas associated with long dry spell and short rainy periods are referred to as arid savanna (Lüttge 1997). Trees in arid savanna have been categorized as deciduous or evergreen and this phenological classification is thought to be mainly dictated by their rooting patterns or localized variations in the soil moisture conditions (Menaut 1983, Goldstein and Sarmiento 1987). Thus, most evergreen formations within the savanna are composed of trees with well-developed root systems, which are able to access stable water sources or they occur on sites with adequate moisture, available during the dry season. Survival of trees in the arid savannas must therefore be associated with availability of favorable local situation, development of xeromorphic aboveground shoot structures such as leaves or well-developed rooting system or both. Success of a given set of characteristics will depend on how they enhance efficient resource use by plants which posses them, rapid growth and reproductive success.

A well-developed root system allows the plants to survive drought by exploring a large volume of soil and ensure adequate water supply despite declining soil water availability. Trees that are successful in

the dry savanna have an elaborate and extensive root system, most of which are found within the upper 30 cm of soil (Menaut 1983). According to Ogle and Reynolds 2004, the ability to access both shallow and deep soil water by trees growing in arid ecosystems is advantageous since it allows plant species to use precipitation pulses of different sizes, duration and timing and, thus, improve their capacity to acquire nutrients for productivity and survival during drought. Studies conducted on *A. tortilis* trees growing in the East African savanna have shown that the trees posses both deep penetrating and shallow spreading root system (Belsky 1994, Ludwig et al. 2003). Walter (1973) has, however, shown that trees in arid savanna tend to have their roots flattening out within the upper soil layers in order to provide the best opportunity to absorb water after a relatively light rain. Studies conducted in West Africa savanna have also shown that almost all the root biomass is found in the upper 50 cm of the soil and tap roots, when present, rarely enter deeply into the soil (Menaut 1983). Rutherford (1980) found both deep and shallow roots in a study conducted in a sandy South Africa savanna even though lateral roots were dominant over taproots. Hopkins (1962) noted that between 10 and 20 cm below the ground surface, the savanna soil is occupied by a network of long twisting roots of trees and shrubs. These results, however, contradict those by Lopez et al. (2001), who showed that lack of water within the upper soil layers restrict root growth despite high nutrient availability in these layers and root distribution may be concentrated in the deeper soil horizons. Thus, controversy still surrounds rooting patterns of the savanna trees and studies are required to better understand water acquisition and survival strategies employed by trees to evade desiccation during the long drought periods.

Accessibility to soil water reserves and effective root water uptake, however, must be accompanied with responsive and effective regulation of water loss to ensure favorable plant water status, given the high atmospheric vapor demand associated with the dry savanna environment. Xeromorphism in the savanna trees thus, manifests itself through a wide range of morphological and physiological adaptations aimed at increasing soil water uptake and reducing water loss. The understanding of root water uptake patterns by savanna trees and the associated shoot responses aimed at regulating water loss during drought must, therefore, provide crucial information with respect to species survival and productivity during declining soil moisture conditions and could shed more light on the general functioning of dryland ecosystems. This is a step towards designing new hypotheses aimed at sustainable management of this valuable ecosystem type. Based on the broad objectives set out for the

entire study, the current experiments conducted in an arid savanna of Kenya were aimed at examining how rooting patterns and root activity of two indigenous *Acacia* species may influence tree growth and plant responses in a changing soil moisture conditions. Implications of species morphological and physiological responses to plant growth, distribution and success in the ASAL are discussed.

Eighty percent (473,000 km^2) of Kenya's total land area lies in the savanna, most of which receives rainfall amounts below 350 mm and are regarded as ASAL (Burley 1984). These are very important areas, supporting up-to six million of Kenya's people (and particularly its poorer people), who derive most of their livelihood, including shelter, food, fuel, timber, medicine and aesthetics from natural resources in the ASAL (GOK 1986). They are also important for Kenya's tourist industry (the country's second biggest foreign exchange earner). Thus, with an economy greatly dependent on an already highly exploited natural resource base, economic and social development in Kenya is inextricably linked to issues of environmental protection and management, and particularly the ASAL, which covers a large portion of the land area.

Currently, most of the Kenyan ASAL is facing degradation as a result of increasing drought intensities and frequencies, over-grazing from both wild and domestic animals, intensive cultivation, expansion of farming and grazing into environmentally sensitive areas and the general over-exploitation of vegetation resources. This degradation process has been aggravated by lack of clear management guidelines for dryland forest resources management and serious constraints to tree establishment (Hector and Ryan 1996). Environmental degradation in areas with such harsh ecological conditions is difficult to reverse, and tree regeneration based on sound and sustainable management practices must play a major role, in order to realize this objective and prevent further degradation. The most promising adaptation to declining tree resources in the ASAL is regeneration of indigenous species (Climate Change 2001). However, success rates of establishing trees in ASAL have been low (Hector and Ryan 1996, Oba et al. 2001), calling for new approaches. Since biomass production in the arid savanna appears to be determined by soil water availability, the diversity of rooting habits, hence water acquisition patterns by savanna plants is the likely explanation for the highly variable productivity patterns in this ecosystem and could be a starting point in deriving new hypotheses related to tree establishment, productivity and survival in arid ecosystems.

1.2.2. Mediterranean Ecosystems

Mediterranean-type ecosystems (MTEs) are distributed worldwide and have regional importance as a vegetation formation adapted to the characteristics of mediterranean climates (Moreno and Oechel 1995). MTEs occur on all the world continents at 30° to 40° latitude; in the Mediterranean basin, in California, in Chile, in the Southern and Southwestern Cape Province of South Africa, and in Southwestern and parts of Southern Australia (Hobbs et al. 1995). The vegetation of the MTEs has often been discussed in terms of convergent evolution with respect to basic form and function (Cody and Mooney 1978, Cowling an Campbell 1980, Milewski 1983). The fundamental characteristic of this ecosystem type is the ability to cope with large changes in climate conditions, e.g., a pronounced drought in summer and cool wet conditions in winter (Nahal 1981). This reflects an inbred capacity to adaptively respond to changing natural conditions and, thus, to survive despite being confronted with unpredictability (Joffre et al. 1999).

A unique Mediterranean-type ecosystem, namely the *dehesa* in Spain and *montado* in Portugal, dominates the southwestern Iberian Peninsula, covering 2 to 2.5 million ha in the southern regions of Spain and Portugal (Joffre et al. 1988, 1999). These are man-made savanna-like ecosystems characterized by an open tree layer, mainly dominated by the evergreen oak species – holm oak (*Quercus ilex* L.) and cork oak (*Quercus suber* L.), and a herbaceous layer composed of grasses and herbs. A structure is achieved through management intervention that mimics the structure of tropical savannas (Infante et al. 1997, Joffre et al. 1999). Nevertheless, tree species establishment, distribution and mortality seems partly controlled by edaphic and climatic factors and could be the product of an ecological optimality with regard to water availability (Joffre et al. 1999, Ribeiro et al. 2003). Soil water is, therefore, recognized as a key factor determining the functioning of the mediterranean ecosystem (Walker and Noy-Mier 1982, Goldstein and Sarmiento 1987, Brown and Archer 1990), while efficiency in soil water uptake by trees is the factor ultimately determining their productivity.

For any given species, the efficiency for soil water uptake, which determines tree water status, primarily depends on root density and distribution (Crombie et al. 1988), the existing water potential gradient between the root cells and soil (Tyree and Jarvis 1982) and the soil characteristics, which determine water flow resistance between soil and tree roots (Rambal 1984, Bréda et al. 1995). Differences in sensitivity to drought among tree species or individuals growing in the mediterranean

ecosystem must, therefore, be the result of differences in access to and uptake of soil water. Understanding the characteristics and patterns of soil water uptake by tree species is then of paramount importance to understanding plant interactions, species distribution range and success in the mediterranean ecosystems.

The mediterranean climate is associated with cold and wet winters and hot and dry summers. Low winter temperatures limit photosynthetic activities and plant productivity confining most of the plant growth activities to the spring and fall periods (Tenhunen et al. 1984, 1987a, 1989a, and 1990). The productive capacity during summer is hindered by the summer drought, associated with high temperatures and low rainfall, leading to high evaporative demand and low soil water availability during most of the potential growing period (Tenhunen et al. 1984, 1987b, 1989b, and 1990). Development of drought is usually a gradual process, providing sufficient time for co-ordinated plant responses; many of, which represent adaptations that enhance survival during stress (Bradford and Hsiao 1982). Success must, therefore, depend strongly on how trees respond to the summer drought since mechanisms employed by different species in response to water stress determine their productivity, distribution and effective competitiveness (Bradford and Hsiao 1982, Cochard et al. 2002). Accurate understanding of the integrated behaviour of trees over the entire drought period must then provide information related to tree productivity and survival in arid environments.

Since tree species in the mediterranean regions have evolved with water stress as one of the major environmental constraints, overall responses to water stress adjust water supply and water use to ensure a productivity that will lead to species propagation (Tenhunen et al. 1990, Sala and Tenhunen 1994, Martine-Vilalta et al. 2002, David et al. 2004). Stomatal closure is regarded as the main mechanism employed by the evergreen tree species in the mediterranean ecosystem to regulate transpiration water loss and avoid dehydration damage to cells and tissues (Tenhunen et al. 1989b, Sala and Tenhunen 1994, Martine-Vilalta et al. 2002). This regulatory mechanism must, however, strike a balance between allowing CO_2 uptake to proceed while restricting excessive loss of water, ensuring that leaf water potential does not become too negative to lead to a breakdown in plant's hydraulic system (Jones and Sutherland 1991, Sperry 2000, Schulz 2003). Thus, stomatal closure occurs when the saving of water, that might be achieved by restricting canopy development or by the maintenance or even enhanced water supply as a result of root development and/or osmotic adjustment, are not enough to prevent leaf water potential from becoming dangerously negative (Bradford and Hsiao 1982). Differences in

sensitivity to water stress among tree species must, therefore, depend on differences in root water supply as well as the capacity to achieve an appropriate balance between canopy and root system extent. Therefore, tree species or individuals with an ample root water supply, as well as a large and effective root system will show no signs or only delayed signs of water stress during the development of drought (Larcher 2003). Deriving maximal productivity during drought, however, depends on timely and effective co-ordination between root and shoot activities, and must ensure success of tree species in mediterranean regions. For example, stomata should remain open for CO_2 uptake as long as root water supply and environmental conditions are favourable, but not so long so that tissue water status is compromised or that damage occurs (Bradford and Hsiao 1982). Root water supply before and during water stress must be regulated to ensure extended soil water availability, possibly through limitation of water use even when soil water is abundantly available.

A number of factors appear to be involved in the regulation of stomatal conductance and leaf water status of plants growing in the mediterranean regions. These include chemical messaging emanating from dehydrating roots (Davies and Zhang 1991) and factors related to plant hydraulics (Nardini et al. 1999, LoGullo et al. 2003). Recently, it has been proposed that stomata may respond to changes in leaf water potentials caused by drought-induced changes in the flow of sap from soil to the leaves (Meinzer and Grantz 1990, Sperry 2000, Cochard et al. 2002, Cruiziat et al. 2002). Changes in stomatal conductance have been equally observed to be associated with changes in environmental factors, especially vapour pressure deficit (VPD) (Schulze et al. 1987, Sala and Tenhunen 1994, Jones and Sutherland 1991). Despite the controversies surrounding stomatal regulation and function, there is a consensus that stomatal closure during water stress has the sole purpose of maintaining integrity in the xylem water transport system and to avoid dehydrative damage of plant cells and tissues (Tyree and Sperry, 1988, 1989, Jones and Sutherland 1991, Sperry 2000, Cochard et al. 2002, Cruiziat et al. 2002).

Regulation of stomatal conductance must be linked to the hydraulic conductance of the soil to leaf pathway (Ks-l) to achieve a balance between water supply from the roots and leaf transpiration in order to maintain Ψ_L at safe levels during the development of drought (Jones and Sutherland 1991, Sperry 2000, Hubbard et al. 2001, Sperry et al. 2002). Under steady-state, the relationship between gs and Ks-l can be described by a simple Ohm's law analogy as:

gs = C[Ks-l]

where $C=[\Psi soil-\Psi_L]/D$. *D* is the vapor pressure deficit and $(\Psi soil-\Psi_L)$ is the water potential gradient between root and leaf driving the flow (Jones and Sutherland 1991). The relationship demonstrates the dependence of stomatal regulation on changes of hydraulic conductance of the soil-leaf pathway, soil moisture and evaporative demand (Salindra et al. 1995, Hubbard et al 2001). Depending on how stomata regulate Ψ_L, different patterns emerge from this relationship (Hubbard et al. 2001). A linear relationship between Ks-l and gs (*isohydric*) would mean a constant magnitude of stomatal control on Ψ_L while a curvilinear relationship (*anisohydric*) means a gradually increasing intensity of stomatal regulation of Ψ_L with increasing drought stress (Hubbard et al. 2001). Differences in the diurnal behavior of Ψ_L during the development of drought between individuals of a species growing at a common location can therefore be used to explain and predict patterns of plant water use with respect to soil water availability as well as localized long-term modifications of the water conducting pathway aimed at optimizing soil water uptake at the root surfaces as well as water transport to the shoots. This has ecological implications with respect to plant productivity, distribution and reproductive success (Meinzer 2002).

How roots and shoots interact during the development of drought must, therefore, determine success of plant species in the mediterranean regions. Since roots and shoots are linked through a hydraulic pathway, characterizing changes associated with plant hydraulics during the development of drought may improve our understanding of coordinated tree responses and the coupling of canopy processes to soil environment and their adaptive significance. It was hypothesized that differences in plant water use as well as stomatal conductance observed among trees growing in the mediterranean regions are the result of differences in soil water access and root water uptake. It was further hypothesized that the co-ordination between the gaseous and liquid phase conductance in trees imposes a limit to tree water use at varying levels of soil water status leading to optimal utilization of soil water resources by *Quercus suber* trees.

1.3. Description of experimental tree species

Trees and shrubs provide ecosystem services of carbon sequestration, storing and transpiring of water required for precipitation, maintaining soil fertility and forming habitats for a diverse array of plants and animals (Climate Change 2001). Also, they provide valuable products that are of economic benefits to the local population, such as food, fuel, medicine, timber, cork etc (FAO 1999).

1.3.1. *Acacia xanthophloea* Benth. and *A. tortilis* Forsk. Hyne

The genus *Acacia* is currently drawing great interest for the improvement of tree cover in the Kenyan arid and semi-arid areas. This is due to their drought stress resistance (Oba et al. 2001) and value for multi-purpose use, e.g., as fodder for animals, sources of wood and non-wood products such as gums, resins and pharmaceuticals, provision of shade and live fencing (Anon 1980) and in maintaining soil fertility through nitrogen fixation (Belsky et al. 1989). Although it is generally known that most *Acacia* trees are drought tolerant (Oba et al. 2001), knowledge is lacking on how different physiological and morphological mechanisms interact to bring about water stress tolerance as well as maintained productivity during drought in indigenous Kenyan species. *Acacia xanthophloea* and *A. tortilis* were selected for detailed examination as prospective candidates for savanna restoration due to their characteristics, *A xanthophloea* being a highly productive species, growing very rapidly when conditions are favorable (Otieno et al. 2001) while *A. tortilis* being drought tolerant, having the ability to hydraulically lift water from the deep soil reserves (Ludwig et al. 2003), a quality that could improve ecosystem and water balance as well as boost productivity and species diversity in the Kenyan savanna.
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The two species naturally have contrasting habitat preferences, with *A. tortilis* found in more xeric eastern and northern provinces of Kenya while *A. xanthophloea* occurs in the mesic lowlands (Noad and Birnie 1987). This distribution pattern could be influenced by differences in abilities to cope with water stress, a fact that can be influenced by differences in rooting patterns and root water uptake. In the recent past, however, distribution of *A. xanthophloea* has been seen to shift to the drier areas, where these trees have been either introduced as ornamentals or become established through natural dispersion, suggesting an ability to adapt to environmental change or perhaps, the result of favorable local situations such as soil quality or improved water balance due to species interactions. In Kibwezi, a

semi-arid part of Kenya, experiment 1 was conducted, the two species were found growing together naturally in the same locality, providing a unique opportunity to investigate their responses to changing soil water under similar natural environmental conditions.

1.3.2.Quercus suber L.

Quercus suber (Cork oak) is indigenous to the Mediterranean region where it occurs in open woodlands on hills and lower slopes. It is the main source of industrial cork and is one of the most important woody species occurring in Portugal, occupying about 713,000 ha (Sauer 1993, Ribeiro et al. 2003). It is described as drought tolerant and can grow on very poor soils due to its structural and physiological adaptations (Oliveira et al. 1992). *Quercus suber* stands dominate the Atlantic-influenced western areas of Portugal, a distribution pattern, which is thought to be influenced by soil qualities, soil water availability and the ability of this species to cope with water stress (Sauer 1993). Ecophysiological responses of the aboveground shoots of *Q. suber* trees in response to summer drought in their natural habitats as well as under controlled greenhouse conditions are well studied and mechanisms underlying these shoot responses are well understood (Tenhunen et al. 1984, 1987a, Oliveira et al. 1992, Nardini et al. 1999, LoGullo et al. 2003). However, detailed studies on coordinated responses between aboveground shoot and belowground root structures of *Q. suber* trees during the development of water stress in their natural environments is lacking. Understanding the role of root processes as well as the link between root and shoot with respect to utilization of soil water resources could shed more light on the responses exhibited by the aboveground shoot structure as observed in this species during the development and throughout drought, as well as its distribution patterns in the mediterranean region of Portugal.

1.4. Research objectives

The main objectives of the study were to:

1. Assess seasonal changes in soil moisture and plant water status

2. Assess root distribution and soil water uptake patterns and how they relate to water use by trees under varying soil moisture conditions.

3. Establish how trees coordinate transpiration with root water uptake in order to avoid the occurrence of catastrophic cavitation during water stress.

4. Establish the broad implications of the above responses with respect to plant productivity and survival in arid environments and how they relate to dryland ecosystem functioning.

5. Establish whether responses exhibited by naturally growing mature trees can be mimicked in a greenhouse situation and whether greenhouse information may be used to design appropriate nursery procedures that will identify and adequately prepare plant materials for out-planting in the aridlands.

Chapter Two

Materials and methods

2.1. Site description, plant material and climate

2.1.1. Experiment 1 - savanna region of Kenya

2.1.1.1. Location

A field site with natural vegetation was identified in Kibwezi, Eastern Province of Kenya, approx. 250 km SE of Nairobi (37^0 88´E, 02^0 35´N). Site selection was dictated by its being within the semi-arid area and having a long history of insufficient and erratic rainfall with well-defined seasonality. An experimental plot measuring 40 m by 25 m was identified within the University of Nairobi's Dryland Research field station 25 km west from Kibwezi town. Both *A. tortilis* and *A. xanthophloea* were growing naturally side by side within the plot. The soils here were undisturbed except for grazing activities from domestic and wild animals. Soils were red in color (rhodic) belonging to the ferrolsols, predominantly sandy-clay-loam (45%) and the remaining was loamy sand (35%), with an average supply of nutrients (high in K and Mg) (Mbuvi 1991).

2.1.1.2. Climate

Rainfall patterns in Kibwezi are seasonal and erratic, being greatly influenced by the Inter-Tropical Convergence Zone (ITCZ), which brings in rainfall from the Indian Ocean through the south-easterly monsoon winds (Kinyamario and Imbamba 1992). The mean annual rainfall for the area over the past 10 years is 450 mm as provided by the Kibwezi weather station of the Kenya Meteorological Services (KMS) located about 10 km away from the study site. Rainfall amounts received in the individual seasons are however, highly unpredictable. Mean annual temperatures are 24 ^{0}C. Figure 2 shows rainfall amounts and distribution between 2000 and 2002. Data was obtained from University of Nairobi's weather station at Kibwezi, located 2 km away from the study site. During this period, the long rains were received between November and January of each year. Short rains occurred between April and May.
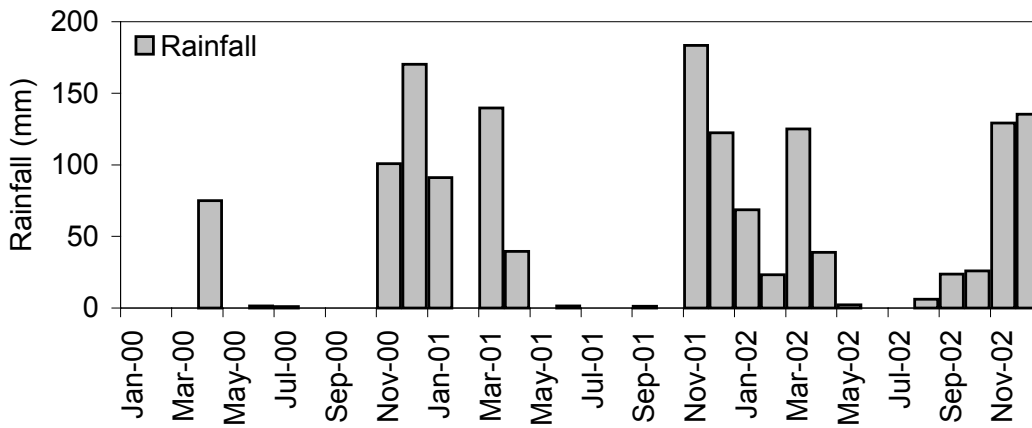
Figure 2. Monthly rainfall recorded in Kibwezi between January 2000 and November 2002.

2.1.1.3. Vegetation

The vegetation type was *Acacia-Cynodon* savanna*,* with sparse tree distribution. The overstorey consisted of *Acacia* trees while the understorey was dominated by the grass *Cynodon dactylon*. Mean tree height and diameter of different tree species found growing within the study plot are shown in table 1 while their heights and tree circumferences at breast height are shown in figure 3. The ground was completely covered with grasses and herbs during the rainy season, but these immediately dried out with the onset of drought, leaving the soil bare, except for the trees, which are perennials. Young trees or seedlings were not found at the site, except for two young *A. tortilis* with mean stem diameter of 2 cm. Lack of regeneration could be the result of grazing or extended drought that hinders growth and establishment of seedlings. Some irrigation was being practiced in the nearby university farm, and a river flows 100 m away, parallel to the plot, on the southern side. The study plot was at an elevation of 5 –10 m above the river surface. The probability of existence of a reachable water table was, therefore, high. Continuous measurements were conducted between December 2001 and June 2002 and discontinuous measurements in December 2002 and January 2003.

Table 1. Tree species, mean tree height and diameter of tree samples within the experimental plot.

Tree Species	Mean height (m)	Mean stem diameter at ground level (cm)	n
Acacia melifera	2.65 (±0.20)	8.28 (±0.90)	2
A.kirkii	6.97 (±4.32)	19.34 (±11.48)	3
A.senegal	4.41	5.40	1
A. tortilis	4.36 (±2.60)	12.37 (±9.70)	8
A. xanthophloea	10.06 (±2.70)	23.53 (±12.60)	11
Commiphora africana	4.18	8.92	1
Dichrostachys cinerea	3.63	11.46	1
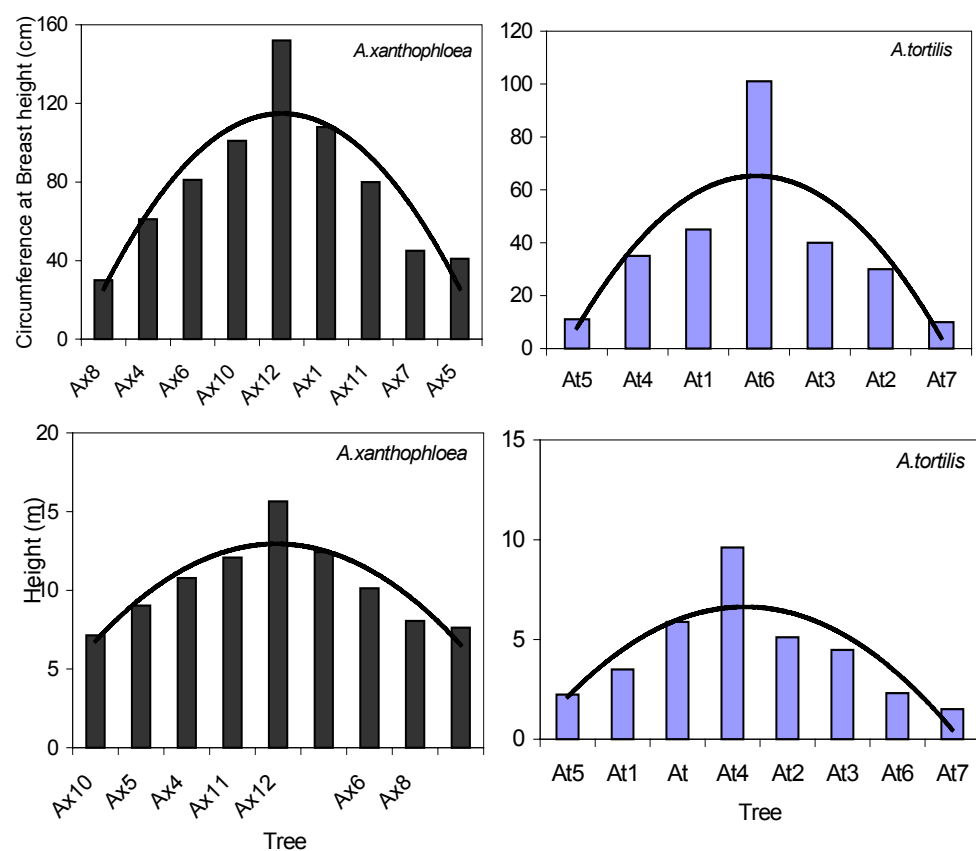
Figure 3. Height and circumference at breast height (CBH) of marked trees within the experimental plot

2.1.2. Experiment 2 - Mediterranean region of Portugal

2.1.2.1. Location

Field experiments were conducted at a site near the University of Evora's Mitra Campus in the rural district of Evora in the centre of Portuguese Alentejo, 12 km south-west from Evora town in southern Portugal. The altitude of the site ranged between 220 to 230 m, (coordinates ca. 38°32'26.549' N and 8° 00'01.424''W).

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2.1.2.2. Climate and soil

The experimental site is submitted to a Mediterranean mesothermic humid climate characterised by hot and dry summers and cool and humid winters (David et al. 2004). For the period covering 1951-1980, the mean annual precipitation reached 664.6 mm mainly distributed between October and May. The mean annual temperatures are 15.4ºC with a maximum in August (31.3ºC) and a minimum in January (3.8ºC) (cf. David et al. 2004). The experimental plot stands on an acid Litholic non-Humic soil derived from Gneiss with a pH of 4 to 6. Key climate variables between 1951-1990 for the Mitra Campus (Herdade da Mitra Meteorological station 38°32'N, 7°54'W altitude 309 m.a.s.l), located ca. 1 km away from the study site are summarized in table 2, while figure 4 shows rainfall patterns and temperatures in Herdade da Mitra between 2001 and 2002

Table 2. Summary of long-term means of weather parameters at Herdade da Mitra, Portugal.

Mean annual temperature (°C)	15.4
Mean Maximum temperatures (°C)	21.5
Mean Minimum temperature (°C)	9.2
Relative air Humidity at 9:00 h (%)	75
Annual precipitation (mm)	665
Most common wind direction	NW
No of days with dew at soil level	58.2

The mean annual evaporation reached 1760 mm. Precipitation and minimum and maximum temperature for the last two years prior to conducting field experiments are presented in figure 3.
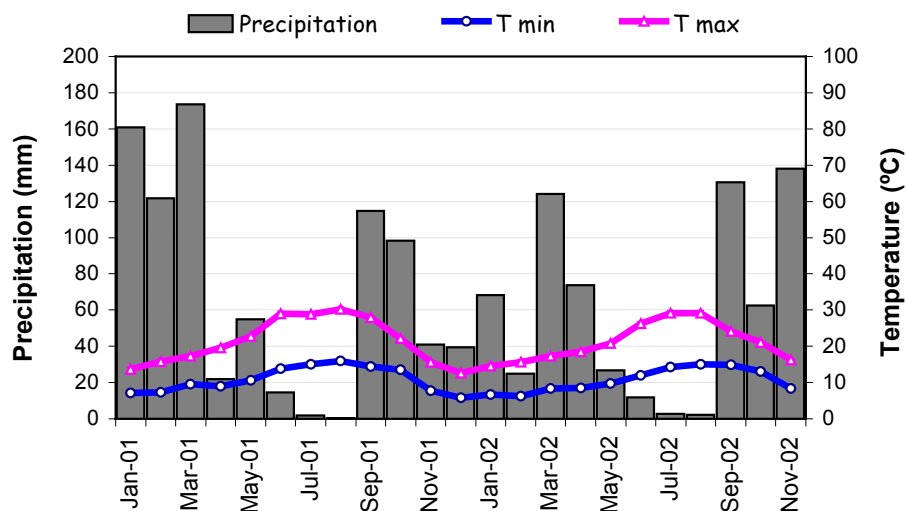
Figure 4. Precipitation and temperature recorded in Herdade da Mitra in 2001 and 2002.

2.1.2.3. Vegetation

The experimental parcel constituted an area of 0.264 ha (46 m × 60 m) exclusively covered by *Quercus suber* trees that were planted in 1988. The herbaceous layer comprised grasses, herbs and shrubs. 484 *Q. suber* trees could be distinguished, from which 168 trees had a mean height greater than 1.30 m with a measurable diameter at breast height (DBH). The mean DBH of the trees was 8.5 cm, with 27% of the trees having a mean DBH less than 5 cm while 45% had a mean DBH of over 10 cm. The mean height of the trees with a measurable DBH was 4.1m with 57% of the trees with a height greater than 5 m. A summary list of species within the study plot is given in table 3 while characteristics of *Q. suber* trees on the study plot is provided in table 4 and distribution of tree diameter class is shown in figure 5. A total of twenty seven trees were selected for measurements, with a representative mean DBH of 11.62 cm and mean height of 5 m. Intensive ecophysiological measurements were conducted on 5 of the 27 trees. Three trees out of these 5 were heavily instrumented.

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Table 3: Species list of the study site in Herdade da Mitra categorized into understorey and canopy.

Canopy	Understorey	
	Grasses	**Herbs/shrubs**
Quercus suber	*Vulpia fasciculata*	*Anthemis maritima*
	Bromus rubens	*Anthyllis hamosa*
	Briza maxima	*Tuberaria suttata*
	Avena barbata	*Cistus salvifolius*
		Echium plantagineum
		Jasione lusitanica

Table 4: Characteristics of trees within the study site in Herdade da Mitra.

Minimum diameter at breast height (DBH) (in cm)	2.7
Maximum DBH (in cm)	21.0
Mean DBH (cm) of all trees in the plot (with diameter>5.0cm) (n=99)	11.1
Mean DBH (in cm) of trees selected for measurements (n=27)	11.6
Mean DBH of neighboring trees (n=53)	11.1
Mean height of selected trees (in m)(n=27)	5.3
Tree density/m^2	2.9
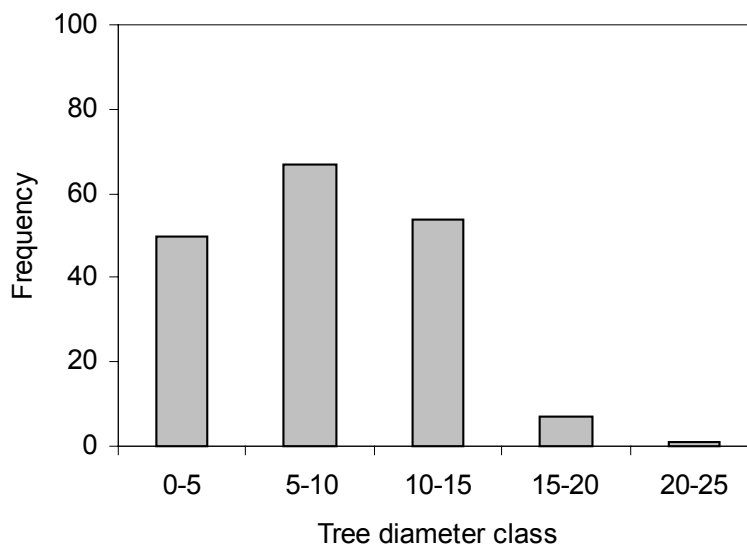
Figure 5. Distribution of tree diameter class. Tree diameter was measured at breast height, except for short trees with diameter less than 5 cm, which were measured at stem base.

2.2. Measurements

2.2.1. Climate and soil water status monitoring

A climate monitoring station was established within the study plots considered in this study (Kenya and Portugal) in an open space to avoid interference from trees.

2.2.1.1. Experiment one (Kenya savanna-Kibwezi).

Weather variables measured included rainfall (Aerodynamic gauge ARG100, Environmental Measurements Ltd., Sunderland UK), air temperature (VAISALA HMP45A, Helsinki Finland), soil temperature at 30-cm depth (Thermistor M841, Siemens Germany), photosynthetic active radiation (LI-Q1217 Quantum sensor, LI-COR USA) and humidity (VAISALA HMP45A, Helsinki Finland). Measurements were taken every 5 minutes, averaged and stored every half-hour with a data logger (Delta-T-Devices Cambridge UK).

2.2.1.2. Soil water content

Soil water content (SWC) in the upper soil layers was monitored using Theta probes (type ML 2x, Delta-T-Devices, Cambridge UK) installed at a depth of 40 cm in the soil at the centre of the study plot. Data was collected every 30 minutes, averaged and logged every hour.

2.2.2.4. Experiment two (Mediterranean region of Portugal–Herdade da Mitra)

Weather conditions were continuously recorded. Parameters monitored included precipitation (ARG100 rain gauge, EM Ltd., Sunderland UK), global radiation, photosynthetically active radiation (PAR) (LI-190 Quantum sensor, LI-COR USA), air humidity and temperature above the canopy (Fischer 431402 sensor, K.Fischer GmbH, Drebach Germany), air and soil temperature profiles (Thermistor M841, Siemens, Munich Germany) outside and under the tree canopy at +200, +10, -15, -40 and −80 cm respectively, wind speed (A100R, Vector Inst. Wind speed Ltd. Rhyl UK) and wind direction (Wind Vane W200P Vector Inst. Wind speed Ltd. Rhyl UK) were recorded. Data was measured every 5 minutes, averaged and logged every half-hour with data logger (DL2e, Delta-T Devices, Cambridge UK).

2.2.2.5. Soil water status

Soil water status near each of the five main experimental trees was monitored using equitensiometers (EQ 15, Ecomatik GmbH, Dachau Germany). For every tree, two sensors were installed at –0.3 m and –1.0 m depth in the soil, on the north facing side, 1 to 1.5 m away from the stem base. The lower section of the sensor casing had circular windows protected by fabric, which provided an uninterrupted link between the sensor body and the external soil. The sensor body consisted of two parts, water content sensor and equilibrium medium (Figure 6). The equilibrium medium consisted of glass fibers with varying diameters that equilibrated to the water potential of the surrounding soil. The changes in electrical conductivity of the glass fiber matrix were monitored with a Time Domain Reflectometry (TDR) sensor, which provided a direct measure of soil water potential. For non-saline soils, the dominant factor determining Ψs is the matric potential Ψm (Radcliffe and Rasmussen 2002). Each sensor was individually calibrated and had a measurement range from 0 to -1500 kPa ± 5 kPa (0 to -15 bar). This could however, be extrapolated to -2500 kPa, but with reduced sensor accuracy. Data was recorded every 5 minutes, averaged and logged half hourly. During installation, care was taken to ensure minimal disturbance to the soil. The recorded data were retrieved using a delta DL2 computer program (Delta-T Devices, Cambridge UK). The mV units were converted into kPa units using WAFLOS-computer program (Ecomatik Dachau Germany).
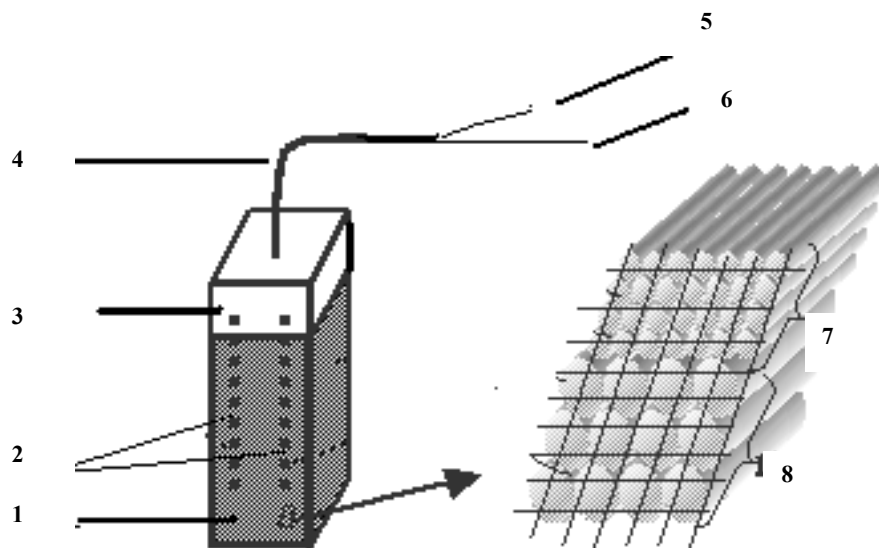
Figure 6. A schematic representation equitensiometer (EQ-15), soil moisture sensor. Labels for components are 1.Equilibrium body, 2. Vertical TDR rods, 3. Soil water content sensor, 4. Cable, 5. Power supply, 6. Signal to logger, 7. Small diameter glass fibres, 8. Large diameter glass fibres.

2.2.3. Morphological and physiological measurements-Experiment 1

2.2.3.1. Plant growth

Trees of *A. xanthophloea* and *A. tortilis* were identified on which growth and also measurements of other morphological features were conducted. Three trees of *A. xanthophloea* and *A. tortilis* labeled as *Ax1, Ax2* and *Ax3* and *At1, At2* and *At3* respectively were identified and from each tree, 2 newly formed (young) shoots, with healthy apices from well exposed branches of each tree were selected for measurements. Monthly measurements on shoot and leaf numbers per branch were conducted. Tree *At2* was smaller and younger than the rest with DBH of 3.2 cm compared to DBH=14.3 and 12.7 cm for *At1* and *At3* respectively. *A. xanthophloea* trees selected for measurements were all relatively large and had a mean DBH of 23.6 cm.

2.2.3.2. Litter fall

Litter was collected in litterbags of diameter 30 cm, suspended below the branches of three trees per species monitored for growth. The litter was removed every month, oven-dried to constant weight and dry weight determined. After emptying their litter contents, the litterbags were replaced to the same positions under the tree branches.

2.2.3.3. Sap flux measurements

Sap flux density (SFD) was measured at the main tree stems of 3 trees per species using the heat dissipation method (Granier 1987). All sensor installations were made on the north-facing side of the trees and covered with a radiation shield (Styrofoam cover with aluminum foil) to prevent thermal load on the sensors. Power was provided by lead-acid batteries that were recharged with solar panels via a charge controller. Each sensor consisted of a pair of 2 mm diameter probes vertically aligned ca. 15 cm apart. Each probe included a 0.2 mm diameter copper-constantan thermocouple. The two thermocouples were joined at the constantan leads, so that the voltage measured across the copper leads provided the temperature difference between the heated upper probe and the lower reference. The heating wire wound around the upper probe was supplied with constant current of 120 mV so that the heaters were powered with 200 mW (Granier 1987). The temperature difference ΔT between the heated and the lower unheated reference probe was measured. Heating of the upper probe was carried out along a 20 mm long winding in all cases (Figure 7).
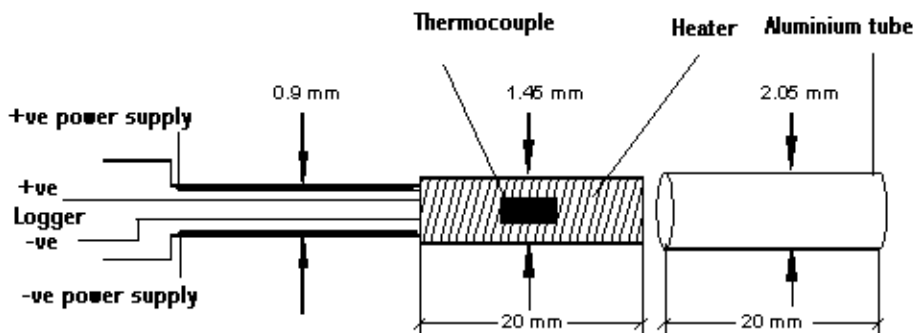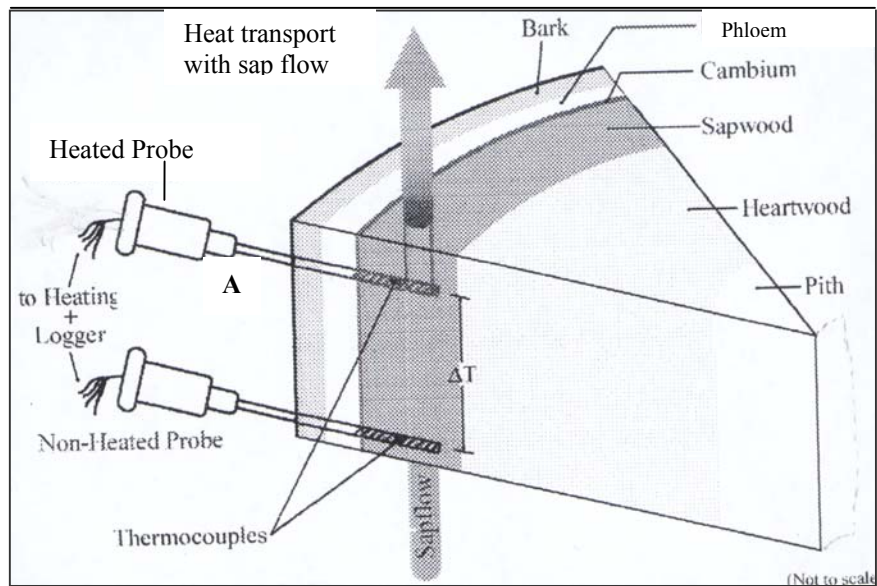
Figure 7. (Above) Schematic representation of sap flow sensor installed on to a tree. The upper probe, labeled A was heated with a constant current power supply while the lower one (reference) was not heated. (Below) Enlarged drawing of the heated probe (A), showing detailed construction and wiring of the sensor. (Courtesy of Ecomatik, Dachau Germany).

In cases where the tree trunk was large with sapwood radius greater than 20 mm, radial sap flow profile was measured in order to allow observation of SFD at different depths. A second and third sensor (when used) was installed on both sides of the first one (annulus 1-20 mm radial sapwood depth). Sensors were spaced 10–15 cm circumferencially, away from the first sensor pair, but still on the same side of the stem to avoid azimuth differences (see Plate 1). The second sensor was implanted 20 to 40 mm into the sapwood and the third (whenever used) 40 to 60 mm deep into the sapwood. To correct for natural temperature gradients in the sapwood between the heated and the reference sap flow probes, heating was turned off so that the measured temperature differences were mainly as a result of natural warming of the sapwood. This was done periodically throughout the season. Data from sap flux probes

(during heater-on) were then corrected for natural temperature gradients for the different time intervals during the season by subtracting the voltage differences resulting from natural heat gradients, measured during the heater-off intervals. Temperature differences were monitored every five minutes and a 30-minute mean value was logged (DL-2 with LAC-1 in single ended mode, Delta-T Devices, England) for each sensor. SFD (g m^{-2} s^{-1}) for each sensor was calculated from ΔT in accordance with Granier (1987), assuming zero SFD (i.e. ΔT_{max}) at predawn and VPD near zero:

$$\text{SFD} = 119 \, K^{1.231} \tag{1}$$

where

$$K = \left[\frac{(\Delta T \max - \Delta T)}{\Delta T} \right] \tag{2}$$

Tree sap flux ($Qtree$, kg h^{-1}) was calculated by multiplying sap flux density by the hydroactive xylem area, i.e., the area over the heated needle, which was determined from the stem dimensions.

$$Qtree = \text{SFD} * \text{SA} \tag{3}$$

where SA is the xylem area over the heated needle. Sapwood area was also determined by taking stem cores at the height of sensor installation and examining the wet cores as well as through staining. This was done at the end of sap flow measurements.

Plate 1. Three sap flow sensors numbered 7, 8 and 9, installed at different depths (0-20, 20-40 40-60 mm respectively) into the trunk of *A. xanthophloea.*

2.2.3.4. Leaf transpiration and stomatal conductance

Between December 2001 and June 2002, monthly measurements of diurnal courses of leaf transpiration and stomatal conductance were carried out using a steady state porometer, model LI-1600, LI COR, U.S.A., with a cuvette for enclosing the *Acacia* leaves. Similar measurements were repeated in December 2002 and January 2003. On each measurement day, three different trees of the same species were selected for measurements. Well-exposed leaves from each of the trees were identified and measurements conducted on the same leaves from sunrise to sunset (7. 00 a.m.- 6.00 p.m. local time). Care was taken not to damage the leaves during measurements by using a soft airtight foam pad placed at the mouth of the cuvette. Each leaf was set in the cuvette so that it received full illumination during measurement. Every turn of measurements lasted one to two minutes to allow the chamber to equilibrate. Alternate measurements were conducted between the two species throughout the day. At the end of the day, the measured leaves were detached and their area determined using a portable leaf area meter CI-202, CID Inc. USA. This was then used to calculate transpiration and stomatal conductance per unit leaf area.

2.2.3.5. Leaf water potential

On the same trees, and during similar periods as indicated in section *2.2.3.4* above, parallel diurnal measurements of leaf water potential (Ψ_L) on triplicate samples were conducted using a pressure chamber, Hayashi Denkho, Tokyo Japan. Water potentials were measured immediately after measurement of leaf transpiration and stomatal conductance with the porometer. During measurements, young shoots with 2-3 leaves were cut and immediately enclosed in plastic bags to reduce further moisture loss during transfer and fixing in the chamber. Green transpiring leaves were also introduced into the chamber to reduce water loss during the measurements. Water potential measurements were carried out on leaves/shoots obtained from the middle and exposed branches. Measurements were conducted between 6.00 a.m. and 7.00 p.m., local time on an hourly interval, with each measurement session lasting less than 30 minutes. Measurements before sunrise, a time when there was still dew on the leaves and no transpiration was expected to have started, were used for pre-dawn values. No further corrections were made on the water potential values. Measurements were conducted twice every month.

2.2.3.6. Estimation of xylem hydraulic conductance

Whole tree hydraulic conductance (K_h) was estimated as:

$$K_h = \frac{F}{\Delta \psi} \qquad (4)$$

where F = current sap flux per unit sapwood area, and

$\Delta\Psi$ = difference between dawn Ψ_s and current Ψ_L. Ψ_s was assumed to be very close to Ψ_{pd}. Direct calculations were made for the days when Ψ_{pd} and Ψ_L were measured. Values around mid-day were taken to avoid effects of stem capacitance.

Estimates of leaf specific hydraulic conductance (K_{s-l}) were derived from equation (4), modified as:

$$Ks - l = \frac{E}{\Delta \psi} \qquad (5)$$

E= Transpiration per leaf area measured by porometer.

K_{s-l} is the reciprocal of the slope of a regression line for Ψ_L expressed as a function of E (Wullschleger et al. 1998)

2.2.3.7. Water use efficiency

Water use efficiency was compared between *A. tortilis* and *A xanthophloea*. Carbon isotope composition of foliage, expressed as $\delta^{13}C$ was used an index of seasonally integrated WUE (Tieszman and Archer 1990). Leaves were collected every month for carbon isotope analysis. Samples were obtained from three trees of each species, with each sample analyzed separately for $\delta^{13}C$. Analysis was done in the isotope analysis lab, Plant Ecology Department, University of Bayreuth.

2.2.4. Physiological measurements-Experiment 2 (Quercus suber)

2.2.4.1. Selection of tree samples

Five trees of *Q. suber* at the Portuguese study site were identified for measurements and marked as *A11, A12, A13, D6* and *D22*. Selection was based on their location, size and stem structure. The trees had stem diameters falling within the same diameter class (from 10 to 15 cm). A summary of morphological tree characteristics is provided in Table 4. From the above selected trees, *A11, A12* and *A13* were heavily instrumented for intensive investigations as described in the following sections.

2.2.4.2. Tree growth

Tree growth between May and October 2003 was monitored by measuring changes in tree stem circumference using circumference dendrometers from Ecomatik GmbH (Dachau, Germany). The sensors were installed at breast height on three trees *A11, A12* and *A13*. Each sensor consisted of a transducer enclosed in a watertight aluminum casing and a beaded metal string that ran around the stem and connected to the pressure sensor. Signals as result of strain exerted (Ohm) were recorded every 5 minutes, averaged and logged half hourly. Increment in stem circumference (Ci in μm) was calculated as:

$$Ci = \left[{Ohm}\middle/{Callibration\ factor} \right] * 11000 \tag{6}$$

The increment in stem circumference during the season was used as a measure of tree growth.

2.2.4.3. Whole tree sap flux

Sap flux density at the tree stems was monitored in trees using SF-L thermal dissipation probe (Ecomatik Dachau Germany) which is a modified form of Granier-type described in section 2.2.3.3. The SF-L type sensor was used to continuously measure sap flux in the trees as well as monitor the background (tree trunk) natural temperature gradients, which may lead to underestimation of sap flux density of trees in dryland environments (see DO and Rocheteau 2002). The advantage of using SF-L sensors in monitoring natural heat gradient over the heater-off experiment described in section 2.2.3.3 is that changes in stem temperature due to natural heating are continuously monitored, providing a more accurate correction for the natural heat gradients. The method also does not require turning off the heaters, so that sap flux can be continuously monitored without interruptions as in the case of heater-off experiments.

The sensor consisted of two components, a normal Granier type component with two probes SH and SO (see section 2.2.3.3 for details) and a second component consisting of two additional probes, S1 and S2. The SH probe was heated from a constant current source while SO, S1 and S2 were not heated. Each probe contained a copper-constantan thermocouple in the middle and connected to the lower reference probe SO in opposition. SO was installed 10–15 cm directly below SH. S1 and S2 were installed at the same height as SH; 5 cm apart on the left side and right sides of SH respectively (see Plate 2). Sensors were installed at breast height on the tree and at similar depths into the sapwood. The

installation was then covered with Styrofoam insulation and then aluminum foil to exclude or minimize the effect of direct insolation on the tree stem.

Temperature differences between SO and S1 and also SO and S2 were influenced by natural temperature gradients of the sapwood on both sides of the tree stem and were referred to as ΔT_{R1} and ΔT_{R2}. During data processing, values of the temperature differences between the heated needle, SH and the sapwood ambient temperature, SO, (ΔT) were corrected by the ΔT_{R1}, and ΔT_{R2}, before calculating sap flux density as shown below

$$SFD = 0.714 \left[\frac{\Delta T \max - \Delta T - (\Delta T_{R1} + \Delta T_{R2})/2}{\Delta T - (\Delta T_{R1} + \Delta T_{R2})/2} \right]^{1.231} \tag{7}$$

Tree sap flux was calculated by multiplying sap flux density by the hydroactive xylem area as described in section 2.3.3.3.
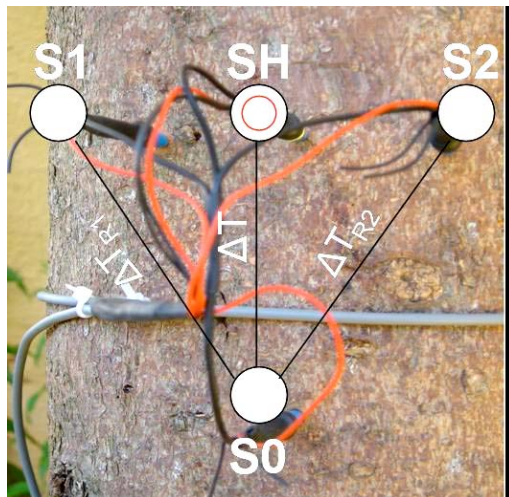
Plate 2. SF-L heat dissipation sensors installed into a tree. The probe SH was heated with a constant current power supply while SO, S1 and S2 were not heated. Wiring was done to measure temperature difference between SO-S1, SO-SH and SO-S2, where SO-S1 and SO-S2 are temperature differences due to natural temperature gradients on both sides of SH while SO-SH is due to sap flux.

2.2.4.4. Branch Sap flux measurements

Sap flux density was monitored on well-exposed south facing branches of the 5 trees *A11, A12, A13, D6* and *D22*, using the Stem Heat Balance method (SHB) described by (Sakuratani, 1981). The gauges were constructed at the electronic workshop of the University of Bayreuth, taking into account the recommendations of Steinberg et al. (1990) for improved accuracy of sap flow determinations. Each gauge consisted of a heating tape (Heater Designs Inc. Bloomington CA, USA) encircling the entire stem circumference mounted on a flexible cork sheet that could be wrapped around the stem segment under study (Figure 8). Copper-constantan thermocouples were positioned within and outside the

mounting cork insulation (Sakuratani 1981, Weibel and de Vos 1994) to monitor temperature gradients. The heater was continuously supplied with a constant voltage (ca. 4.5 V) from a battery, which was constantly re-charged from an AC current source. Current to the heaters was continuously logged to eliminate errors due to voltage fluctuations. The logged voltage was used for calculating sap flow (F).

Heat flows from the heater in different directions, namely vertical direction due to heat conductivity (Qv), radial direction (Qr) and by the convection of the sap flow (Qf) (see Figure 8), were estimated from temperature gradients measured between thermocouple junctions at strategic locations on the mounting cork. The installed sensors were insulated with thick closed-cell foam jackets and then with aluminum foil to prevent external heating by solar radiation. Signals from the thermocouple junctions were recorded every 5 minutes averaged and stored every 30 minutes on a Delta-T logger. Radiation incident at each branch studied was monitored with small photodiodes and similarly recorded. Measurements were conducted on the trees selected for growth measurements. The energy budget equation for the heated stem section was expressed as:

$$Qf = Pin - Qr - Qv \qquad (8)$$

where Pin is the electrical power to the heater (W) and is calculated as:

$$Pin = \left(\frac{V^2}{R} \right) \qquad (9)$$

where V is the voltage of the heater (V) and R its resistance (Ω).

Branch sap flow rate (F) was calculated as:

$$\text{F} = \frac{Qf}{Cp \Delta Tsap} \qquad (10)$$

where Cp is the heat capacity of water (J g^{-1} K^{-1}), and $\Delta Tsap$ the temperature difference of the sap measured below and above the heater (K). Since the measured branches had diameters not greater than 13 mm, no significant storage was anticipated, hence F (g h^{-1}) is the transpiration rate at branch level.

For more details on the SHB method see Sakuratani (1984), Weibel and de Vos (1994) and see Appendix 1.

At the end of measurements, sap flow gauges were removed from the branches and branches were examined for any defects or damages, which might have arisen during measurements. Each branch was then carefully cut about 5 cm below the sensor position and immediately immersed into a stain solution (acid fuchsin) with leaves still attached. The branches were left standing in the stain to allow transpiration for a period of 15 – 20 minutes before being removed. A series of sections below the position occupied by the heater were made using a sharp and clean woodcutting knife. The stained sections were then photographed using a digital camera and the pictures transferred onto a computer for analysis of wood structure and to quantify the amount of conducting vessels relative to the stem surface area. Total leaf area (LA) of all the leaves on the measured branch, ahead of the heater were then immediately measured using a leaf area meter (CI-202 CID Inc. USA). Sap flux per unit leaf area was determined using the measured LA values.
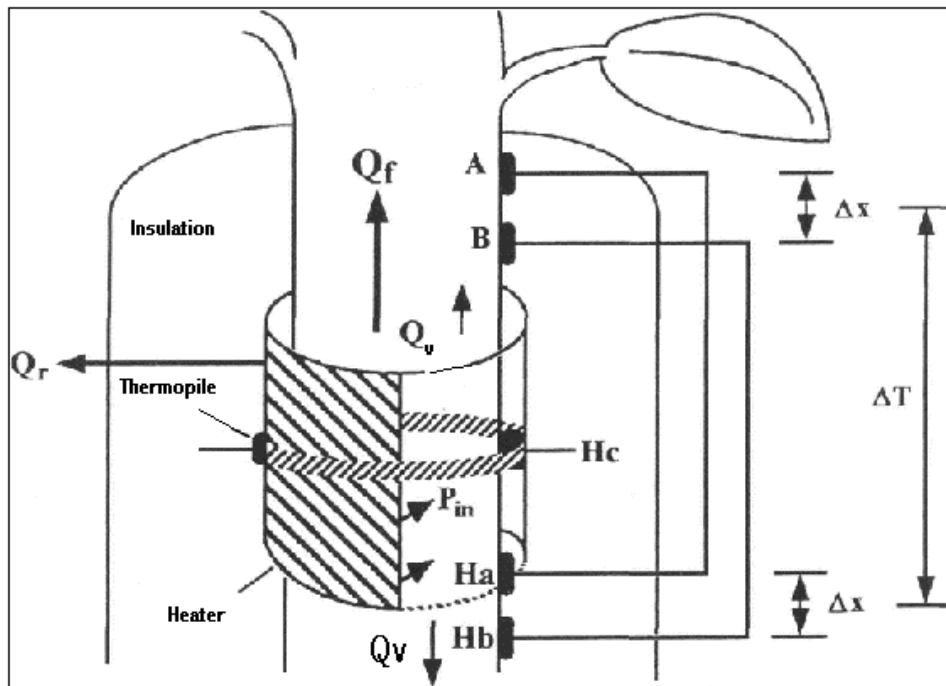
Figure 8. A schematic representation of a branch sensor (SHB method), showing arrangement of the thermocouples around the investigated stem as described in the text. Ha, Hb and Hc represent the respective temperature differences recorded at the logger

2.2.4.5. Leaf water potential

Between May 2003 and October 2003, a series of measurements of diurnal courses of leaf water potential (Ψ_L) on duplicate samples were conducted using a portable pressure chamber (PMS Instruments Co. Corvallis, OR, USA). Measurements of predawn leaf water potential (Ψpd) were carried out between 4:00 and 5:00 hours local time on all the 5 experimental trees, while measurements during the rest of the day were conducted on trees *A11, A12* and *A13*. At least 4 complete diurnal course measurements were conducted every month except during the month of August when only one measurement was carried out. During measurements, young shoots with 2-3 leaves from well-exposed exposed east and west facing branches respectively were cut, then enclosed in a plastic bag to reduce further moisture loss during transfer and fixing into the chamber. Shoots were used because of the short nature of the leaf petiole. Moist tissue paper was introduced into the chamber to reduce water loss during the measurements. Hourly measurements were conducted on the respective trees and each round of measurements lasted less than 15 minutes.

2.2.4.6. Plant water relations

At sunset on the following days during the months of June (9, 10, 11), July (7, 8, 9) and September (19, 20, 21) 2003, ten young shoots were excised from each of the 3 intensively investigated trees, namely *A11, A12* and *A13* on separate, consecutive dates respectively. The shoots were excised and re-excised under distilled de-ionized water and left to re-saturate in a dark chamber overnight. During re-hydration, they were wrapped in plastic bags to prevent any evaporative water loss and ensure full re-hydration. The following morning 5-7 shoots were removed, one at a time, from water and their fresh saturated weights and water potential (Ψ) determined. The shoots were then left to transpire freely under ambient conditions on a bench set up at the field site. At periodic intervals (3 minutes), weights and ψ of each shoot were measured. On each occasion, the pressure was increased slowly to prevent tissue damage until water/air bubbles appeared at the distal end of the cut shoot (Tyree and Jarvis, 1982). During each pressure chamber measurement, two weights were taken, one before and another immediately after water potential determination. The two weights were used in subsequent calculation of relative water content (R*) as:

$$R^* = 100.\left[\frac{FreshWt. - DryWt}{SaturatedWt. - DryWt}\right] \qquad \text{(Kramer 1980).} \qquad (11)$$

Measurements were continued until the shoots were beyond their wilting points i.e. no further weight change. The shoots were then oven -dried at 80^0C for 48 hours, before obtaining their dry weights, used in the determination of R* as above (equation 10). To develop the pressure-volume curves (*p-v*), the reciprocal of tissue water potential was plotted against relative water content R* (%) for each shoot.

Estimation of tissue water relations variables

Osmotic potential at full turgor (Π^{100}), relative water content at turgor loss point (R*tlp) and water potential at turgor loss point (Ψ_{tlp}) were derived from *p-v* curves by considering a regression line between the inverse of the final balancing pressure points and R* (Tyree and Hammel 1972). Turgor potential (P) was estimated as the difference between Ψ and Π

$$P = \Psi - \Pi \qquad (12)$$

Bulk modulus of elasticity (ε) was calculated as the change in P per unit change in R*

$$\varepsilon = \frac{\Delta P}{\Delta R^*} R^* \qquad (13)$$

where: ΔP = change in P, and

ΔR^* = change in R^* over the same interval.

Osmotic adjustment was calculated as the difference in mean Π^{100} between measurements conducted in June when soil moisture conditions were favorable, and subsequent measurements when soil water was declining.

2.2.5. Influence of tree roots on the rhizosphere

2.2.5.1. Diel fluctuations in soil water potential

Soil water potential was monitored continuously at two different depths, 0.3 and 1 m, at the root bases of 5 main experimental trees of *Q. suber* standing in the experimental plot using Equitensiometers-(EQ15 Dachau, Germany) as described in section 2.2.2.5. Each EQ15 was individually calibrated before it was installed in the soil and allowed to equilibrate for 1 week before the data could be used for the examination of hydraulic lift. At a similar soil depth at which the EQ15 were installed, soil temperatures were monitored using thermistors M841 (Siemens, Germany). This allowed monitoring the soil temperatures around EQ15 so that any arbitrary measurements of Ψs that could be caused by

changes in soil temperature could be revealed and EQ15's soil water potential output corrected for temperature. Data from both EQ15 and thermistors were recorded every 5 minutes, averaged and logged every 30 minutes using data logger (Delta-T-Devices Cambridge London).

2.2.5.2. Stable isotope sampling

Fresh plant and soil samples at the experimental site were obtained; water extracted from them and stable isotope composition determined as explained below. Since there is no isotopic fractionation between the roots and the twigs (Emerman and Dawson 1996), the isotopic signatures of the twig water provided information of the source water for the trees. On each tree two 10cm-lignified twigs were sampled at a minimum distance of 10 cm from the leaves. Twigs were sampled at midday on 27 trees, before the drought period in late June and at the end of the drought period in early September (when minimum leaf predawn water potential was reached). Soil samples were collected at the end of August, to avoid any influence of rain events on soil isotopic signature. Samples were collected at 10cm interval from the surface down to 1m depth. Three soil profiles were collected on a vertical transect across the study plot, running from the river valley to the top of the plot as illustrated in Figure 9. One profile was collected in the vicinity of big trees (height >4 m), a second profile was sampled in-between small trees (height <1 m) in the middle of the parcel, and the last profile was done at one corner of the plot where there were no trees but only grasses. Grass on the study site dried out and died by end of June.

Precipitation samples were collected once a month (When it rained) using a decantation flask filled with a layer of liquid paraffin and provided with a funnel collector. Ground water samples were collected in June and September at 12 m depth in a well located at about 500 m from the parcel (see David et al. 2004 for description). River samples were collected in June before it dried out to compare isotopic signature with twig xylem water. Twigs collected for xylem water extraction were only the non- green sections (see Dawson 1993 for details). All water, mineral and organic samples were collected in plastic test tubes closed with a plastic stopper and hermetically sealed with parafilm to avoid any alteration of the isotopic signature due to evaporation. Samples were kept in a freezer (-18ºC) until water extraction and mass spectrometry analyses were conducted.

Water was extracted from samples in a cryogenic vacuum distillation apparatus (Ehleringer and Osmond 1989). The final extract was transferred into a glass tube connected to a vacuum line and immersed in warm water (80ºC) to induce water evaporation. In the vacuum line under a maximum pressure of 5.10^{-2} mbars, evaporated water was trapped in a U-tube plunged in liquid nitrogen, where it was immediately frozen. Water extraction lasted at least 2 hours for soil samples and 3 hours for twig samples. At the end of every extraction, the U-tube was removed from the line and the melted ice transferred into 0.3 ml glass vial. The vials were then closed with rubber stoppers and aluminium caps.

Mass spectrometry analysis

The vials were then transferred to the mass spectrometer autosampler for pyrolysis and analysis. The water samples were analysed for their $^{18}O/^{16}O$ and $^{2}H/^{1}H$ isotope ratio via high temperature pyrolysis with an Isotope Ratio Mass spectrometer (IRMS), Delta Plus XL (Finnigan, Bremen Germany). All isotopic analyses were organised and conducted from Lisbon University; Portugal and details for the discrimination process are provided in Kurz-Besson et al. (in preparation). δD are expressed in delta notation (‰; ppt or "per mil") relative to an accepted standard (Dawson 1993)
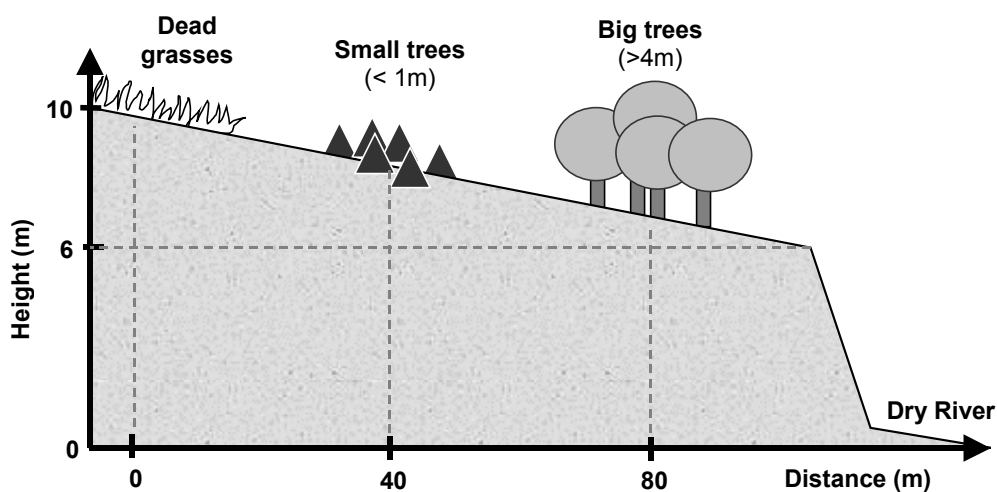
Figure 9. Schematic representation of the vertical transect along which samples were obtained for isotope analysis. The vertical dashed lines represent locations at which the soil profile samples were obtained.

2.3. Greenhouse experiments

2.3.1. Plant culture and experimental design

Seeds of *Acacia xanthophloea* and *A. tortilis* (Kibwezi provenance) previously obtained from the Kenya Forestry Research Institute (KEFRI), Nairobi, Kenya was germinated on May 23, 2001 in a greenhouse at the University of Bayreuth, Germany. The seeds were first soaked (pre-treated) in hot water (100^0C) and left to imbibe in the water overnight as it cooled down, before being transferred into germination trays with vermiculite and incubating at 27 ^{0}C. Most of the seeds germinated on the third day after planting. The germinated seedlings were then placed into plastic pots where they grew for a month with regular watering to saturated soil capacity before being transferred into larger containers (18 cm high by 14 cm diameter). Seedlings were transferred into final pots (V=1.3 m^3) on September 13, 2001. The potting mixture was 2:1 forest soil and sand respectively. The pots were arranged on a greenhouse bench in two blocks labeled as stress and controls. Each block comprised 24 trees or pots per species, randomly arranged within the blocks. To eliminate the possibility that regular watering of the controls might influence the stress-treated plants (e.g., through overflow, since pots had holes at the bottom), stress-treated pots were placed on a slightly raised bench top and at a distance of ca. 2 m between the treatments, with a gap between the benches to drain away any running water.

Differences in the watering treatment commenced on September 16, 2001. All the pots were first watered to saturated soil capacity. After this, the controls received water regularly, but the stress treatments were progressively stressed by withholding watering, until a 6-day water-stress cycle was attained on October 3, 2001. After this, stress-treated seedlings were watered to saturated soil capacity every 7 days while controls received water every other day. Stress was induced slowly in order to allow seedlings to adjust accordingly and to avoid losing seedlings. The treatments were continued for a period of one year, during which various physiological and morphological studies were carried out on them at varying time intervals. During this period, greenhouse temperatures were maintained between 25 and 30^0C while mean daily photosynthetic active radiation (PAR) ranged between 500 and 800 µmol m^{-2} s^{-1}. During winter, natural light was supplemented with artificial light of 300 µmol m^{-2} s^{-1} generated by electrical lamps.

2.3.2. Plant growth and morphology

On a monthly basis, three randomly selected seedlings from each treatment per species were harvested and separated into leaves, stems and roots. Leaf area was measured using a portable leaf area meter CI-202, CID Inc USA. The parts (roots, stems and leaves) were dried separately at 70 - 80^{0}C for 24 hours and their dry weights determined. Total dry weight (Tdwt), leaf dry weight (Ldwt), root dry weight (rdwt), leaf area (LA) and total root: shoot dry weights (r:s), i.e., root to shoot ratios, were then determined for every treatment per species.

2.3.3. Whole plant Sap flux

Sap flux was measured on one-year old plants subjected to the prescribed water stress treatments in the greenhouse using the stem heat balance (SHB) method described in section 2.2.4.4. Measurements were conducted on two plants from every treatment per species. Sensors were installed at 0.6 m above the ground on the main plant stems. Table 4 shows the mean stem diameters of the plants measured.

Table 4 Mean stem diameters of plants selected for sap flux measurements.

	A. tortilis		*A. xanthophloea*	
	Controls	Stress treatments	Controls	Stress treatments
Mean stem diameter (mm)	10.2	9.4	12.8	12.3

2.3.4. Water relations

Diurnal courses of leaf water potentials were observed one day following re-watering with a pressure chamber (PMS instruments, Corvallis Oregon) between 8:00 and 18:00 hours. The days selected for measurements were those when re-watering for stressed and control plants coincided. Due to the small leaf size of the *Acacia* trees, young shoots bearing 2-3 leaves were used during each measurement. Measurements were conducted between July and September when maximum air temperatures and light intensities were realized. On measurement days, the glasshouse roof was left open throughout the day for maximum insolation.

2.3.5. Pressure volume (p-v) curves

Measurements of tissue water relations were commenced two months after imposition of cyclic water stress. In total, five measurements were conducted on monthly intervals during the entire experimental period. Five young shoots per treatment per species were excised under distilled de-ionized water early in the morning. The shoots were left to re-saturate in a dark chamber for a period of 24 hours. The chamber was sealed to prevent any evaporative water loss and ensure full re-hydration. After 24 hours, procedures outlined in section 2.2.4.6 were undertaken to determine R^*, Π^{100}, R^*tlp, Ψ_{tlp}, respectively. Osmotic adjustment was estimated as the difference in mean Π^{100} between water-stressed plants and controls of the same species.

2.3.6. Hydraulic conductance

Leaf specific hydraulic conductance (Ks-l) was estimated for each treatment per species from the reciprocals of total flow resistance (R). A simple Ohm's Law analogy described in section 2.2.3.6 relating E and Ψ_L was employed to estimate total flow resistance (R) from soil to leaf.

2.3.7. Severe water stress and post-stress recovery

Three plants per species from both stressed and non-stressed treatments were subjected to severe water stress by withholding water until they were all wilted overnight. This was considered the maximum level of stress that could be tolerated by plants without inducing mortality. Watering was terminated for these plants on August 25, 2002. During the dry-down procedure, a series of measurements were conducted. Light, humidity and temperature conditions within the glasshouse during this period were monitored since the glasshouse roof was left open during the day to allow for measurements under natural light conditions. Mean daily PAR recorded over this period was 600 μmol m^{-2} s^{-1}.

2.3.7.1. Leaf water potentials (Ψ_L)

After withholding water, regular measurements of leaf water potentials (Ψ_L) were carried out. Measurements were conducted on the 3 replicate samples from every treatment early in the morning before any significant transpiration was realized and also at midday when plants experienced highest stress conditions. During measurements, young shoots with 2 to 3 leaves were used.

2.3.7.2 . Leaf transpiration and stomatal conductance

On similar trees as above, diurnal courses of leaf transpiration (E) and stomatal conductance (gs) were carried out on days 4 and 5 after imposing severe water stress, using steady state porometer, model LI-6400, LI COR, U.S.A. Measurements were conducted under natural light conditions. After day 5, leaves of control plants were wilted most of the day and no further gas exchange measurements could be conducted on them. At the end of the measurements, the measured leaves were detached and their area determined. This was then used to calculate transpiration per unit leaf area.

Chapter Three

Results

3.1. Experiment 1: Savanna region of Kenya

3.1.1. Weather

Figure 10 shows the prevailing daily weather conditions and changes in soil water status at the Kibwezi study site between November 2001 and June 2002, during the time when field measurements were conducted. Precipitation during 2002 was above mean average for the region. The total amount of rainfall received between September 2001 and June 2002 was 544.3 mm and was well above mean values recorded in the past three years, before the start of experiments, which was 483.6 mm per annum, as provided by Kenya Meteorological Services (KMS). Highest amount of rainfall during the experimental period was received between November 2001 and January 2002. Some significant rainfall also occurred between March and May 2002, but this was of a lower magnitude compared to the long rains, amounting to 165 mm. Air temperatures were stable and daily maximum temperatures remained fairly constant at around 30 °C during the year. Lowest mean daily maximum air temperatures recorded during the entire study period was 25 °C and occurred between December and January coinciding with the rainy seasons. Night temperatures averaged 15.7 °C and did not fall below 10 °C during the study period. Changes in VPD resembled those of air temperature. Lowest maximum VPD occurred between November and January and also during some days in April when it rained. Minimum VPD during the night approached zero and dew formation occurred at dawn during most of the days when the study was conducted. Highest VPD of 40 hPa was observed during this period and occurred in June. Light conditions were favorable for most of the period, with the average total of light during the day, for the entire study period being 35 mol m^{-2} d^{-1}.

3.1.2. Soil water content

Soil water content within the upper soil layer (0-40 cm) was significantly influenced by rainfall (Figure 10a). Moisture content at 40 cm depth increased within a period of 12-24 hours after an event of rainfall. Highest soil water content (SWC) recorded at this depth was 0.34 m^3 m^{-3} and was during the rainy season (in December). There was a dry spell between January and April and this was accompanied with a decline in SWC. Rainfall events in April, however, restored SWC at 40 cm depth

to 0.3 m³ m⁻³. No significant rainfall occurred after April and SWC within the upper soil layers significantly declined thereafter with the lowest value of 0.11 m³ m⁻³ observed in June.
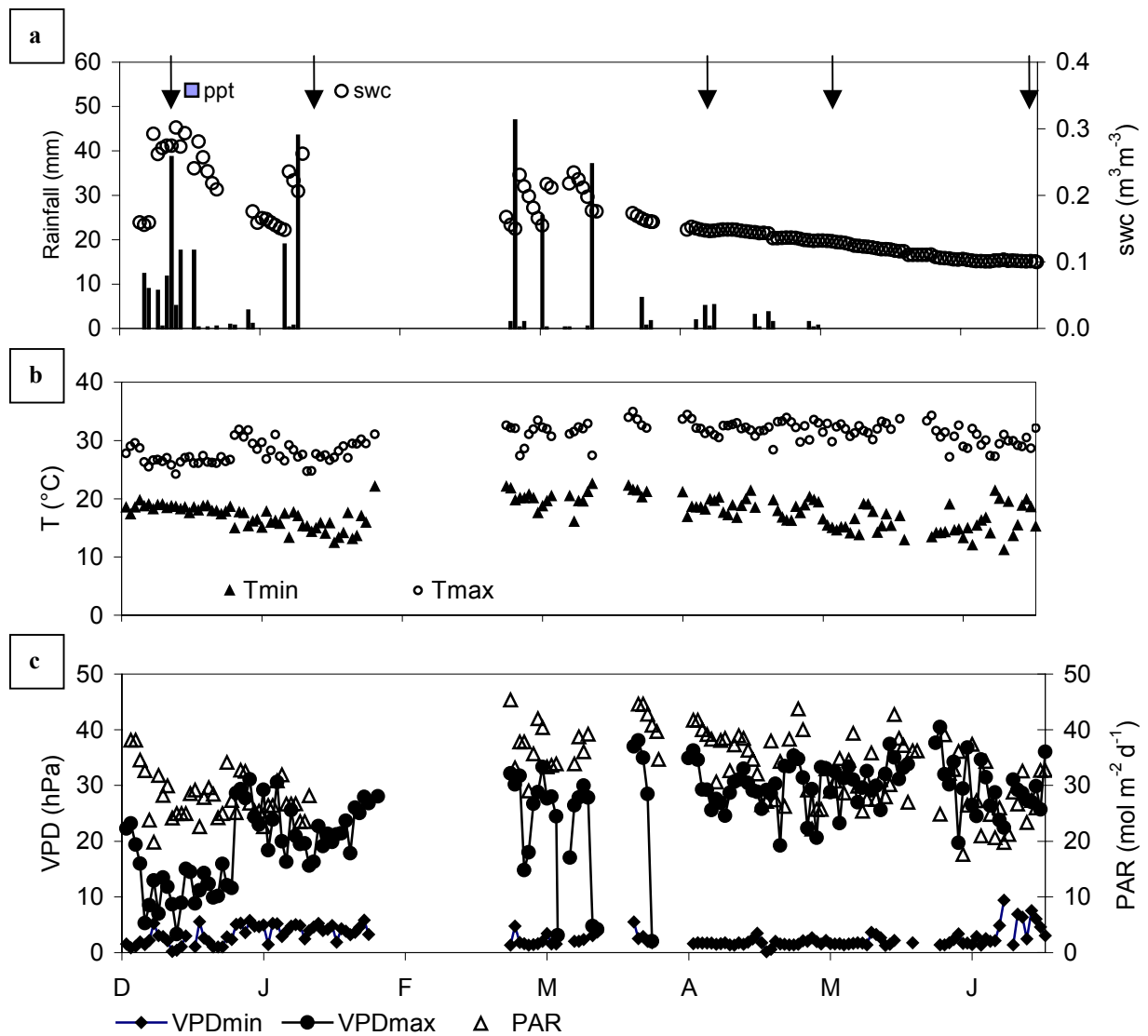
Figure 10. (a) Precipitation and soil water content (b) Daily minimum and maximum temperatures and (c) Daily minimum and maximum VPD and photosynthetic active radiation (PAR) recorded at the study site between Dec. 2001 and June 2002 when measurements were conducted. Periods when intensive field campaigns were conducted are indicated by arrows on the upper graph.

3.1.3. Shoot growth, leaf initiation and shedding.

Shoot extension and leaf growth occurred only during and shortly after the rainy periods except for mature *A. tortilis* (*At1* and *At3*) where it continued long after rains had stopped (Figure 11). For *A. xanthophloea* (*Ax1*, *Ax2* and *Ax3*) and the young *A. tortilis* (*At2*) studied, significant shoot extension

occurred only during and shortly after the long rains after which trees remained dormant. It was evident that water stress greatly affected leaf initiation and growth more than shoot extension. Monthly records of tree leaves showed that there was continuous leaf shedding (Figure 11) and that canopy leaf area gradually declined as water stress intensified. It must be reported here that massive leaf fall also occurred immediately following a rain event that occurred after an extended period of water stress, after which leafout again occurred. Close monitoring of representative branches showed very few leaves remaining in June and this was typical of the entire crowns.
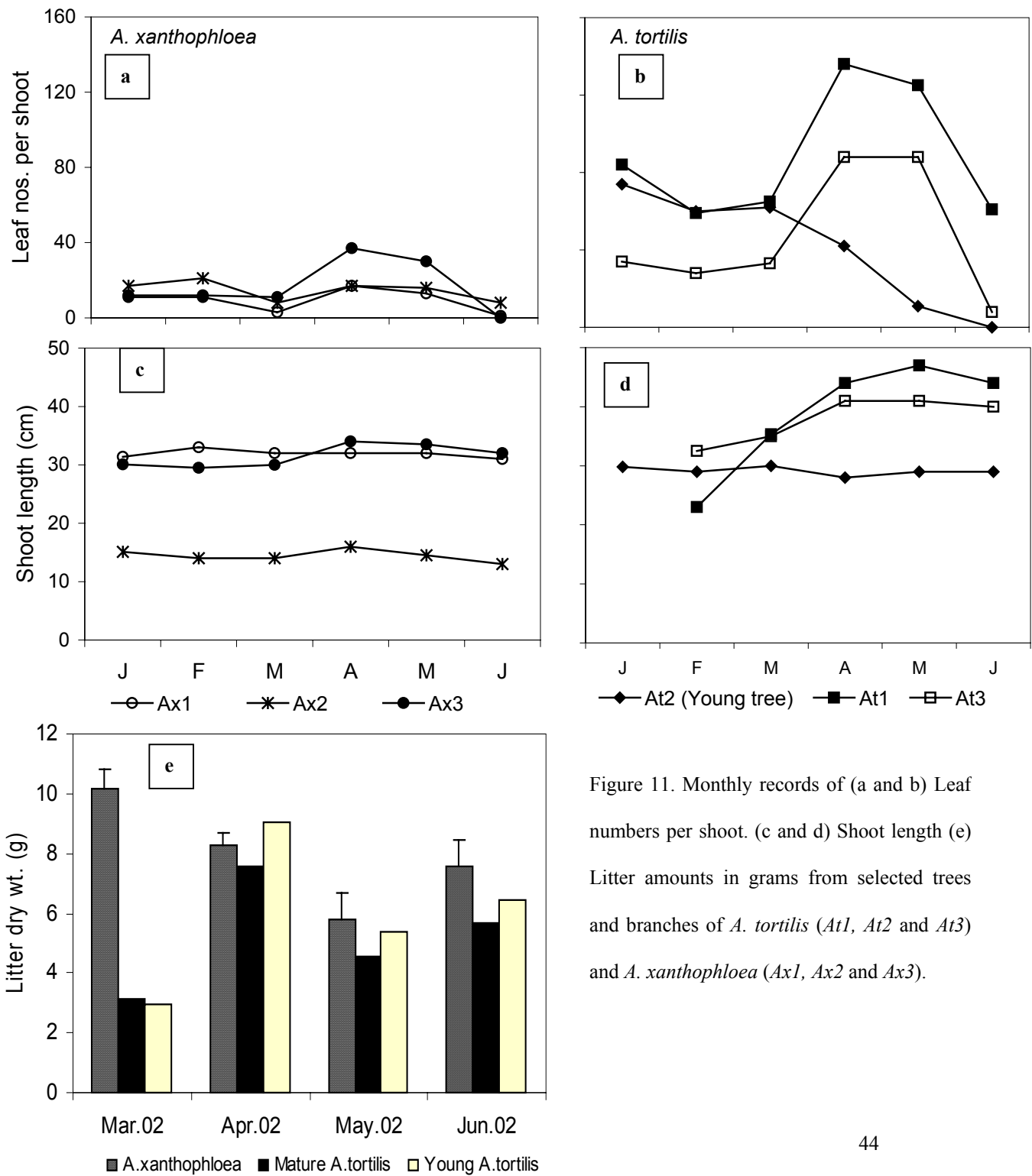
Figure 11. Monthly records of (a and b) Leaf numbers per shoot. (c and d) Shoot length (e) Litter amounts in grams from selected trees and branches of *A. tortilis* (*At1, At2* and *At3*) and *A. xanthophloea* (*Ax1, Ax2* and *Ax3*).

3.1.4. Seasonal changes in leaf water potential

Seasonal changes in predawn leaf water potential (Ψpd) are shown in Figure 12a. Highest Ψpd were reported for all the trees during December, after successive rain events. Favorable tissue water status was also observed after the April rainfall events. Plant water potential significantly declined between April and July, with *A. xanthophloea* showing the largest drop in Ψpd (-2.0 MPa) compared to mature *A. tortilis* (-1.2 Mpa). Young *A. tortilis* showed intermediate response (Ψpd = -1.4 MPa). After November 2002 rains, all plants increased Ψpd to a similar value (-0.5 MPa). Decline in Ψpd for *A. xanthophloea* corresponded to the changes in SWC observed at 40 cm soil depth and a strong linear relationship existed between Ψpd for *A. xanthophloea* and SWC measured at this depth (Figure 12b). The weakest relationship between Ψpd and SWC at this depth was observed for mature *A. tortilis*, while that for young trees of *A. tortilis* was intermediate. Lowest Ψpd values recorded during the study period were between May and June, a time when the SWC was 0.1 $m^3 m^{-3}$. During this time, there were significant differences in Ψpd values among the three tree classes.
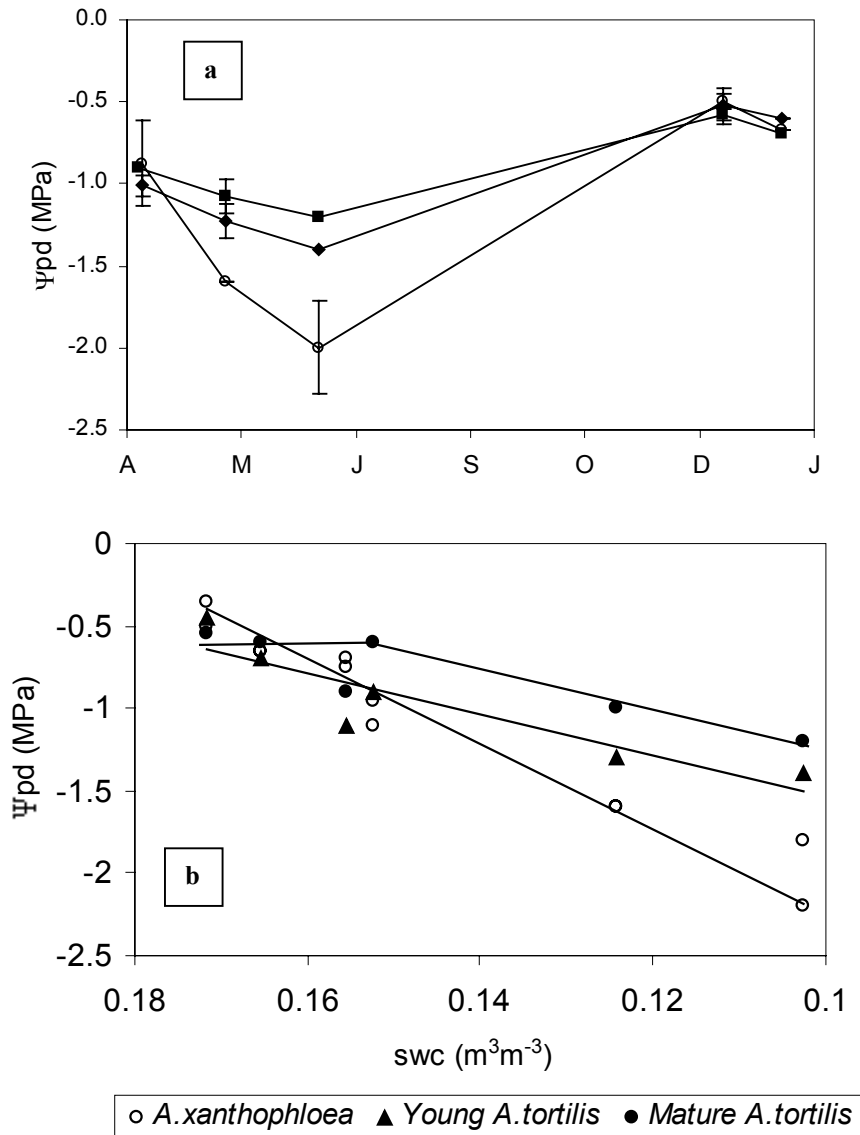
Figure 12. (a) Seasonal changes in leaf predawn water potential (Ψpd) for *A. xanthophloea,* young and mature *A. tortilis*. Deviation from the mean are represented by error bars and n=3. (b) Relationship between predawn leaf water potential for *A. xanthophloea*, young and mature *A. tortilis* and soil water content measured at 40 cm soil depth

Similarly, midday Ψ_L also declined with increasing drought. However for mature *A. tortilis* a stable value of midday Ψ_L (around –2.0 MPa) was maintained despite the declining SWC measured at 40 cm soil depth (Figure 813a). Figure 13b shows that *A. xanthophloea* exercised no control on water loss during favorable soil water conditions (assuming that Ψpd is an indicator of Ψs at the rooting zone), until Ψpd reached –0.8 MPa, after which it strongly regulated water loss. Conversely, *A. tortilis*

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consistently regulated water loss irrespective of soil water status, with consistent diurnal fluctuations in Ψ_L. Combining Figures 13a and b, it appears that the differences in response as shown in Figure 13a are due to differences in water accessibility and supply to the shoots.
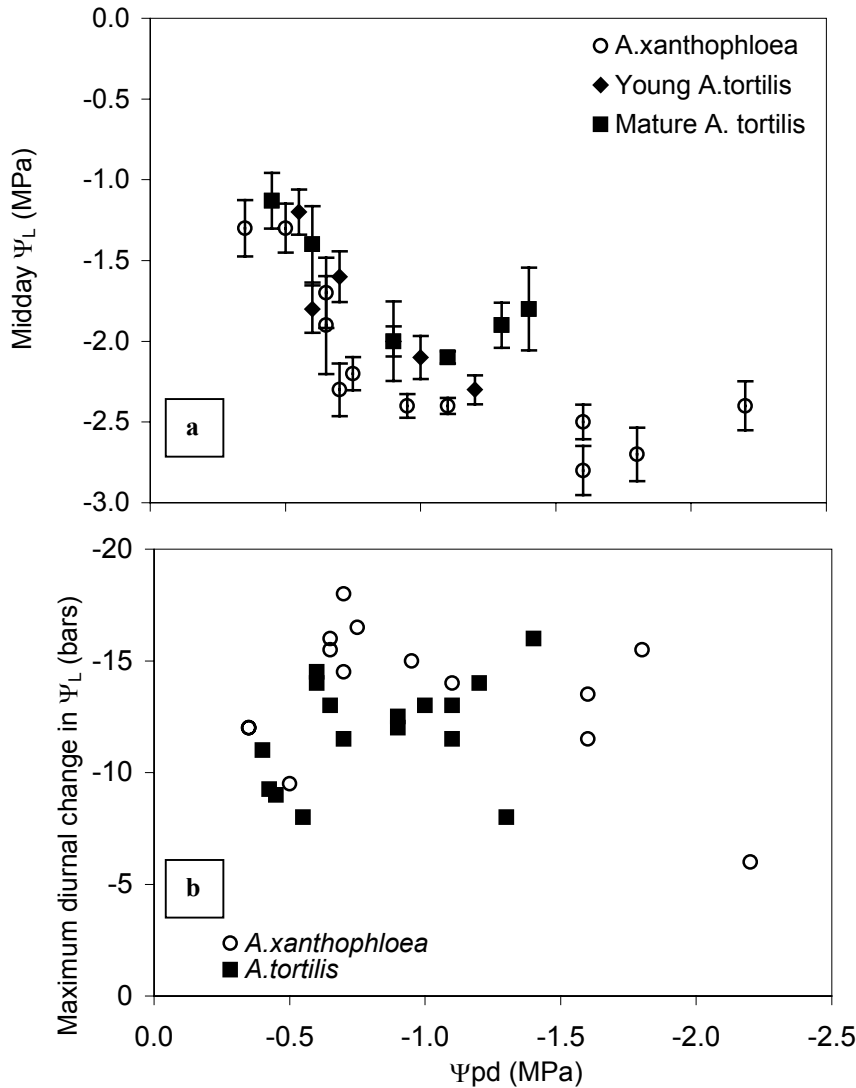
Figure 13 (a) Relationship between midday leaf water potential (Ψ_L) and Ψpd and (b) Relationship between maximum diurnal change in leaf water potential and Ψ_L for the trees measured for *A.xanthophloea*, young and mature *A. tortilis* trees.

3.1.5. Seasonal changes in sap flux and tree transpiration

Maximum daily water use of 35.8 ± 5.4 kg d^{-1} and 11.3 ± 2.1 kg d^{-1} were recorded for trees of *A. xanthophloea* and those of mature *A. tortilis* respectively. The *A. xanthophloea* trees considered for the study had larger stem diameters as well as crown sizes compared to *A. tortilis* (see Figure 3) and this might have contributed to the differences in total daily water use. Declining soil water content led to decline in daily sap flux (*Qtree*) in both species but the magnitude and time at which this decline commenced varied significantly (Figure 14). Whole tree daily sap flux declined by about 40% in *A.*

xanthophloea trees, 60% in young *A. tortilis* while a non-significant decline was recorded in mature *A. tortilis*. For *A. xanthophloea* and young *A. tortilis*, *Qtree* started to decline when SWC at 0-40 cm was around 0.1 and 0.15 m^3m^{-3} respectively. Sap flux in mature *A. tortilis* was less affected by changes in SWC at this depth. Decline was however more rapid in *A. xanthophloea*. Daily sap fluxes also declined as VPD increased except in mature *A. tortilis*, where no change was observed (Figure 15).
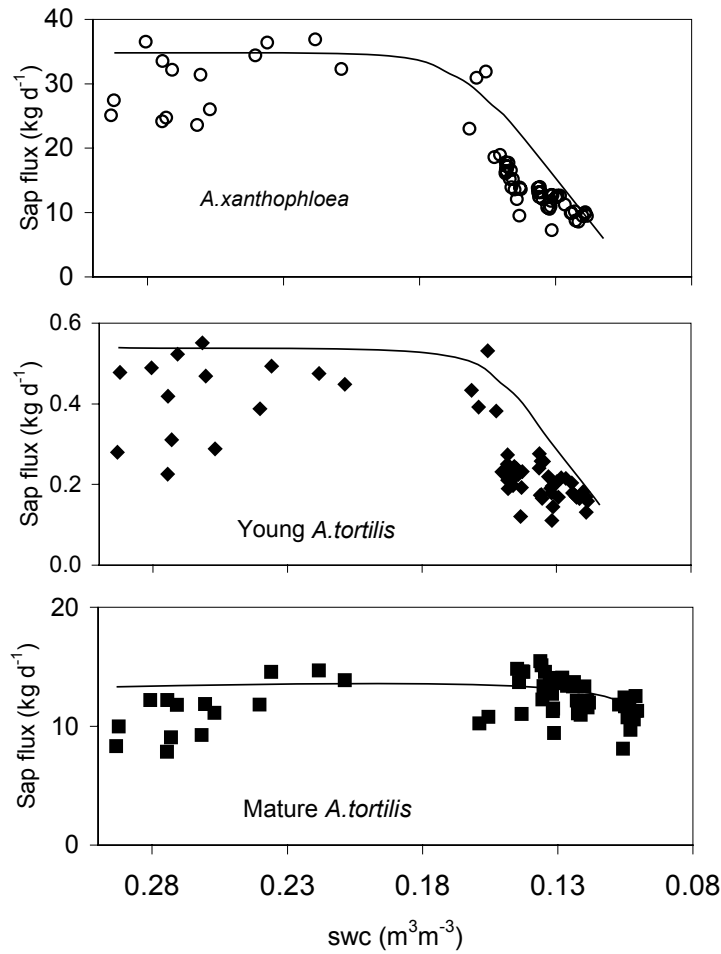
Figure 14. Total daily sap flux per tree for *A. xanthophloea*, young and mature *A. tortilis* under changing soil water content (SWC). Soil water content was measured at 40 cm soil depth.
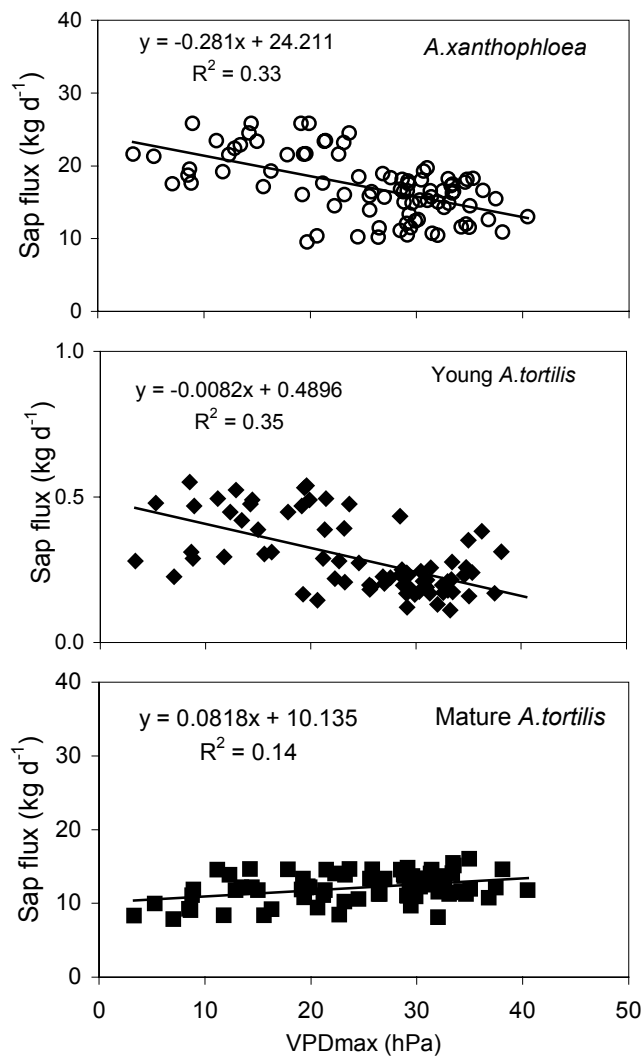
Figure 15. Daily sap flux per tree in *A. xanthophloea,* young and mature *A. tortilis* trees in relation to daily maximum vapor pressure deficit (VPDmax). VPDmax was used as a measure of atmospheric drought

3.1.6. Seasonal patterns of leaf stomatal conductance

Seasonal patterns of maximum leaf stomatal conductance (gs_{max}) are shown in Figure 16. Maximum leaf stomatal conductance was highest in both species (350 mmol m^{-2} s^{-1}) during favorable soil water conditions. Lower conductance rates (300 mmol m^{-2} s^{-1}) observed in young *A. tortilis* during this period was attributed to shading by the mature and taller trees, since they occurred within the understorey. Declining soil water led to a drop in stomatal conductance with significant differences occurring between the two species. *A. xanthophloea* showed a more rapid decline in stomatal conductance, attaining a mean minimum gs_{max} of 100 mmol m^{-2} s^{-1} at the end of the dry season. This was similar to the pattern of changes in Ψpd. Trees of *A. xanthophloea* were more sensitive to declining SWC and gs_{max} was at its lowest when SWC was about 0.15 $m^3 m^{-3}$ (Figure 17a). Mature *A. tortilis* however, maintained gs_{max} nearly twice as high during this period (Figure 17b). The relationship between Ψpd

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and gs_{max}, thus separating effects of differences in SWC at the root zone showed a similar response pattern in both young and mature *A. tortilis*, Figure 17d. It was therefore assumed that the differences observed in Figure 17b were mainly due to differences in soil water availability. This was with the assumption that under similar conditions, both young and mature *A. tortilis* will have similar gs. A clear distinction appeared between the two species, with *A. xanthophloea* showing an early decline in gs (Figure 17c). At Ψpd (surrogate for Ψs) of -1.00 MPa, when *A. tortilis* experienced stomatal conductance rates less than 200 mmol m^{-2} s^{-1} (Figure 17d), gs_{max} was 100 mmol m^{-2} s^{-1} in *A. xanthophloea* (Figure 17c).
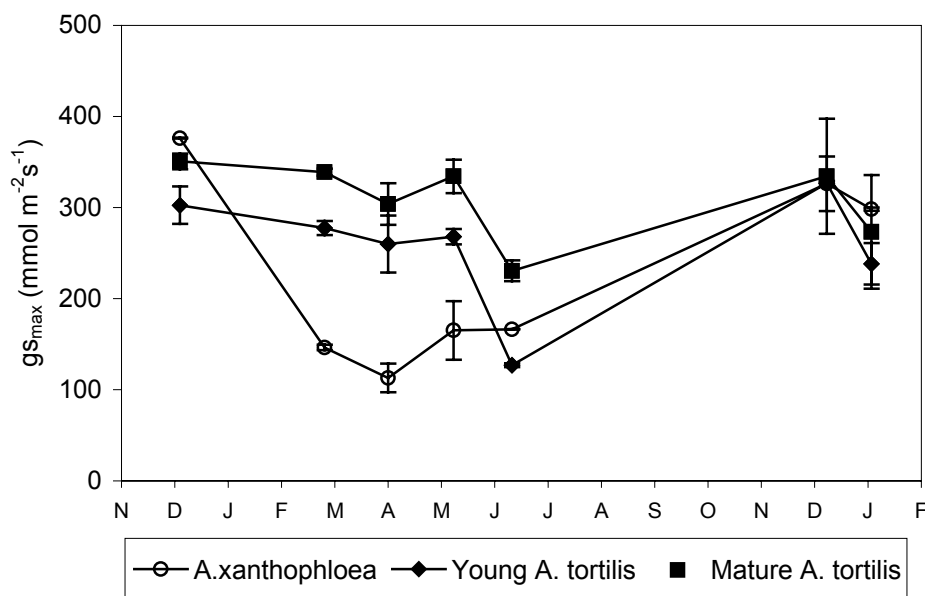
Figure 16. Seasonal changes in maximum leaf conductance (gs_{max}) measured in the sun crown of *A. xanthophloea*, young and mature *A. tortilis*. Bars indicate standard error of the mean and each point represents mean of 3 measurements per tree.
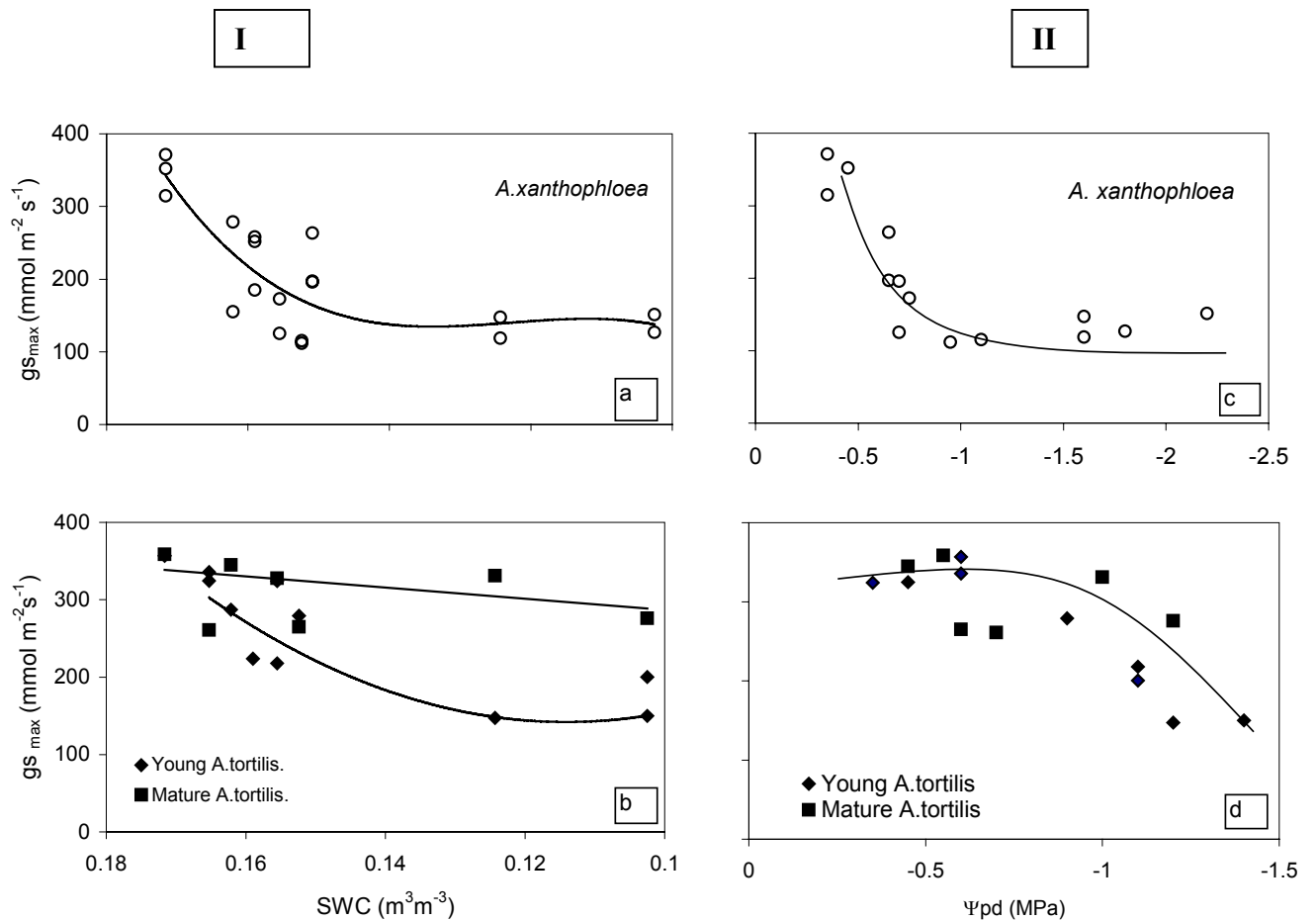
Figure 17. (**I**) Responses of maximum leaf conductance (gs_{max}) to changes in soil water content (SWC) at 40 cm in (a) *A. xanthophloea* and (b) young and mature *A. tortilis.* (**II**) Relationships between maximum leaf conductance (gs_{max}), with predawn leaf water potentials (Ψpd) of (c) *A. xanthophloea* and (d) young and mature *A. tortilis* trees.

3.1.7. Diurnal changes in leaf stomatal conductance, transpiration and leaf water potential

During favorable soil water conditions (December), gs in *A. xanthophloea* rose to maximum during morning hours but declined significantly before midday with a brief resumption later in the day (Figure 18, Panel a). For *A. tortilis* however, there was a brief decline in gs around midday, but this later resumed to morning values (Figure 18, Panel a). A similar pattern was exhibited by E, leading to a decline in Ψ_L during the morning and a recovery later in the day after E declined (Figure 18, Panel a). Recovery of Ψ_L was, however, slower in *A. xanthophloea*. Also for *A. xanthophloea*, a brief depression in E was observed around midday even when soil moisture was high (December). In many cases, *A. xanthophloea* experienced higher fluxes (40 kg d^{-1}) than *A. tortilis* (20 kg d^{-1}). However, when E was

expressed per unit leaf area, there was no significant difference between the two species, suggesting that high daily sap flux in *A. xanthophloea* was mainly due to its large crown. With increased water stress, trees showed an early morning peak in gs followed by a decline before noon (Figure 18, Panels b and c – April and May). This was more pronounced in *A. xanthophloea*, which retained near zero gs after midday. During this time, E in *A. xanthophloea* significantly dropped in the afternoon. Diurnal changes in Ψ_L were closely linked to transpiration, steeply declining when E was high and recovering when the rate declined. In the case of *A. xanthophloea,* however, it was surprising that at lower SWC, decline in E as a result of stomatal closure was not followed by recovery in Ψ_L, suggesting that there was an interruption of water supply to the leaves. This was different in *A. tortilis*, where E significantly increased over the season due to increased VPD but still fully recovered its Ψ_L by dusk after the occurrence of stomatal closure (Figure 18, Panels b and c).
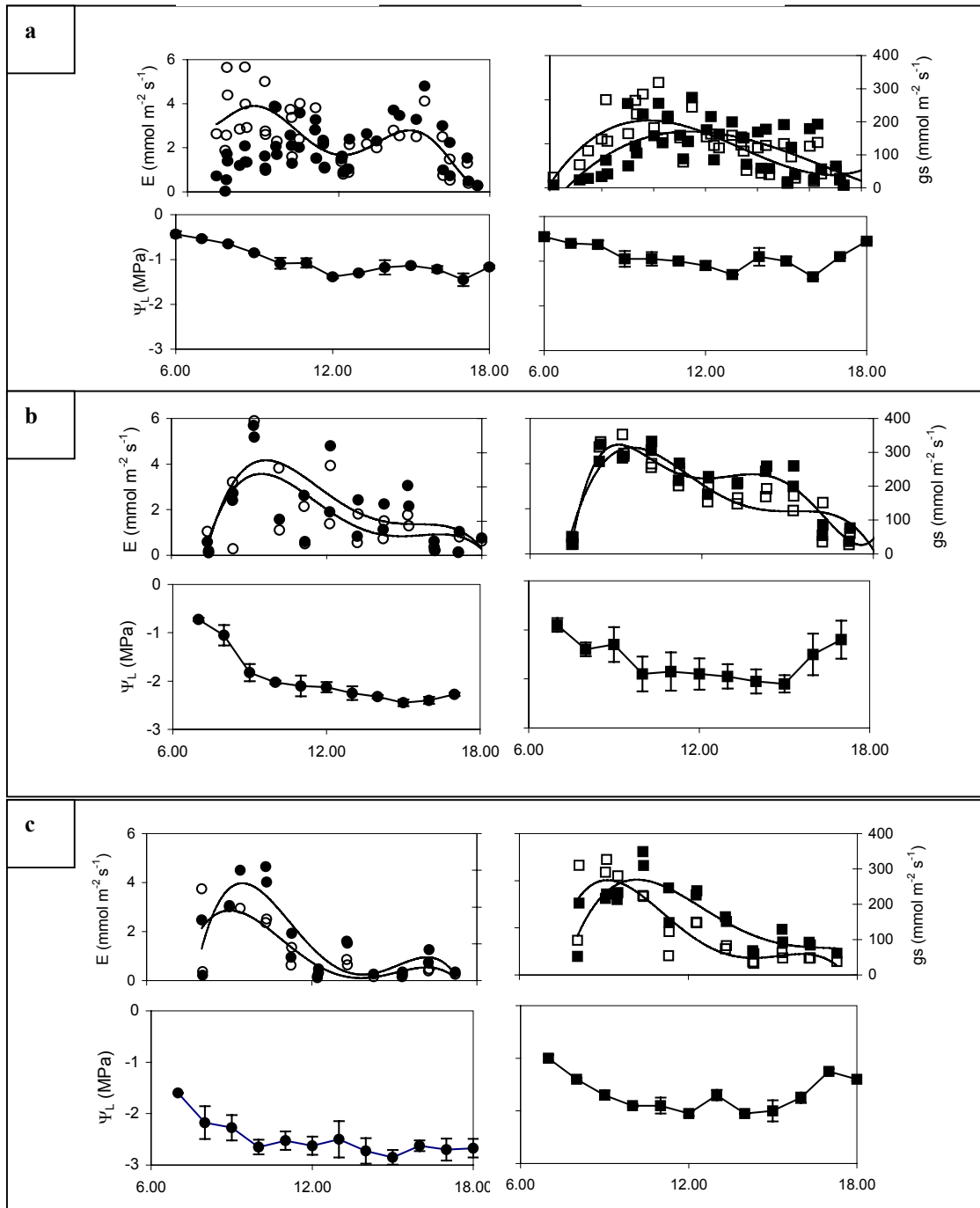
Figure 18. Diurnal changes in leaf transpiration, E (open symbols), stomatal conductance, gs (closed symbols) and leaf water potentials, Ψ_L (bottom) for *A. xanthophloea* (left) and *A. tortilis* (right), during three periods of the year, December (Panel **a**), April (Panel **b**) and May (Panel **c**), experiencing varying intensities of soil and atmospheric drought.

3.1.8. Hydraulic conductance

Figure 19 shows changes in whole tree hydraulic conductance (K_{plant}) in both species at different levels of soil water content. During the period when measurements were taken, declines in K_{plant} between the wettest and driest months were 50, 27 and 83% in *A. xanthophloea*, mature and young *A. tortilis*, respectively. Under favorable soil water conditions, significant difference occurred between mature and young *A. tortilis* (K_{plant} = 46.97 and 18.18 kg m^{-2} h^{-1} bar^{-1} respectively). Mature *A. tortilis* also had higher hydraulic conductance compared to *A. xanthophloea* (46.97 and 28.82 kg m^{-2} h^{-1} bar^{-1} respectively). Under similar conditions leaf specific hydraulic conductance (Ks-l) was 3.94 and 2.8 μg bar^{-1} cm^{-1} s^{-1} in *A. xanthophloea* and *A. tortilis,* respectively. A significant drop in K_{plant} occurred in trees of *A. xanthophloea* between SWC of 0.18 and 0.16 m^{3}m^{-3}. This coincided with the drop in gs as well as E.
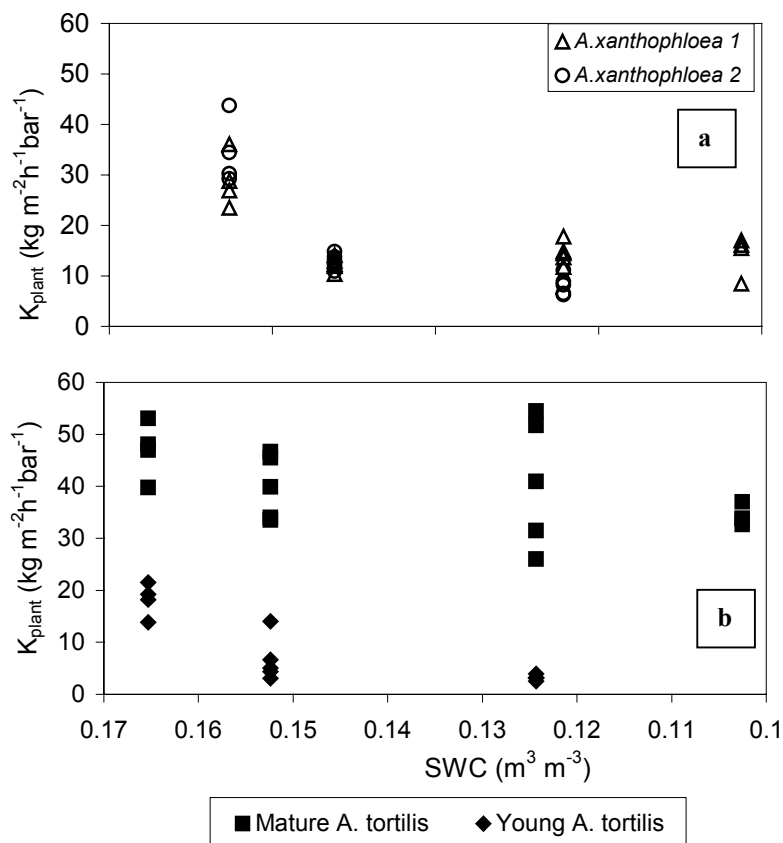
Figure 19. Relationship between whole tree hydraulic conductance (K_{plant}) and soil water content (SWC) for (a) *A. xanthophloea* (b) mature and young *A. tortilis* trees.

3.1.9. Water use efficiency

Less negative δ^{13}C was observed for *A. xanthophloea* than for *A. tortilis* during favorable soil water condition (Table 5). δ^{13}C also increased with increasing water stress in *A. tortilis*, but no significant

change occurred in *A. xanthophloea,* suggesting that *A. xanthophloea* was not able to alter water use with increasing water stress.

Table 5. Monthly records of $\delta^{13}C$ discrimination for leaves of *A. tortilis* and *A. xanthophloea*

Month	$\delta^{13}C$ values	
	A. xanthophloea	*A. tortilis*
March02	-27.64 (±0.50)	-27.95 (±0.40)
April02	-27.42 (±0.50)	-28.20 (±0.58)
May02	-26.61 (±0.49)	-27.59 (±0.31)
June02	-26.37(±0.52)	-27.67 (±0.77)
January03	-26.64 (±0.40)	-28.40 (±0.53)

3.2. Experiment 2: Mediterranean region of Portugal

3.2.1.Weather conditions

Figure 20 shows daily amounts of rainfall received in Herdade da Mitra study site between January and December 2003. Prevailing weather conditions during the study period are shown in Figure 21 Measurements commenced in May 2003 after the winter and spring rainfall. Total amount of rainfall recorded between January and December 2003 was 656 mm, not significantly different from the mean annual precipitation of 665 mm recorded between 1951 and 1990. However, summer 2003 was drier than usual since rainfall seized earlier than usual. After May, no significant rainfall was realized until beginning of September. Major rainfall events however commenced in October 2003. Mean maximum and minimum air temperatures during summer 2003 were 30.6 (± 6.8) and 14.3 (± 3.4) °C respectively. Highest temperature recorded during this period was 45.8 °C and occurred in the month of August. Mean daily temperature fluctuations were 23.4 °C. Soil temperature declined with increasing depth, lowest depth monitored being 0.8 m. Mean daily temperatures recorded at this depth during summer were 22.9 (± 2.2)°C. Temperatures at this depth were stable, with less fluctuation compared to the shallow soil layers. Highest temperature fluctuations occurred at the soil surface (+10 cm to -15 cm).

Both air and soil temperatures fell drastically in October, with the onset of rains. Changes in VPD mirrored those of air temperature, mean maximum VPD (VPDmax) in summer being 31.5 (± 17.9) hPa while mean minimum VPD (VPDmin) was 1.6 (± 2.8) hPa. As in the case of air temperature, highest

VPD recorded (50 hPa) was in August. Significant increase in VPD occurred between May and September, however on most of the days during summer 2003, dew formation occurred between 6:00 am and 7:00 am (i.e. VPD = 0). VPD declined significantly with the onset of October rains and remained at lower values, with maximum values recorded averaging 5 hPa. Favorable light conditions prevailed during summer 2003, PAR reaching 1,500 μmol m^{-2} s^{-1} on most of the days. Mean daily maximum PAR between May and October was 1,200 μ mol m^{-2} s^{-1}. Significant decline in PAR occurred between October and November, reaching a mean daily maximum of 500 μmol m^{-2} s^{-1}. Average wind speed was 0.93 (± 0.60) m s^{-1}, most winds during summer originating from N and NW direction.

Figure 20. Daily amounts of rainfall received in Herdade da Mitra between January and December 2003.

Figure 21 (a) Daily temperatures and maximum VPD and (b) Daily maximum PAR in Herdade da Mitra during summer 2003 and the following winter months. PAR data are records from six sensors placed at different locations within the plot.

3.2.2. Seasonal changes in soil water potential

Soil water potential (Ψs) measured at 0.3 m and 1 m depths significantly declined during summer 2003, the mean lowest value of −2.0 MPa being recorded at the end of August when maximum drought was experienced (Figure 22a). There was high heterogeneity in soil water distribution at both depths, within the study plot. Soil water potential at 0.3 m depth declined rapidly between May and July and lowest Ψs values of −2.3 MPa were observed in soils around trees *A11* and *D6* in September when maximum drought was experienced. Mean minimum Ψs attained at this depth around tree *A12, A13* and *D22* was −1.75 MPa. Trees *A11* and *D6* were more isolated with high density of grass, herb and shrub growth around them compared to *A12, A13* and *D22.* Tree *D6* had dense growth of grass (*Vulpia faciculata*) during and shortly after the rainy season while around tree A11 was a higher distribution of the shrub *Cistus salvifolius,* which, maintained its vegetation phase into July. After July, decline in Ψs at the 0.3 m depth was slow and uniform around all the 5 trees studied. Soil water potential at 0.3 m depth returned to zero or near zero values at the end of November after a rain event.

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Soil water potential at 1.0 m depth remained at ca. 0 MPa until late June when differences among trees started to appear (Figure 22b). Soils around trees *D22* and *D6* showed earlier decline in Ψs at this depth compared to the rest. Decline however, was more rapid in *D22*. Latest onset of decline in Ψs at 1 m depth was observed around *A11*, commencing in mid July, at the middle of the summer season. Over the entire summer period, a decline of only 0.7 MPa was reported for soils around *A11*. Soils at 1 m depth around trees *A12* and *A13* showed similar patterns in Ψs decline. Mean minimum Ψs observed during summer at this depth was –2.0 MPa and was reported in September around trees *A12, A13* and *D22*. Most favorable Ψs at this depth was recorded in soils around *A11* and was –0.75 MPa. Unlike Ψs at 0.3 m, Ψs at 1.0 m did not immediately return to zero values after the rainfall events at the end of September. Also significant was the lack of response in Ψs at this depth to short rains, which occurred at the end of August.
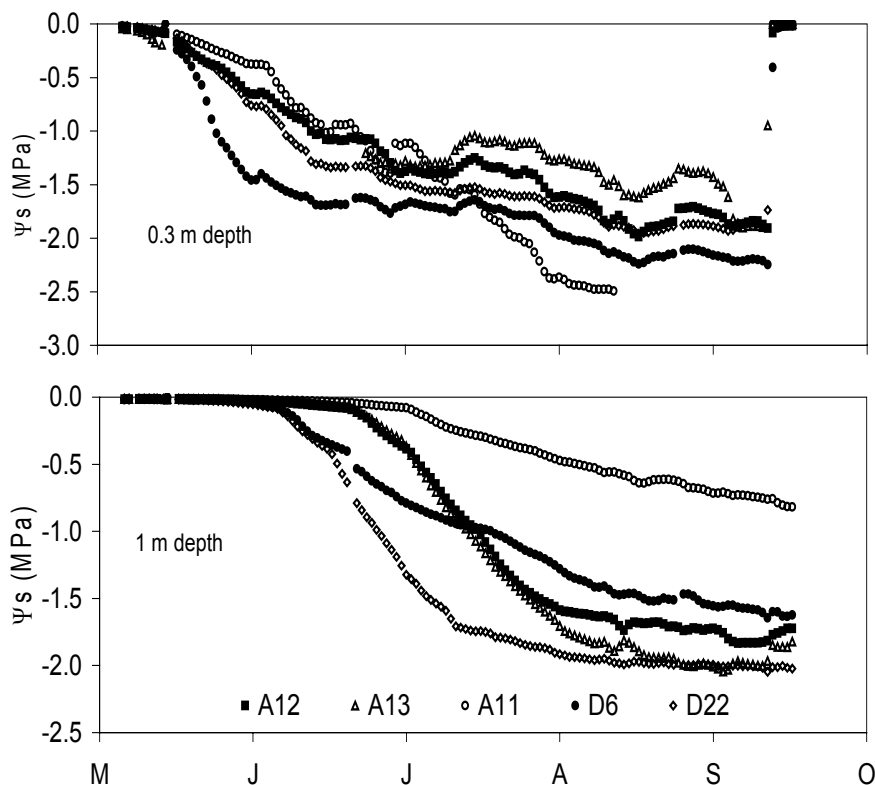
Figure 22. Mean daily soil water potential (Ψs) measured at 0.3 m and 1 m soil depth for the study stand of *Q. suber*, measured around 5 main trees, *A11, A12, A13, D6* and *D22* during summer 2003.

Trees experienced favorable tissue water potentials (Ψpd ca. -0.1 MPa) between May and July, following the winter and spring rainfall, corresponding with Ψs. There were no significant differences among trees between May and July (Figure 23). Ψpd however, declined markedly between July and September associated with the summer drought, reaching the lowest value of -1.9 MPa recorded during this study period. After July, significant variations in Ψpd occurred among trees and tree *A12* appeared to be more vulnerable, having the lowest mean Ψpd values (Ψpd ca. -1.9 MPa) at the end of summer. *A13* and *D22* showed similar changes in their Ψpd, attaining the lowest values of -1.53 MPa in September when maximum drought was experienced. Tree *D6* experienced the most favorable tissue water status and Ψpd declined to only -0.8 MPa at the end of summer. Intermediate responses were reported for tree *A11*, attaining minimum Ψpd values of -1.2 MPa during summer. Similarly, midday leaf water potential (Ψmd) declined at the beginning of July, corresponding with the decline in Ψpd. Despite significant differences in seasonal Ψpd values, there was no significant differences among trees in their seasonal Ψmd values, suggesting possibly a common regulation mechanism.
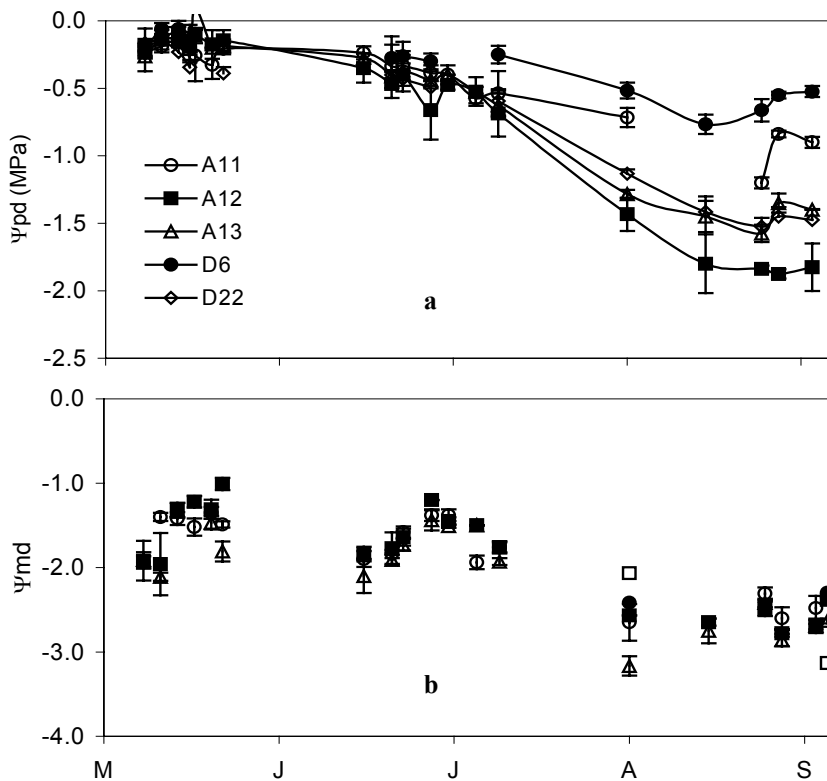
Figure 23. Seasonal changes in (a) Predawn (Ψpd) and (b) midday (Ψmd) leaf water potential measured respectively on five and three main experimental trees of *Q. suber* at the experimental site respectively.

3.2.3. Relationship between soil and plant water status

Correlation between Ψpd and Ψs at 0.3 m revealed significant differences among the trees (Figure 24). From this correlation, a clear distinction appeared between two sets of trees in which trees *A11* and *D6* showed less response to changes in Ψs at this depth compared to trees *A12, A13* and *D22*. Although Ψpd for trees *A12, A13* and *D22* were also less influenced by changes in Ψs at the 0.3 m depth, their Ψpd started to decline at much higher Ψs (1.0 MPa) and the decline in Ψpd was more rapid compared to *A11* and *D6* which retained near zero Ψpd values until Ψs at 0.3 m depth was -1.7 MPa.

Plotting tree Ψpd against Ψs at 1 m depth showed a strong relationship between Ψpd for trees *A12, A13* and *D2*2 and Ψs at this depth. Water status of tree *A12* appeared to be more closely related to Ψs at the 1 m depth compared to *A13* and *D22*. However, the difference was not statistically significant. There were significant differences between *D6* and *A11* at 1 m soil depth, with Ψpd of tree *A11* being significantly influenced by changes in Ψs at this depth while Ψpd for *D6* was less affected. Although Ψpd for tree A11 appeared to be influenced by Ψs at 1 m depth, similar to trees *A12, A13* and *D22*, there was apparently very slow seasonal changes in Ψs at 1 m depth around this tree and consequently, Ψpd declined rather slowly during summer compared to the rest of the trees (Figure 24, upper panel). Despite strong influence on Ψpd of most trees by Ψs at 1 m depth, measurements conducted between 13-24 September, that is after rain events between 31 August and 2 September, showed an increase in Ψpd in all the trees. Only Ψs at 0.3 m depth increased after the rains while there was no change at 1 m depth.
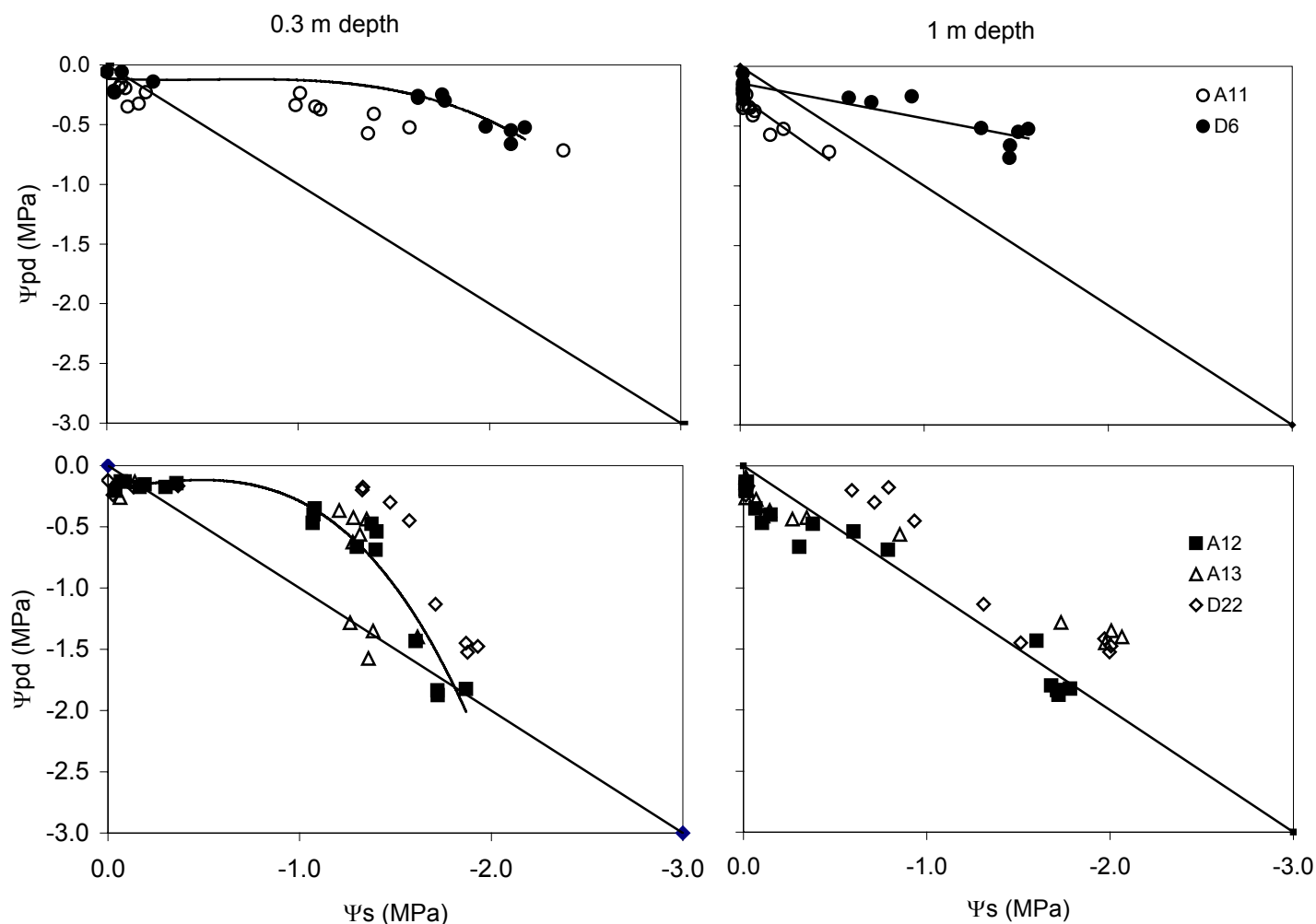
Figure 24. Predawn leaf water potentials (Ψpd) of five trees of *Q. suber* as a function of the soil water potential (Ψs) measured at 0.3 and 1 m depth soil layers around the trees. The upper panel is for trees *A11* and *D6* while the lower panel for trees *A12, A13* and *D22*. Separations were based on clear differences among the tree groups.

3.2.4. Seasonal changes in sap flow

Figure 25 shows estimates of daily amounts of sap flow (*Qtree,* kg cm^{-2} d^{-1}) expressed per unit area of active xylem transported across the main stems (breast height) of five experimental trees measured with the heat dissipation method during summer 2003. The estimates represent daily plant water use expressed per unit active xylem area, transpired by each tree. Similar results measured at the branches (*QBranch* kg cm^{-2} d^{-1}) using branch sensors are shown in Figure 26. Separation of trees into two groups was based on their significant differences (p=0.05). Although higher (ca. 3 times) estimates of water use were obtained from branch sensors, there was good agreement between the two sensor types in terms of seasonal patterns of tree response and also differences among trees were clearly shown by the two methods. High estimates of daily water use by the branch sensors were attributed to lack of leaf

shading since the measured branches were well exposed. Except for days when there were overcast sky, maximum tree water use occurred between May and July when mean daily water use of 0.35 kg cm^{-2} d^{-1} were recorded. Water use however declined by ca. 30-50 % as a result of drought. Throughout this period, trees with favorable tissue water status recorded higher transpiration rates. Significant (p> 0.05) differences occurred between trees *A11* and *D6*, on the one hand, and (*A12, A13* and *D22* on the other. Also, differences between *A11* and *D6* were significant. Differences were more pronounced between July and August.
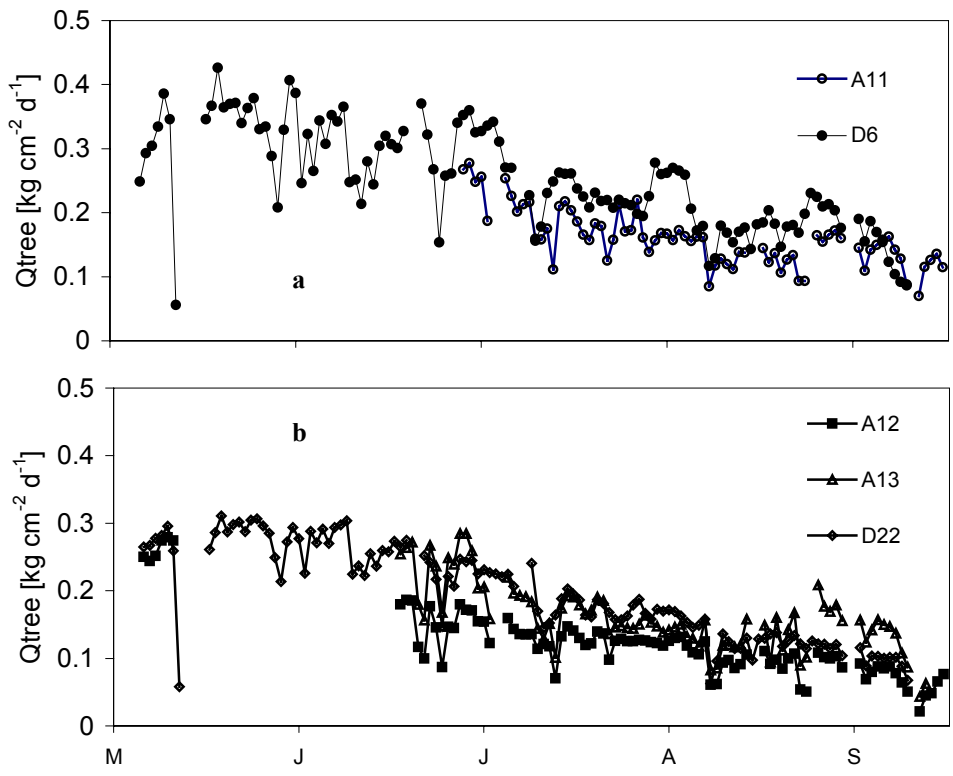
Figure 25. Seasonal changes in daily tree water use (Qtree) measured at the main tree stems with the heat dissipation method. Trees are grouped as **a** (*A11* and *D6*) and **b** (*A12*, *A13* and *D22*). Data is missing for *A11*, *A12* and *A13* as a result of technical field problems.
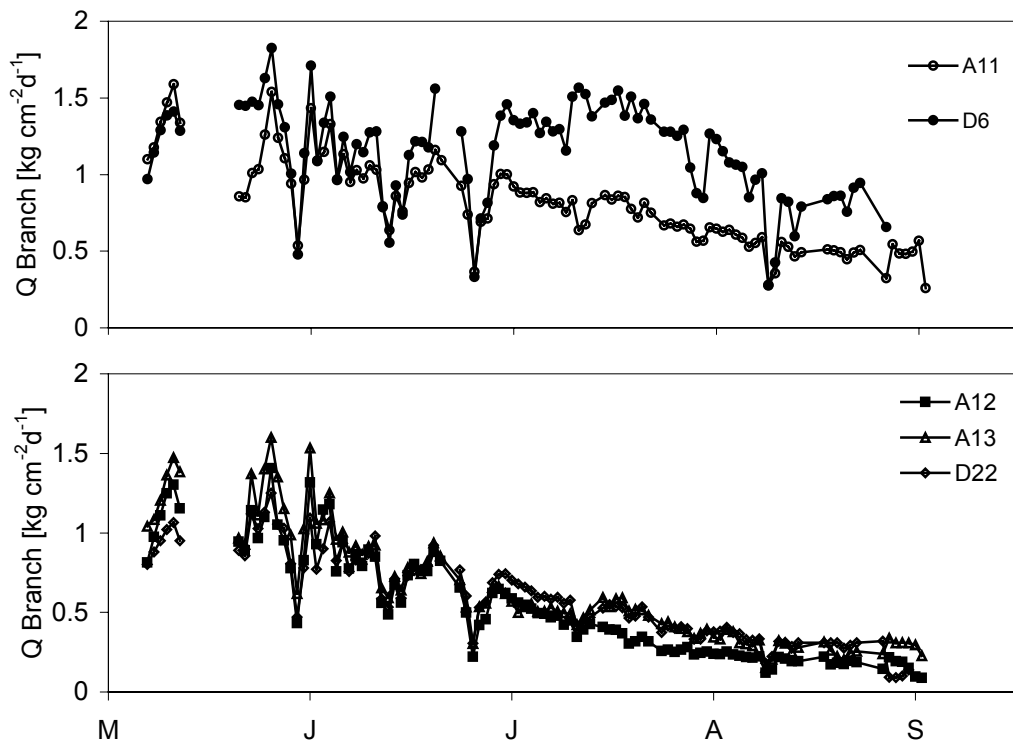
Figure 26. Seasonal changes in daily sap flux expressed per unit xylem area (*QBranch*) measured at the branches of 5 *Q. suber* trees with the heat balance method. Sensors were installed on well-exposed south facing branches. Trees are grouped as (*A11* and *D6*) and (*A12, A13* and *D22*) according to significant differences (p=0.05).

In contrast to the significant differences of sap flux rates per unit active xylem between the trees, the relationship between maximum daily sap flux rates expressed per unit LA and Ψpd (Figure 27) revealed similar pattern of response in all the trees. This suggests that trees will behave in a similar manner when subjected to similar levels of water stress. Since they were of the same species and also exposed to similar atmospheric conditions, the only likely source of variation as observed in their tissue water status could be in the soil environment.
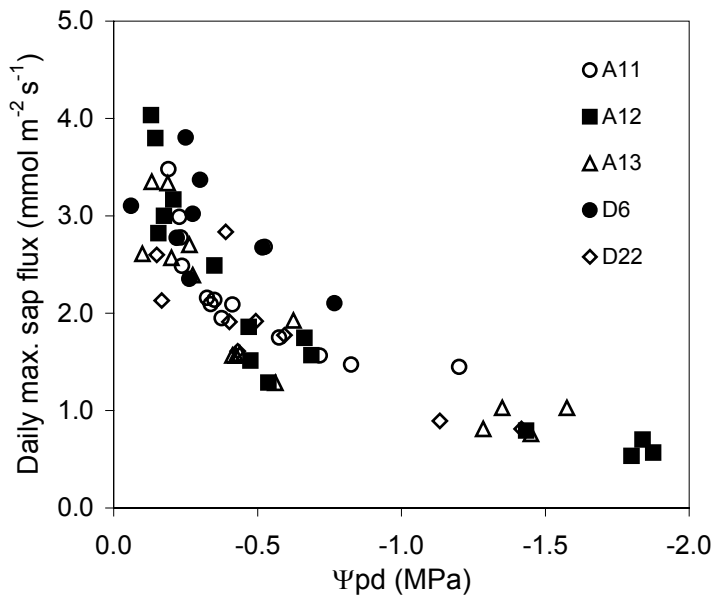
Figure 27. Daily maximum sap flux (per LA) as a function of predawn leaf water potential (Ψpd) for *Q. suber* trees under declining soil water conditions.

Relationships between daily maximum sap flux rates and soil water status at 0.3 and 1 m depth for different study trees are shown in Figure 28. Results show significant differences among trees at both soil depths. A weak relationship existed between maximum sap flux rates and Ψs for tree *D6* at both depths. Relationship between Ψs and sap flux rates were stronger ($R^2 = 0.5$) at 1 m soil depth for tree *A11*. Strongest relationships between diurnal maximum sap flux rates and Ψs were observed for trees *A12* and *D22* at 0.3 and 1 m soil depths respectively. Response in *A13* was intermediate.
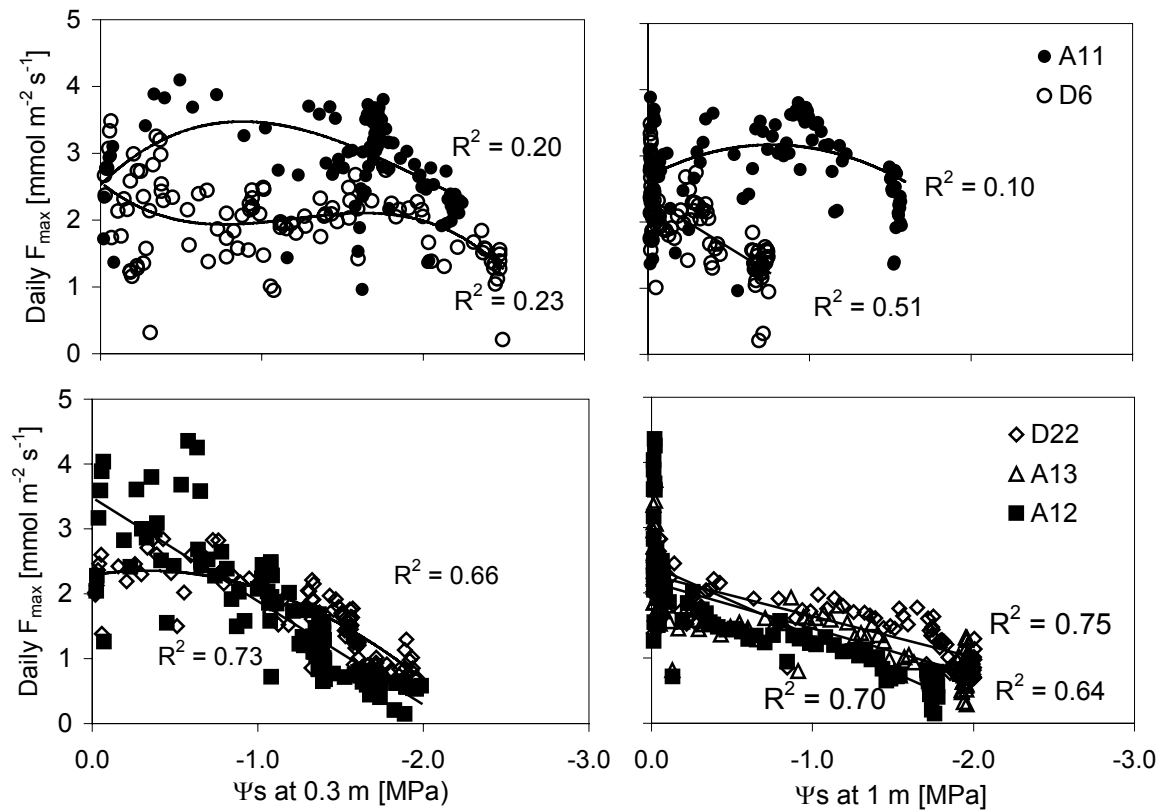
Figure 28. Daily maximum sap flux rates (Daily F_{max}, leaf area basis) as a function of soil water potential (Ψs) at 0.3 (left) and 1 m (right) depth for five trees *A11* and *D6* (upper panel) and *A12, A13* and *D22* (lower panel).

3.2.5. Shoot water relations.

Summary of water relations parameters for tree *A12* derived from the analysis of *p-v* curves are shown in Table 6.

Table 6. Osmotic potential at full saturation (Π^{100}), water potential at turgor loss point (Ψtlp), relative water content at turgor loss point (R*tlp) and maximum bulk modulus of elasticity derived from *p-v* curves constructed from measurements on tree *A12*. Significant difference is indicated by (*). $p < 0.05$, ±SD.

	Π^{100}(MPa)	Ψtlp(MPa)	R*tlp(%)	ε_{max}(MPa)
June	-1.2 (±0.1)	-2.0 (±0.3)	76.0 (±1.6)	5.70
July	-1.9 (±0.1)*	-2.40 (±0.04)*	72.5 (±6.5)	22.41*
September	-1.2 (±0.1)	-2.10 (±0.04)	79.7 (±1.5)	7.62

Between June and July, Π^{100} declined significantly by about 60%. This was associated with increasing drought stress experienced by trees over this period. Similarly, Ψtlp declined from -2.0 MPa to –2.4 MPa but R^*tlp was not significantly affected by the declining soil water availability. ε_{max} increased four fold over the same period. Measurements conducted in early September, a few days after rain events showed all parameters (Π^{100}, Ψtlp, R^*, and ε_{max}) were restored to their pre-stress values (June) despite the persistently low Ψs at 1.0 m depth. It is evident that Ψ_L was above turgor loss point (TLP) most of the time during summer except in September, after the first rain events.

3.2.6. Growth

Figure 29 shows cumulative increase in stem circumference (Ci) of three *Q. suber* trees during 2003. Increase in stem circumference, which in this study was representative of tree growth, occurred between May and July in trees *A12* and *A13* but continued until August in tree *A11*. Increase in stem circumference was recorded only during favorable soil and plant water status, and declined as drought increased. Although stem expansion ceased much earlier in *A12*, it had a relatively higher rapid stem expansion when water was available compared to *A11* and *A13*.
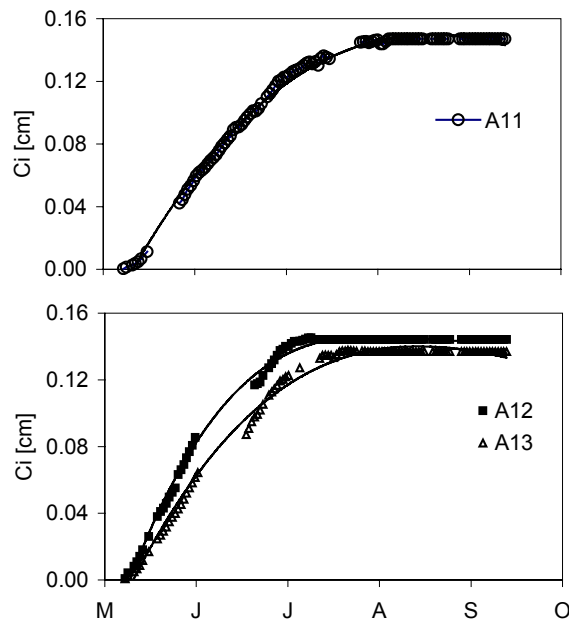
Figure 29. Cumulative stem circumference increment (Ci) of three *Q. suber* trees (*A11, A12* and *A13*) measured with circumference dendrometer. Data gaps are the result of equipment failure during measurements.

3.2.7. Seasonal changes in leaf transpiration, stomatal conductance and water potential.

Results for seasonal changes in leaf transpiration and leaf stomatal conductance are shown in Figure 30. Seasonal course of E and gs were similar, with respective mean maximum rates of 6 and 300 mmol m^{-2} s^{-1} being experienced between May and mid-June. E and gs then rapidly declined to lowest maximum values of 2 and 50 mmol m^{-2} s^{-1} respectively recorded during the summer season in August. Seasonal changes in E and gs resembled those of Ψpd (Figure 23a). During favorable soil water availability, the respective mean values of E and gs were similar among trees. This, however, changed with increasing drought and trees *A12* and *A13* showed significantly lower E and gs compared to tree *A11*.
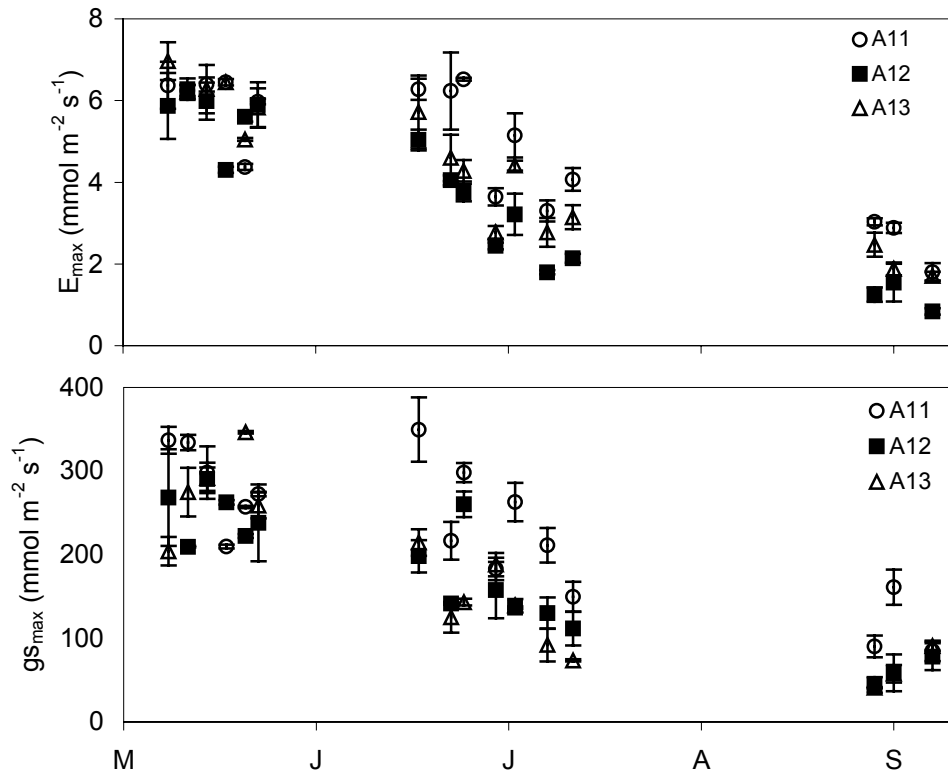
Figure 30. Seasonal course of (a) maximum leaf transpiration (E_{max}) and (b) maximum leaf stomatal conductance (gs_{max}) measured in the sun crown of 3 *Q. suber* trees experiencing varying intensities of water stress. Bars indicate deviation from the mean and n= 4

3.2.8. Responses of leaf transpiration and stomatal conductance to changing soil and plant water status

Responses of leaf transpiration and stomatal conductance to changing soil water status at 0.3 m depth were different among trees. Thus, E_{max} and gs_{max} declined more rapidly in both trees *A12* and *A13* compared to *A11*, in response to declining Ψs at 0.3 m depth (Figure 31). Decline in Emax and gsmax were slower in tree *A11* compared to trees *A12* and *A13*. Trees *A12* and *A13* however, did not differ from each other (p=0.58). Differences among trees indicated variations on influence of water availability in soil layers above and around 0.3 m soil depth on their functioning. Plotting E_{max} and gs_{max} against Ψs at 1 m depth however, revealed similar response patterns in all the trees considered (Figure 31), an indication that they were accessing soil water at this depth. Water availability at 1 m depth influenced both E and gs in a similar manner and Ψs at this depth had a strong influence on tree responses.
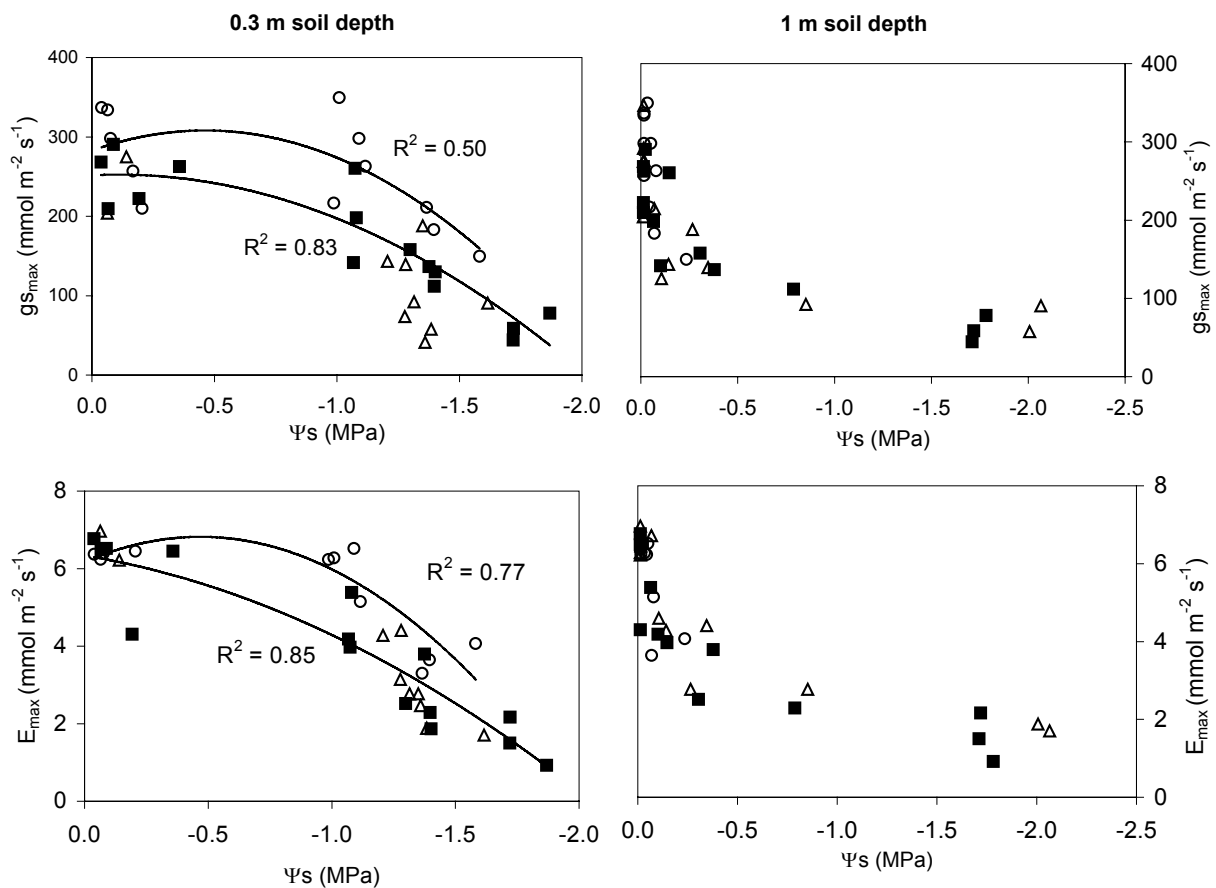
Figure 31. Relationship between maximum leaf stomatal conductance and soil water potential (Ψs) measured at both 0.3 and 1 m depth (upper panel) and maximum leaf transpiration vs soil water potential (Ψs) at 0.3 and 1 m depth.

Relationship between gs_{max} and Ψpd and also E_{max} and Ψpd (Figure 32) equally showed similar responses to water stress among the trees and that gs and E declined with increasing drought intensity. An exponential decay functions $\left[g_{max} = a\exp(-b\Psi pd)\right]$ and $\left[E_{max} = a\exp(-b\Psi pd)\right]$ were fitted to data plot relating gs_{max} and Ψpd as well as E_{max} and Ψpd respectively (Figure 32), using non-linear least square analysis (SIGMA PLOT 8.0, SPSS Inc., Chicago, IL, USA). The functions were first fitted to grouped data from the three trees and then to separate data from the respective trees. There were no significant differences ($p>0.05$) among the trees. This suggested that differences in gs and E among trees emerging during drought were the result of differences in soil water availability at the rhizosphere, which affected Ψpd. Despite seasonal differences in tree transpiration (Figure 30a) and Ψpd (Figure 23a), there were no significant difference in daily minimum leaf water potential (Ψmd) attained by trees during the day (Figure 23b). It was also observed that during ample soil water availability, Ψmd did not drop below -2.0 MPa. This lower limit however dropped to -2.4 MPa between July and September (Figure 23b). Similarly, water potential at turgor loss point (Ψtlp) was -2.0 when soil water supply was abundant but later dropped to -2.4 MPa between June and July (Table 6) and perhaps further into August.
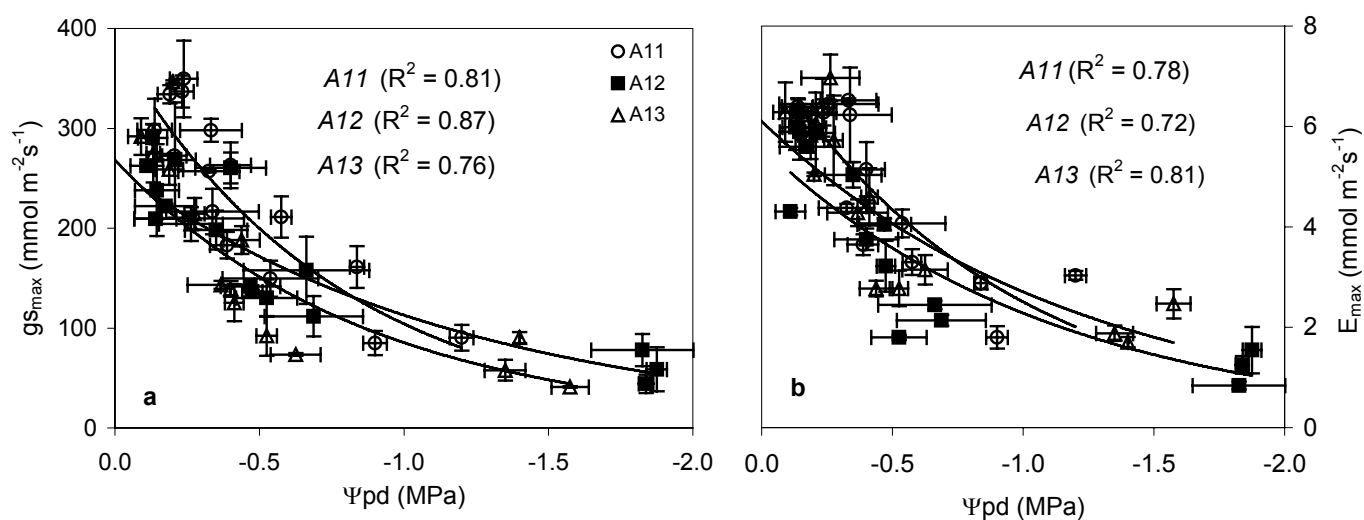
Figure 32 (a) Maximum leaf stomatal conductance (gs_{max}) and (b) maximum leaf transpiration (E_{max}) as a function of predawn leaf water potential (Ψpd) in *Q. suber* three trees. Measurements of E and gs were conducted on well-exposed crown leaves. Bars indicate deviation from the mean and n= 4

3.2.9. Diurnal changes in leaf stomatal conductance, transpiration and water potential

Patterns of diurnal gs, E and Ψ_L of two trees *A11* and *A12* during different times of the year, with varying drought intensities, are shown in Figure 32. Corresponding air temperatures and VPD are shown in the upper panel. Results from these two trees are considered for further comparisons because of their significant seasonal differences in Ψpd with *A11* showing consistently higher Ψpd values. Diurnal patterns of gs and E changed strongly over the season following changes in Ψs and Ψ_{pd}. Between May and early June, when Ψs was favorable (Ψpd = ca. 0 MPa), gs rose with increasing light and VPD, attaining mean maximum rates of around 280 mmol m^{-2} s^{-1} by 10:00 hours and remained at these high rates as long as light conditions were favorable. Conductance rates declined later in the day around 17:00 hours, when light intensity had declined to lower values. Leaf transpiration followed similar pattern exhibited by gs, associated with decline in Ψ_L during the early part of the day. Mean diurnal minimum Ψ_L attained during this period was -1.2 MPa. Recovery of Ψ_L occurred as soon as transpiration rates decreased and Ψpd values recovered by dusk. Although slight depression in gs occurred around midday in *A12*, trees attained similar maximum gs and E and minimum Ψ_L during the day.

Soil water potential at 0-30 cm depth significantly declined by July (Figure 22) and both *A11* and *A12* had similar Ψpd values (-0.5 MPa), which was the mean Ψs measured at 1 m depth. During this period, maximum gs rates declined to around 200 mmol m^{-2} s^{-1} and 150 mmol m^{-2} s^{-1} in *A11* and *A12*, respectively. Maximum rates of E in *A11* were 5.2 mmol m^{-2} s^{-1}, similar to values recorded during full soil water saturation. However E declined by half in *A12*. Both *A11* and *A12*, however, attained similar minimum Ψ_L during the day. Also during this period, gs rose to a maximum early in the morning, reaching maximum at around 10:00 hours but significantly declined before midday and slightly resuming later in the day. Both *A11* and *A12* exhibited similar pattern of responses.

In September, when lowest Ψs were experienced and significant differences in Ψpd were observed between *A11* and *A12* (-0.85 and −1.65 respectively). The rates of gs were twice as high in *A11* compared to *A12* (gs$_{max}$ ca. 160 and 68 mmol m^{-2} s^{-1} respectively). This coincided with the periods when lowest gs rates were observed during the summer. Rates of stomatal conductance rose to maximum early in the morning, but significantly declining before noon and remained at very low

values during the rest of the day. Despite significant differences in Ψpd, trees however, attained similar midday Ψ_L of around –2.7 MPa. Recovery of Ψ_L later in the day, when transpiration rates had declined was more rapid in *A11*. Throughout summer, Ψ_L declined rapidly after sunrise, attaining minimum values by 11:00 hours and remaining relatively stable for the rest of the day, recovering later in the day when E declined.
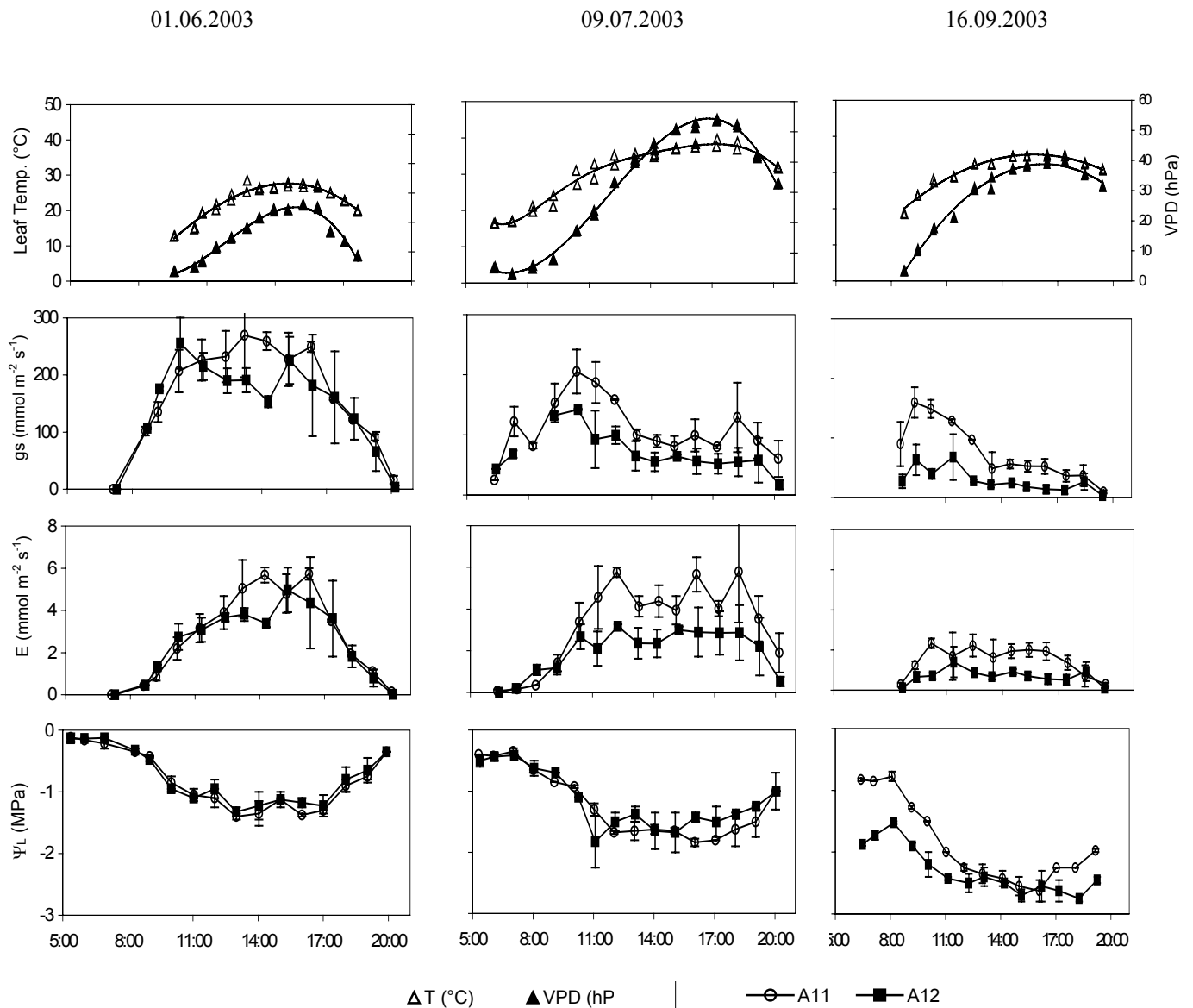
Figure 33. Diurnal courses of vapor pressure deficit (VPD) and air temperature (panel 1), Stomatal conductance (gs) (panel 2), Leaf transpiration (E) (Panel 3) and Leaf water potential (Ψ_L) (Panel 4) of two *Q. suber* trees growing under similar atmospheric weather conditions but different soil moisture content. Results are from 3 different times of the year with different drought stress intensities.

3.2.10. Leaf specific hydraulic conductance (Ks-l)

Examples of relationships between leaf transpiration (E) and leaf water potential (Ψ_L) for tree *A12* at different times during summer season are shown in Figure 33. Leaf specific hydraulic conductance (Ks-l) was derived from this relationship. Results show consistently increasing gradients, hence decreasing Ks-l with increasing drought intensity.
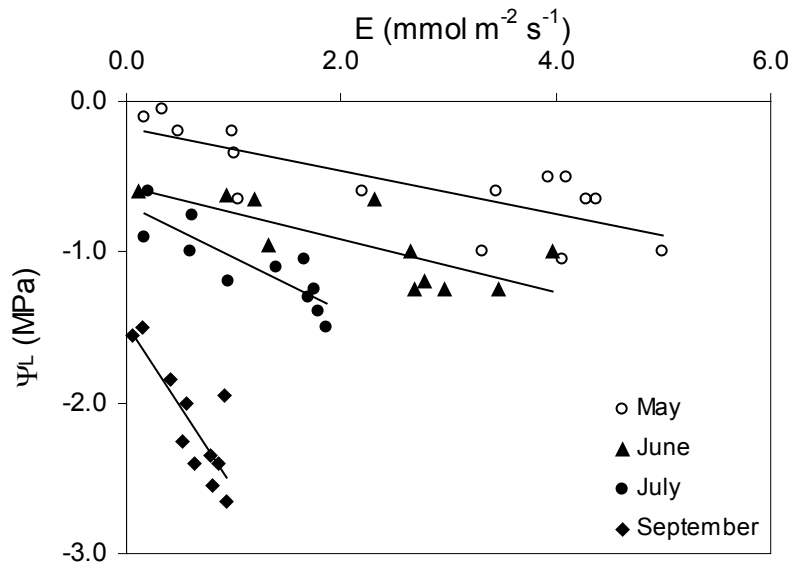
Figure 34. Relationship between leaf transpiration rate (E) and leaf water potential (Ψ_L) of tree *A12* during different times of the season experiencing different levels of drought. Only values till midday when minimum Ψ_L was attained, are considered for the plot. Lines are linear regressions.

Results showed that changes in Ks-l were more responsive to changes in Ψs at 1 m depth than at 0.3 m depth (Figure 35). Response patterns among trees were similar to those observed for stomatal conductance and leaf transpiration. Significant differences occurred among trees when changes in Ks-l were compared to changes in Ψs at 0.3 m soil depth. These differences however, disappeared when comparisons were made at 1 m soil depth.
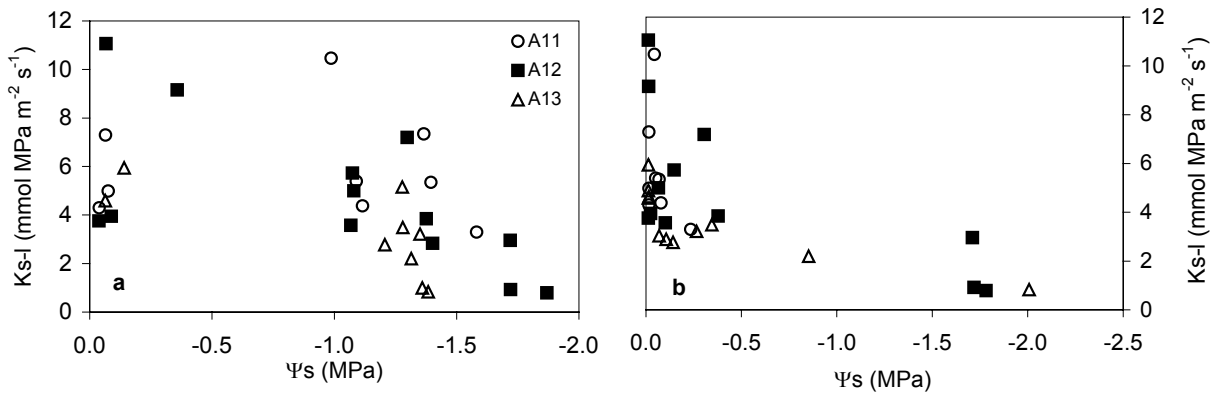
Figure 35. Relationship between leaf specific hydraulic conductance and soil water potential at (a) 0.3 m and (b) 1 m soil depth at the rhizosphere of three *Q. suber* trees.

Figure 35 shows relationship between Ks-l and predawn leaf water potential (Ψpd) of the *Q. suber* trees. Trees exhibited similar responses to water stress. Changes in Ks-l with respect to plant water status (Ψpd) were similar to responses of Ks-l to changes in Ψs at 1 m depth. A rapid drop in Ks-l occurred between Ψpd = 0 and Ψpd = -1.0 MPa. This was similar to the pattern of response to Ψpd exhibited by gs and E. Mean maximum hydraulic conductance was 6.5 mmol MPa^{-1} m^{-2} s^{-1} during favorable soil water conditions. Ks-l, however, declined to around 2 mmol MPa^{-1} m^{-2} s^{-1} at the end of summer drought when Ψpd values of –2.0 MPa were recorded.
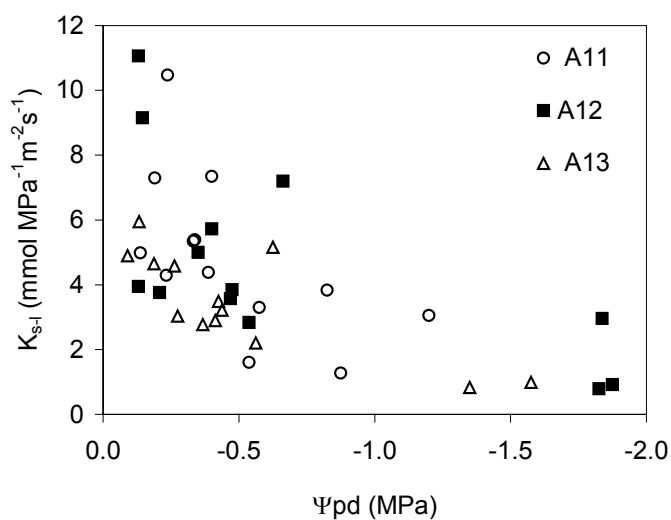
Figure 36. Leaf specific hydraulic conductance (Ks-l) as a function of predawn leaf water potential (Ψ_L) for three *Q. suber* trees experiencing varying levels of water stress.

3.2.11. Constraints on transpiration water loss

The results showed a consistent decline in maximum E attained during the day as drought stress increased. Mean maximum transpiration rates (E_{max}) attained during favorable soil water status were 8 mmol m^{-2} s^{-1}, associated with high Ks-l but E_{max} declined to ca. 1.5 mmol m^{-2} s^{-1} between August and September, a time when maximum drought stress was experienced. The relationship between gs and Ks-l and also between E and Ks-l were curvilinear, with gs and E saturating at higher Ks-l values, while the gradients were steeper at lower Ks-l values (Figure 36).
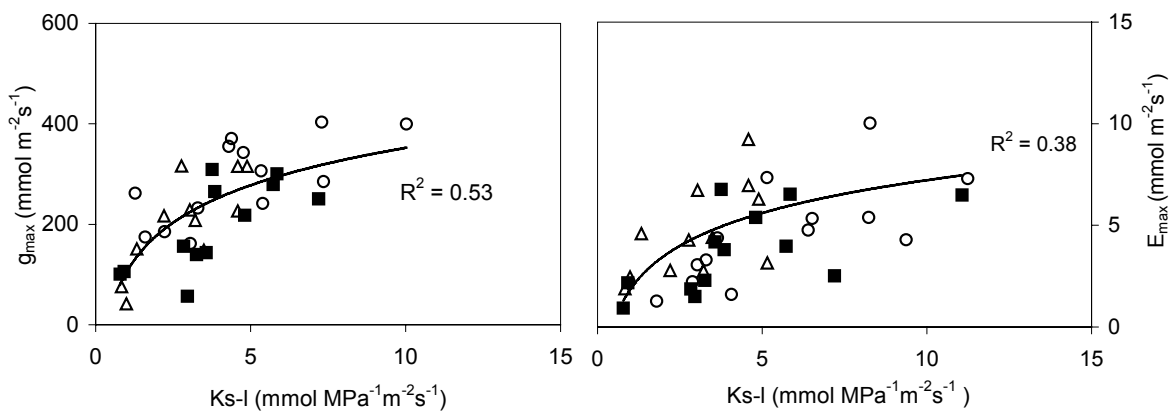
Figure 37. (a) Maximum stomatal conductance (gs_{max}) and (b) maximum leaf transpiration (E_{max}) as a function of leaf specific hydraulic conductance (Ks-l) for three *Q. suber* trees experiencing varying levels of water stress.

There was an upper limit to sap flux rates at saturated light (Figure 37). Thus, rate of sap flux increased during the day as light and VPD increased, reaching a maximum. The maximum rates attained during the day varied seasonally depending on soil and plant water status (Figure 27). Maximum sap flux rates declined with increasing drought stress. Once the maximum sap flux rates were attained, they remained stable for the rest of the day despite increasing VPD and light (Figure 37, lower panel). Stomatal conductance was at its maximum until VPD of 30 hPa. Maximum stomatal conductance rates ranged 350 mmol m^{-2} s^{-1} during ample water supply but declined significantly during drought (Figure 32). Stomatal conductance also declined at VPD values greater than 30 hPa (Figure 38).
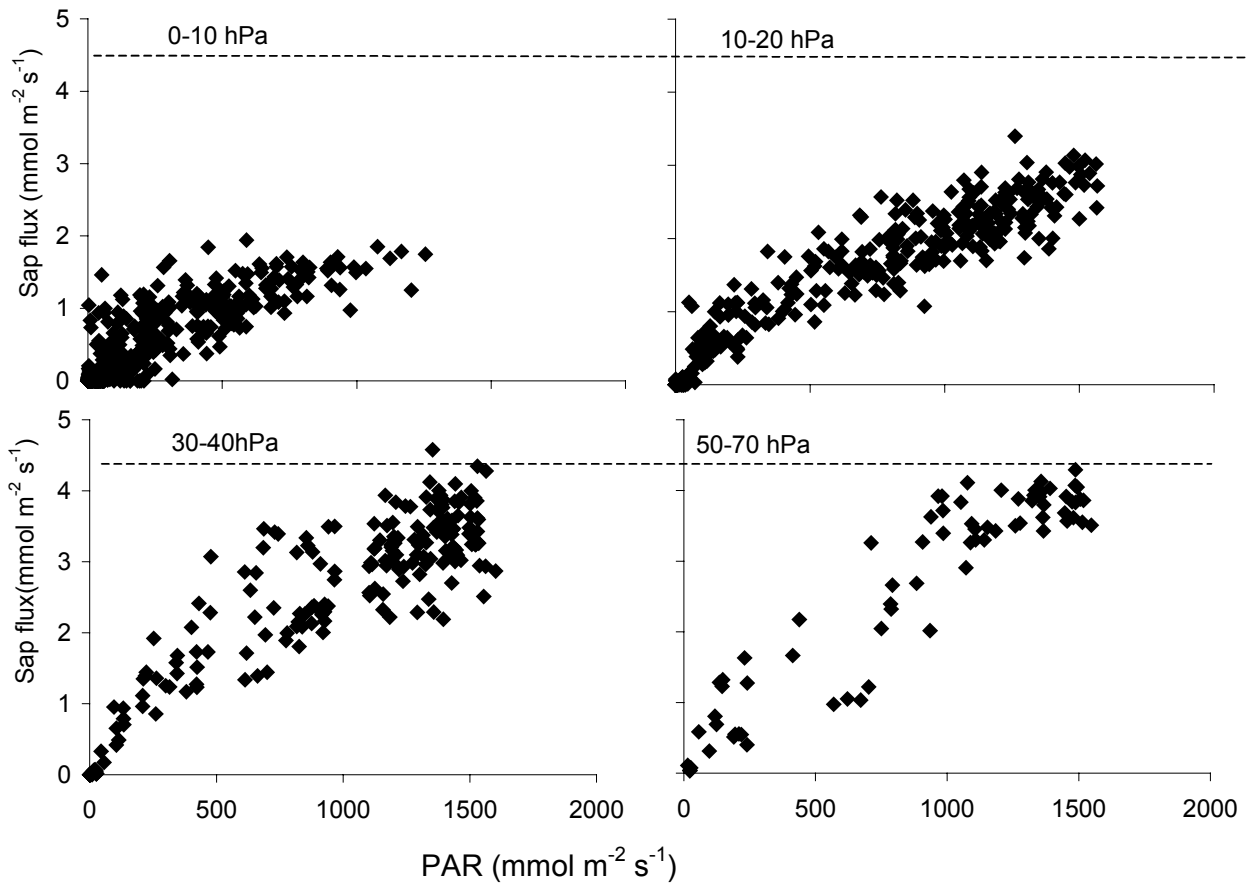
Figure 38. Relationship between sap flow rate and solar radiation (PAR) at different intensities of vapor pressure deficit (VPD) for *Q. suber* trees under conditions of favorable soil water content. Dotted lines show the upper limit of sap flux rate.

Relationship between stomatal conductance and VPD showed that plants maintained maximum stomatal conductance rates of ca. 300 mmol m^{-2} s^{-1} as long as VPD was below 30 hPa and light and soil moisture conditions were suitable. Further increase in VPD led to progressive decline in stomatal conductance associated with stomatal closure (Figure 36). Similar observations were made for all the trees studied.
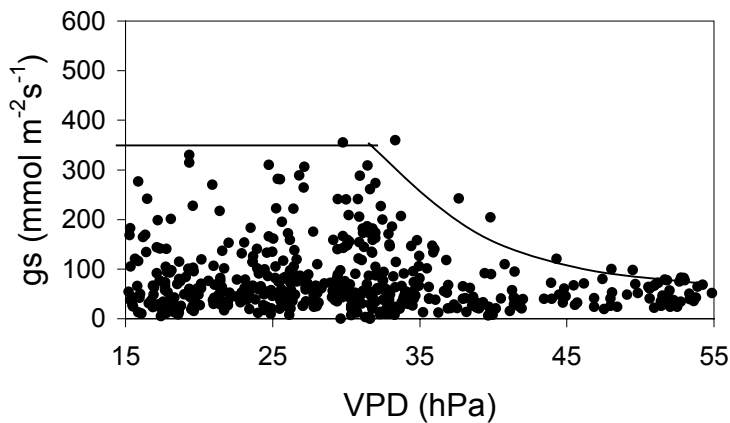
Figure 39. Relationship between stomatal conductance (gs) and vapor pressure deficit (VPD) during the entire summer 2003 period for all the trees studied.

3.2.12. Influence of Q. suber trees on the water content of the rhizosphere.

Changes in soil water potential measured at 0.3 m and 1 m soil depth at the rhizosphere of the five trees studied are shown in Figure 22. Distinct and repeatable dial fluctuations were observed at 0.3 m soil depth at the rhizosphere of tree *D6*, which commenced late during the month of July and continued till mid August. Pronounced fluctuations between 0.025 and 0.03 MPa were observed at 0.3 m soil depth during the early part of August as shown in Figure 37. Soil water potential rapidly declined during the day, commencing soon after sunrise (6.00 a.m.) and increased during the night, soon after sunset (8.00 p.m.). The net Ψs at this depth however, continued to decline indicating that water lost during the day was far more than the amount that could be lifted to the surface layers at night. Fluctuations at 1 m soil depth occurred after 1st September (not shown), when some rain events occurred. This was accompanied with increase in Ψs at this depth, which continued for 2 – 3 days after the rain events. Surprisingly, no such rapid reaction was observed at the beginning of October, when large rain events were realized and Ψs at the deeper soil layers remained unchanged for a couple of days after the first rain event.
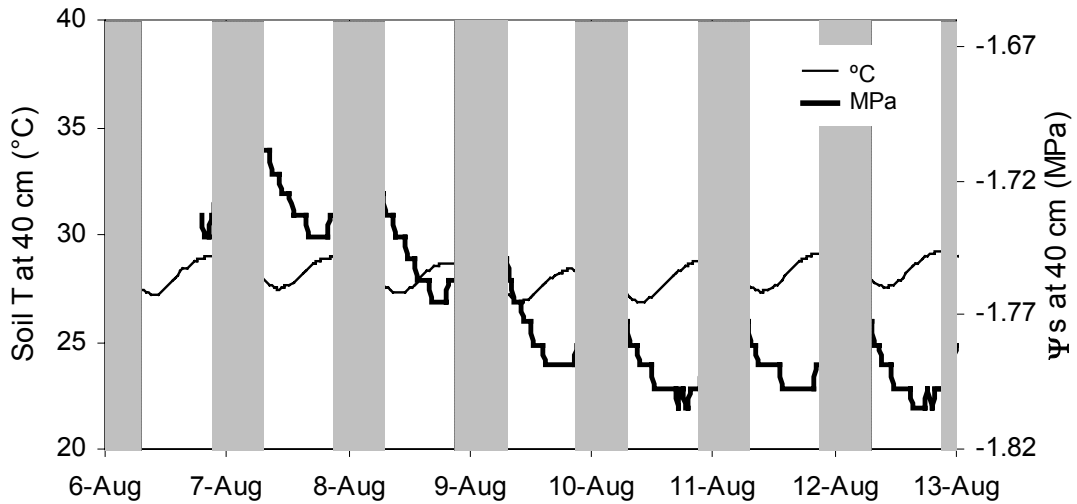
Figure 40. Time course of soil water potential (Ψ_s: thick line) measured at 40 cm depth under *Quercus suber* tree (*D6*) showing hydraulic lift. Nighttime (shaded) was occurring between 20:30 to 6:40 (shaded areas). Vertical lines indicate midnight. Soil temperature (T: thin curve) is represented to show that EQ15 signals were not influenced by natural soil temperature gradients.

3.2.13. Soil and xylem water isotope signatures

Most isotopic variation is explained by the type of rainfall event. Significant variation occurred in the δD values of precipitation received on the study site with values ranging between −40 and −12‰ during winter and −35 and −4‰ during summer. δ^{18}O values equally showed more enrichment in the summer than in winter precipitation. Results from monitoring of Ψs and stable isotope analysis showed a marked gradient in both the stable isotope composition and soil water and that a positive Ψs gradient existed with increasing soil depth (Figure 22). Until mid July, there was a significant drop in the Ψs of the upper 0.3 m soil profile, while Ψs at lower soil depths remained unchanged. This was similarly associated with isotopic enrichment of soil water within this layer (Figure 38). During this time, changes in isotopic composition were similar for soil samples taken among large trees, small trees and open space with grasses up to 0.25 m. From 0.25 m down to 1m depth, δD trends differed according to vegetation type, showing more depleted value (-45‰) for soil collected close to trees than close to dead grasses (-35‰). Isotope analysis taken during September 2003, before the long rains however, showed varied results among the three locations with different vegetation types at all soil depths. Samples taken at 0.25 m depth, close to the large trees showed isotopic signatures closer to those of ground water (ca.

-38‰). Isotopic signatures were also more depleted in soil water further away from the stem bases, especially, those of trees with favourable Ψ_L. Intermediate signature occurred in the twigs during this period (-38±3‰).
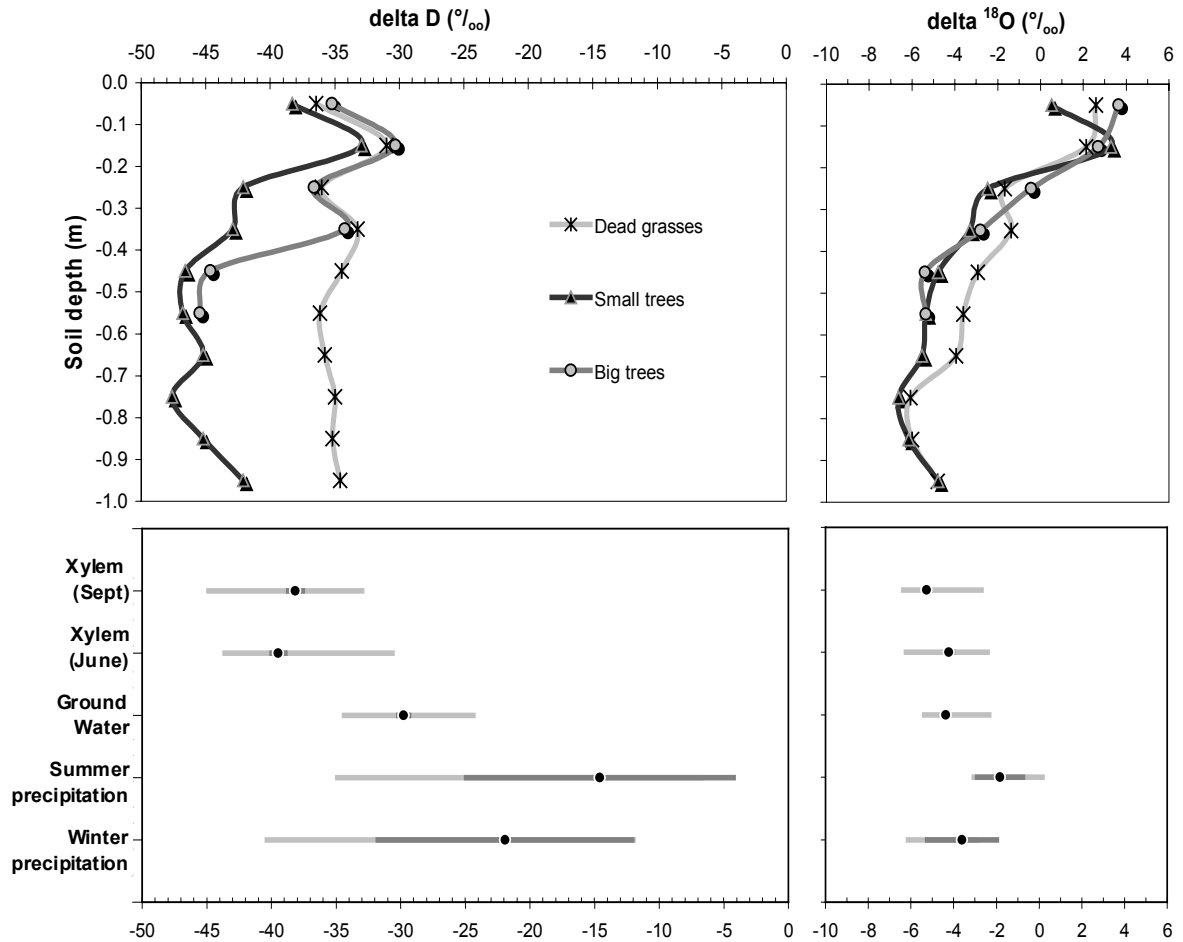
Figure 41. (Upper panel) soil water isotopic signature ($\delta^{18}O$ and δD) versus soil depth at three different locations in the experimental plot: Close to big trees (circles), small trees (triangles), and dead grasses (crosses) during the month of July 2003. (Lower panel) water isotopic signature in tree xylem (in June 2003 and September 2003), ground water, summer precipitation (June-September 2003), and winter precipitation (September 2002-May 2003). Black square indicates mean value. Dark grey bars indicate standard error of the mean. Light grey bars indicate minimum and maximum value observed from March 2002 to September 2003.

No relationship was established between twig xylem δD and Ψ_L in June, but significant negative linear relationships were found in early September for all sampling occasions, with a best fit obtained from samples obtained between 10.00 am and midday, with $\delta^{18}O$ (Figure 39).
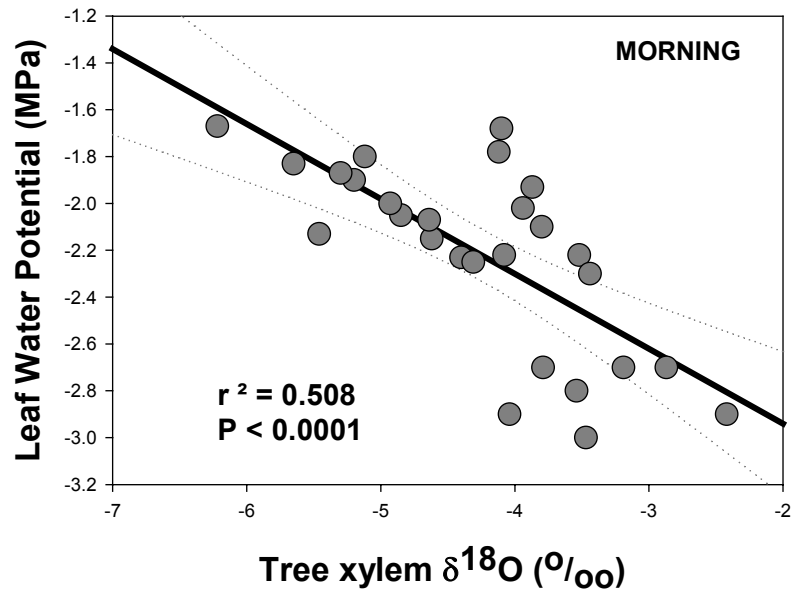
Figure 42. Relationship between Leaf water potential measured at 10:00 AM and twig xylem isotopic signature in September 2003 (n=27). Trees that have the more negative twig xylem signature possess the best water status.

3.3. Greenhouse results

3.3.1. Plant growth

A summary of parameters measured after a growth period of 15 months is shown in Table 7.

Table 7. A summary of growth parameters (root dry weight (Rdwt), Leaf area (LA) and Total dry weight (Tdwt)) measured for controls and repeatedly stressed *A. tortilis* and *A. xanthophloea* seedlings. Data are from the final harvest after 15 months of growth in the greenhouse. Each value is a mean of 3 plants; ± is the standard error of the mean. Significant differences p =0.05 between the means are shown by (*) and p=0.001 by (**),±SD.

	A. tortilis		*A. xanthophloea*	
	Stress	Controls	Stress	Controls
Rdwt/LA	0.013**	0.008	0.011*	0.001
Tdwt (g)	147.0(±0.8) **	243.0(±0.7)	222 0(±2.6)*	403.0(±6.1)
LA(m^2)	0.29(±0.01) **	0.34(±0.04)	0.42(±0.04) *	0.57(±0.08)
r/s ratio	0.67(±0.04) **	0.52(±0.01)*	0.190 (±0.002)	0.220(±0.001)

Control plants showed more rapid growth rates and accumulated more biomass than the stressed ones (Figure 40). Under adequate water supply, *A. xanthophloea* and *A. tortilis* accumulated mean Tdwt of 403.0 (± 6.1) and 243.0 (±0.7) g/plant respectively, after 15 months of growth (Table 7). Repeated 6-day water stress however impacted negatively on plant growth. Tdwt declined by 45% in stressed *A. xanthophloea*, while a similar stress magnitude contributed to a 40% decline in Tdwt of *A. tortilis*. Significant differences (p<0.05) between treatments and also between species occurred 6 months after imposition of water stress. In both treatments, *A. xanthophloea* however accumulated more Tdwt.
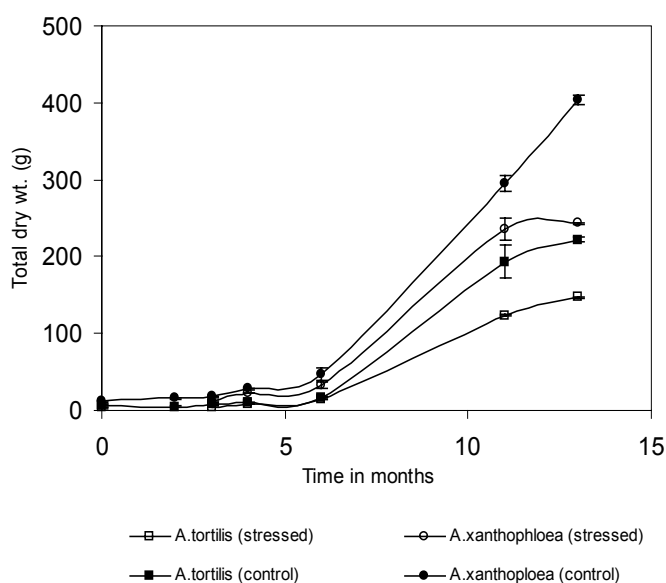
Figure 43. Long-term changes in Total dry weight (Tdwt.) of seedlings of *A. tortilis* and *A. xanthophloea* grown under two different watering regimes for a period of 15 months. Deviation from the mean is indicated by error bars and each point represent mean of 3 plants.

Patterns of total leaf area (LA) development over time resembled that of Tdwt (Figure 41), with final LA (m^2) of 0.57 and 0.34 in control and 0.42 and 0.29 in stressed *A. xanthophloea* and *A. tortilis*, respectively. LA development was more rapid in the control treatments than in stressed plants. Under favorable water supply *A. xanthophloea* showed rapid leaf growth and accumulated higher (LA) compared to *A. tortilis*. After 15 months of growth, controls of *A. xanthophloea* had 40% more LA compared to *A. tortilis*. However, repeated water stress resulted in a 26% reduction in LA in *A. xanthophloea* while similar stress level caused 15% LA reduction in *A. tortilis*. Thus repeated water stress caused 11% more LA reduction in *A. xanthophloea* than in *A. tortilis*. LA development was more sensitive to water stress, with significant differences between treatments and species evident during the second harvest (Figure 41). Repeated water stress caused significant leaf senescence and shedding. There was also reduced leaf initiation and expansion as the pots dried, contributing to the overall reduction in total LA in the repeatedly stressed plants.
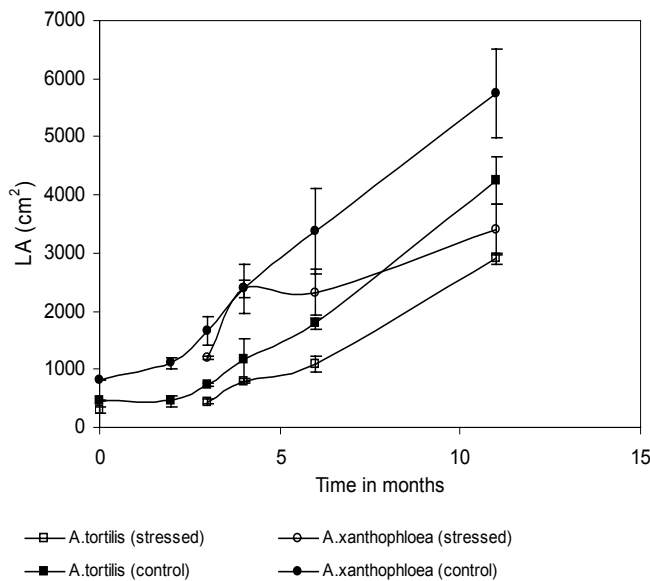
Figure 44. Leaf area development (LA) in *A. tortilis* and *A. xanthophloea* plants subjected to two different watering regimes for a period of 15 months. Error bars indicate deviations from the mean and each point represent mean of 3 plants

The relationship between LA and Tdwt was analyzed by log plots of mean values of accumulated LA versus Tdwt. A linear relationship irrespective of species existed between log LA and log Tdwt for both stressed and control plants (Figure 42), suggesting that Tdwt depended on LA development. For *A. tortilis*, there was a significant change in the slope of the relationship when seedlings were repeatedly stressed.
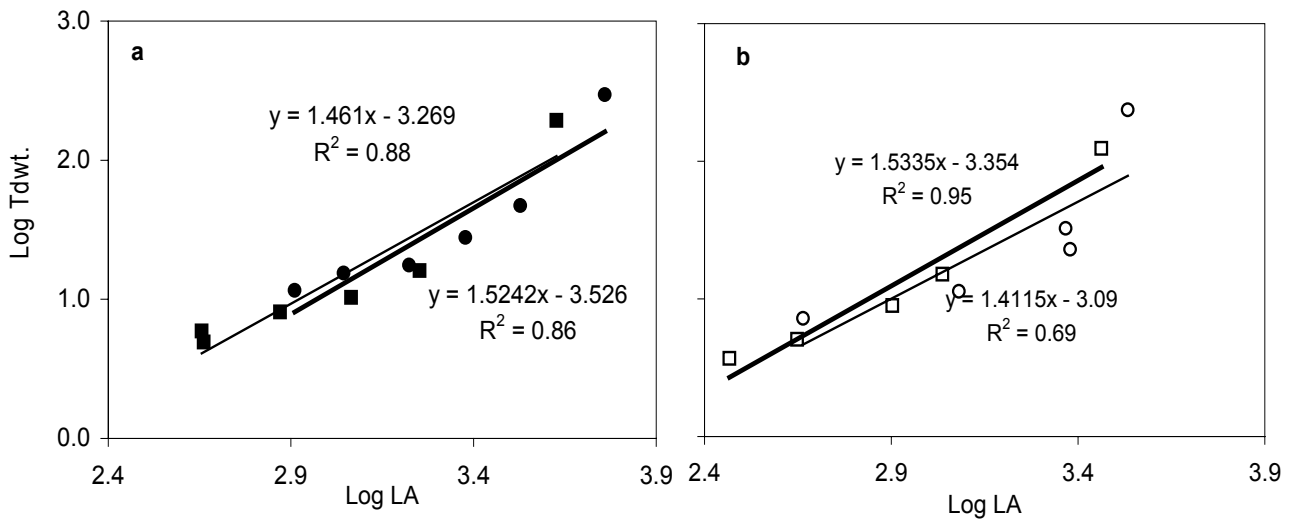
Figure 45. Transformed plot of total dry weight (Tdwt) versus leaf area (LA) of (a) Controls and (b) stressed *A. tortilis* (thin line) and *A. xanthophloea* (thick line). Controls were watered every other day while stressed seedlings were watered every 7th day.

3.3.2. Carbon partitioning

Repeated 6-day water stress resulted in a significant increase in r:s ratio of *A. tortilis*, having a mean ratio of 0.67 ($\pm$.04) compared to 0.52 ($\pm$0.01) for the controls after 15 months of growth. Irrespective of treatment, *A. tortilis* adjusted its r/s ratio upwards over time with a 2 and 3 fold increase in controls and stressed plants, respectively (Figure 43a), suggesting an intrinsic capacity to balance root biomass with transpiring leaf area with respect to available soil water and atmospheric vapor demand. The ratio (r/s) is probably higher in stressed *A. tortilis* since some fine roots were lost during washing. *A. tortilis* developed a significant amount of fine roots as water stress increased some of which could not be recovered.

No significant difference (p=0.51) was, however, observed between controls and stressed *A. xanthophloea* (Figure 43a). *A. xanthophloea* showed a declining r/s ratio and after 15 months of growth, r:s ratio had decreased by 14 % and 3% in both controls and stressed plants respectively. Root dry weight to leaf area ratio (rdwt/LA) increased in both species over time (Figure 43b). Stressed *A. tortilis* had higher rdwt/LA (38%) compared to controls, while an insignificant difference (9%) existed between controls and stressed *A. xanthophloea*. The increase in rdwt/LA as observed in *A. xanthophloea* could mainly be associated with leaf shedding. The two species also differed

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significantly in their rooting patterns. *A. tortilis* tended to develop a stronger tap root system and rooting depth increased significantly in the repeatedly stressed plants. Under adequate water supply, most of the roots of *A. xanthophloea* were only found in the upper soil layers, forming a dense fibrous rooting system. Rooting depth, however, increased in repeatedly stressed plants although the development of a deep tap root system was not as pronounced as in *A. tortilis.* (Plate 3).
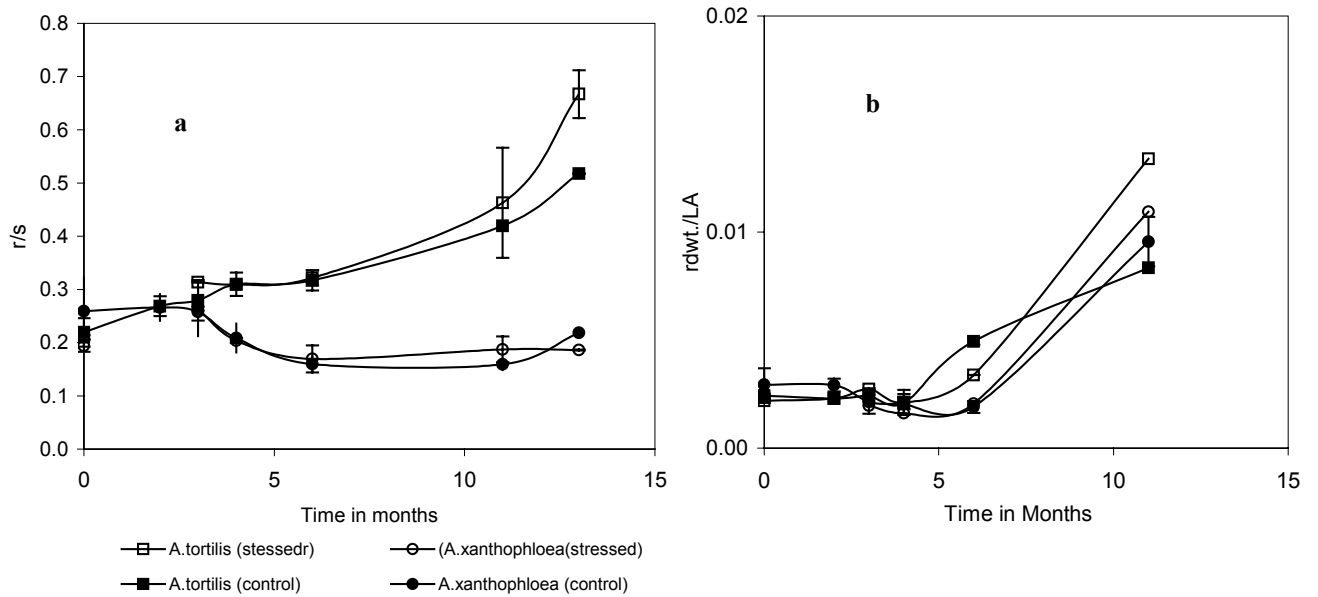
Figure 46. Progressive long-term development of (a) root shoot ratio (r:s) and (b) Root dry weight to leaf area ratio (rdwt/LA) of controls (closed) and repeatedly stressed (open) of *A. tortilis* (squares) and *A. xanthophloea* (circles) plants grown under glass conditions over a period of 15 months. Measurements were conducted every month by destructive harvesting. Each data point represents means of 3 plants in every treatment. Error bars show differences between treatments and species.

A. xanthophloea (Control)　　　　　　A. xanthophloea (Stress)

A. tortilis (Control)　　　　　　A. tortilis (Stress)

Plate 3. Root system structure in controls and repeatedly stressed plants of *A. xanthophloea* and *A. tortilis* after 12 months of growth. Stress was achieved by withholding water for 6 days as described in the text.

3.3.3. Sap flux and leaf water potential

Sap fluxes were higher in controls compared to stressed plants (Figure 44a). Percentage decline in sap flux of about 33 and 25% as a result of 6-day cyclic stress were recorded for *A. xanthophloea* and *A. tortilis* respectively. Controls of *A. xanthophloea* also showed higher (mean diurnal max = 100 g h^{-1}) flux rates compared to *A. tortilis* (mean diurnal max = 80 g h^{-1}). Expressing sap flux per unit LA (Figure 44b) revealed no significant differences between controls of *A. tortilis* and *A. xanthophloea* suggesting that the initial difference in sapling sap flux was due to differences in total LA. However, differences between controls and stressed plants still persisted. Six-day water stress had significant

effect on whole-plant sap flux, with flux rates being reduced to half at the end of water stress cycle (Figure 45). Although whole plant sap flux rates were higher in *A. xanthophloea*, stressed *A. tortilis* showed higher flux rates per unit LA compared to *A. xanthophloea*. Recovery of transpiration after re-watering occurred within 1-2 days. *A. xanthophloea* showed more rapid resumption in sap flux after re-watering compared to *A. tortilis*. Since no substantial amount of stem water storage was anticipated, due to age and sizes of the plants (Sakuratani 1981), sap flux was equivalent to transpiration water loss.
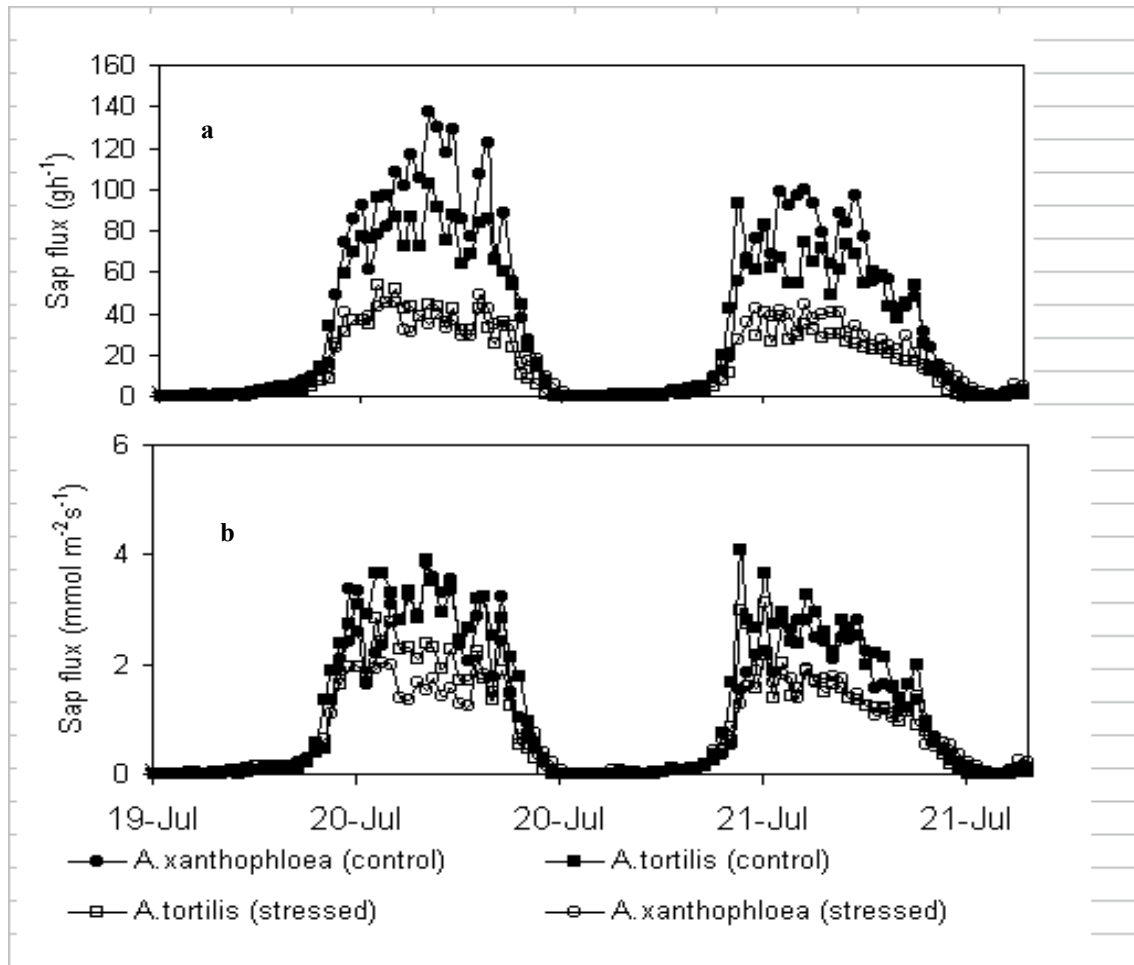
Figure 47. (a) Mean whole plant sap flux rates and (b) Sap flux rates expressed per unit leaf area, for one year old stressed and control plants of *A. tortilis* and *A. xanthophloea*. Data represent means of two plants from each treatment per species.
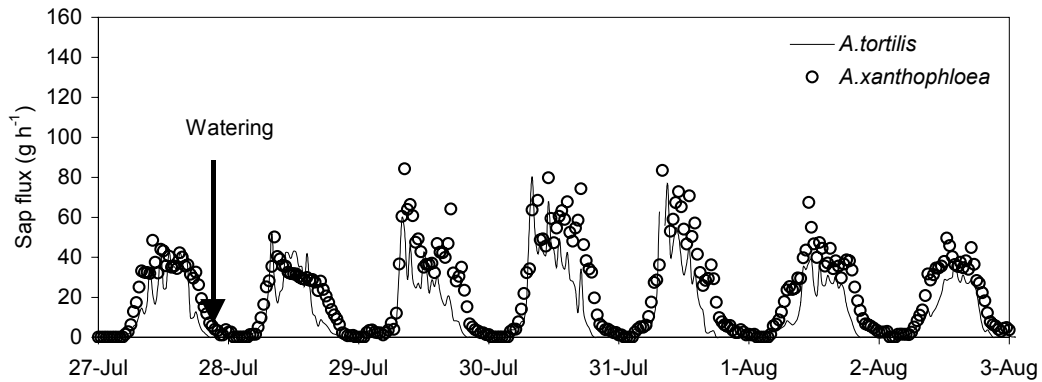
Figure 48. Effects of six-day water stress on sap flux (transpiration) rates of repeatedly stressed *A.tortilis* and *A.xanthophloea* plants grown in a glasshouse.

During favorable soil water conditions, increasing transpiration during the early part of the day led to decline in Ψ_L (Figure 46). Controls of *A. xanthophloea,* which exhibited higher transpiration rates, experienced steeper drop in Ψ_L reaching a mean diurnal minimum value of -2.2 MPa. The decline in Ψ_L was, however, less in repeatedly stressed *A. xanthophloea* compared to control plants as well as rapid recovery in Ψ_L at the end of the day, when transpiration rates declined. Withholding water significantly reduced soil water availability and also Ψ_L of plants. *A. xanthophloea* however suffered more stress compared to *A. tortilis,* experiencing much lower Ψ_L throughout the day (Figure 47). A strong regulation in decline of water potential was observed for both species after midday when soil water was limiting.
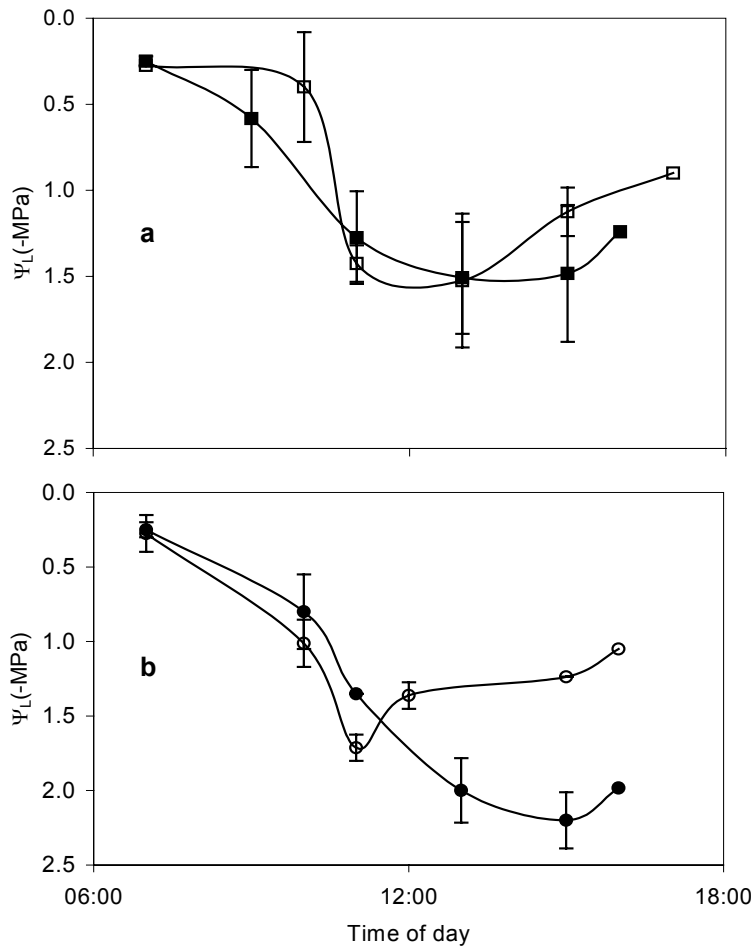
Figure 49. Diurnal changes in leaf water potential Ψ_L of controls (closed symbols) and repeatedly stressed (open symbols) (a) *A.tortilis* and (b) *A.xanthophloea* seedlings. Plants were watered to container capacity the previous night before conducting measurements the following day. Measurements were conducted on bright sunny days. Error bars show deviation from the mean n=3.
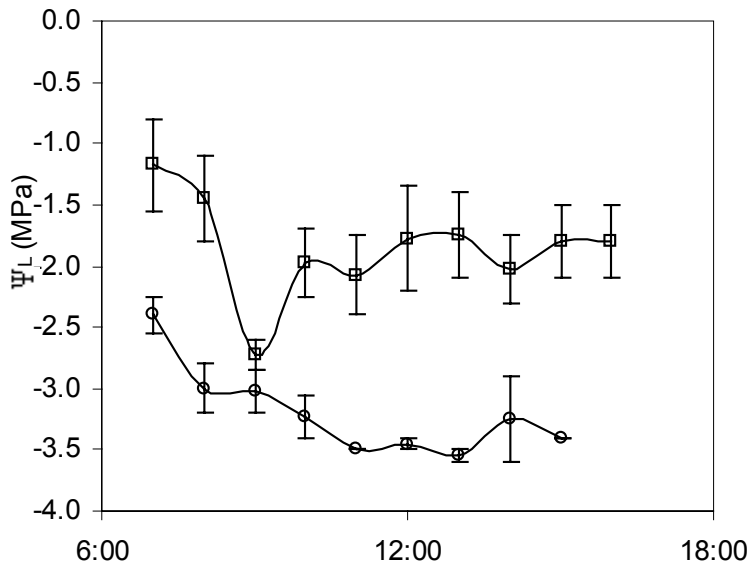
Figure 50. Diurnal course of leaf water potential (Ψ_L) of repeatedly stressed *A.tortilis* (squares) and *A.xanthophloea* (circles) plants on the 6th day of cyclic water stress. Data points are means of 3 measurements taken from different plants and deviations from the means are shown by error bars.

3.3.4. Hydraulic conductance

Mean Ks-l values for controls were 4.73 and 3.48 mmol MPa^{-1} m^{-2} s^{-1} for *A. tortilis* and *A. xanthophloea*, respectively. Mean values estimated for repeatedly stressed plants were 4.45 and 3.13mmol MPa^{-1} m^{-2} s^{-1} for *A. tortilis* and *A. xanthophloea*, respectively. In both treatments, *A. tortilis* exhibited higher hydraulic conductance compared to *A. xanthophloea*. There was no major difference in Ks-l values of stressed and control plants.

3.3.5. Tissue water relations

Parameters derived from P-V curves are shown in Table 8. Osmotic potential at full turgor (Π^{100}) was significantly lower in stressed seedlings of *A. tortilis* compared to both its controls and *A. xanthophloea*. Thus *A. tortilis* had a mean osmotic adjustment of 0.48 MPa. Slight osmotic adjustment also occurred in seedlings of *A. xanthophloea* subjected to cyclic water stress but this was of a lower magnitude (0.16 MPa) compared to *A. tortilis*. Water potential at turgor loss point (Ψtlp) was higher in the controls compared to stressed seedlings of both species. Stressed seedlings of *A. tortilis,* however, showed a significantly lower Ψtlp compared to both its controls and *A. xanthophloea*. A strong correlation ($r^2 = 0.96$) existed between Π^{100} and ψtlp, irrespective of species and treatments (Figure 48) underscoring the role of osmotic adjustment in water stress tolerance in trees. Control seedlings also had a higher R*tlp compared to stressed seedlings. A significant difference occurred between ε of stressed and control seedlings of *A. xanthophloea*, suggesting some considerable elastic adjustment as a result of repeated water stress. However no change in ε was observed between controls and stressed *A. tortilis*.

Table 8. Water potential at turgor loss point (Ψtlp, MPa), osmotic potential at full turgor (Π^{100}, MPa), bulk modulus of elasticity (ε, MPa) and relative water content at turgor loss point (R*, %) for controls and repeatedly stressed *A. tortilis* and *A. xanthophloea* plants. Values are means (± standard errors) for *n*. Significant difference between means is shown by (*) for p =0.05 and by (**) for p=0.001

	A. tortilis			*A. xanthophloea*		
	Stress	Control	*n*	Stress	Control	*n*
Ψtlp (-MPa)	1.4 (±0.1) **	1.1(±.11)	10	1.24 (±.07)	1.10 (±0.16)	9
ε (MPa)	11.2 (±2.3)	10.4(±3.1)	10	9.91 (±1.32)**	18.70 (±2.05)	9
Π^{100} (-MPa)	1.3 (±0.2) **	0.9 (±0.1)	10	0.97 (±0.12)	0.81 (±0.18)	9
R*tlp (%)	85.0(±0.5) *	87.0(±1.0)	10	85.0 (±0.8) *	88.00 (±0.80)	9
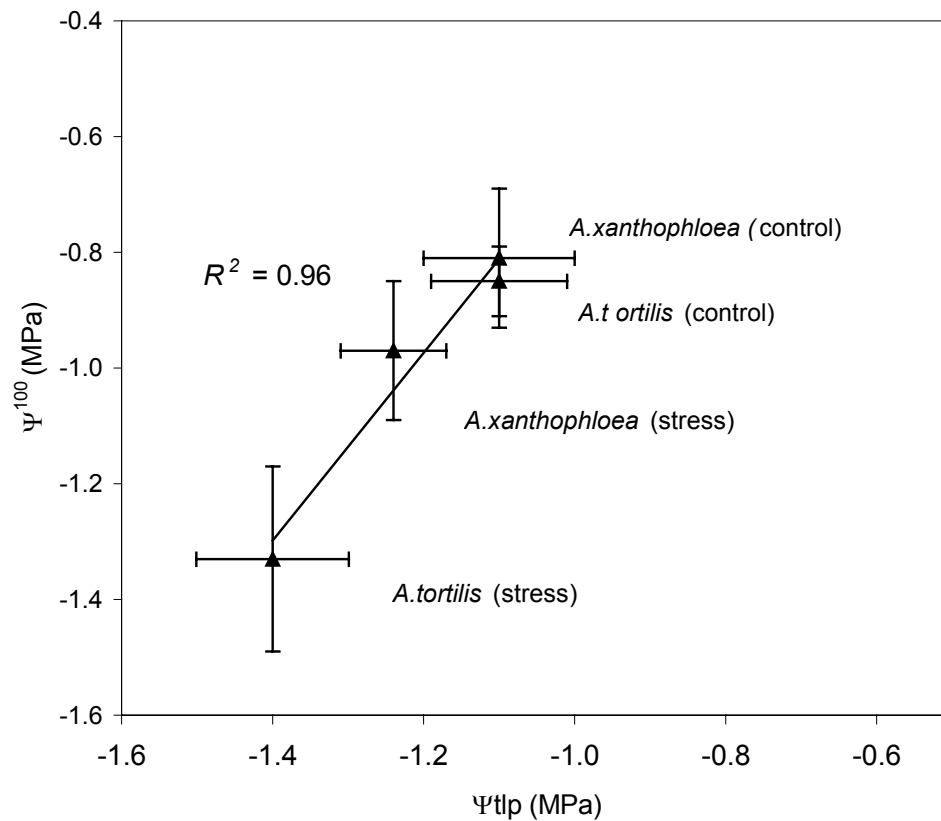
Figure 51. Relationship between osmotic potential at turgor loss point (Π^{100}, MPa) and water potential at turgor loss point (Ψtlp, MPa) of controls and repeatedly stressed *A. tortilis* and *A. xanthophloea*. Values were derived from *p-v* curves and they represent mean of n=8 to 10 measurements.

3.3.6. Severe water stress

Withholding water resulted in decline of Ψ_L in both controls and previously stressed plants of both species but the rate of decline differed as shown in Figure 49. Previously stressed plants maintained favorable Ψ_L and were able to withstand water stress for a much longer period before showing any signs of water stress compared to the controls. For example, leaves of control plants of *A. xanthophloea* were wilted overnight only 3 days after imposing severe water stress while leaves on previously stressed plants were wilted overnight after 8 days of withholding water. Similar observations were made for *A. tortilis*, with stressed plants surviving 11 days without water before wilting overnight compared to the 7 days of control plants. Controls of *A. xanthophloea* lost turgor by midday after the second day of severe water stress (Figure 50). The plants had a mean Ψ_L of -1.5 MPa, which was well below Ψtlp. After reaching Ψtlp, further decline in Ψ_L as stress progressed was accompanied by leaf senescence and drying of branches and this was more severe in the control plants. Previous water stress cycles extended the time before reaching Ψtlp by 5 days, and also minimized the effect of water stress in *A. xanthophloea*
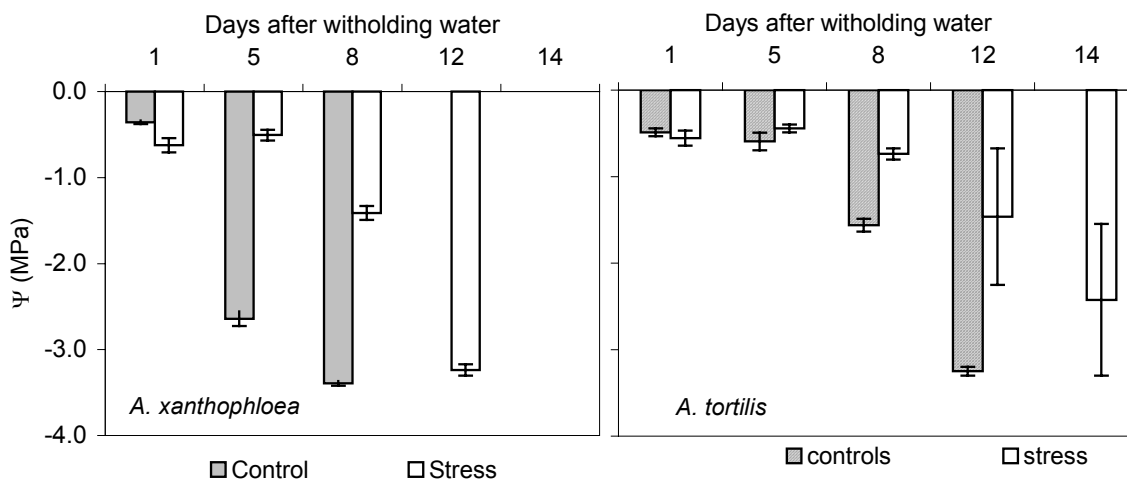
Figure 52. Progressive changes in Ψ_L of previously stressed and controls of *A. tortilis* and *A. xanthophloea* when plants were subjected to severe water stress. Plants were first watered to container capacity before withholding water until they were wilted overnight. Measurements of Ψ_L were conducted in the morning before any significant transpiration was recorded. Data are means of 3 plants per species per treatment. Deviations from the means are shown by error bars.

A. tortilis survived much longer when subjected to severe water stress (7 and 11 days for controls and previously stressed plants respectively) before reaching ψtlp (-1.1± 0.1 and -1.4 ± 0.1 MPa respectively). Results showed that previous stress cycles greatly improved survival under conditions of drought in *A. tortilis* compared to *A. xanthophloea* as shown during severe stress; however in general, water stress pre-conditioning improved plant survival under conditions of limited water supply in both species. For *A. tortilis,* previously stressed plants also retained their leaves and leaf drop occurred after re-watering, when new ones started to appear, but leaf sprouting and plant recovery was slow.
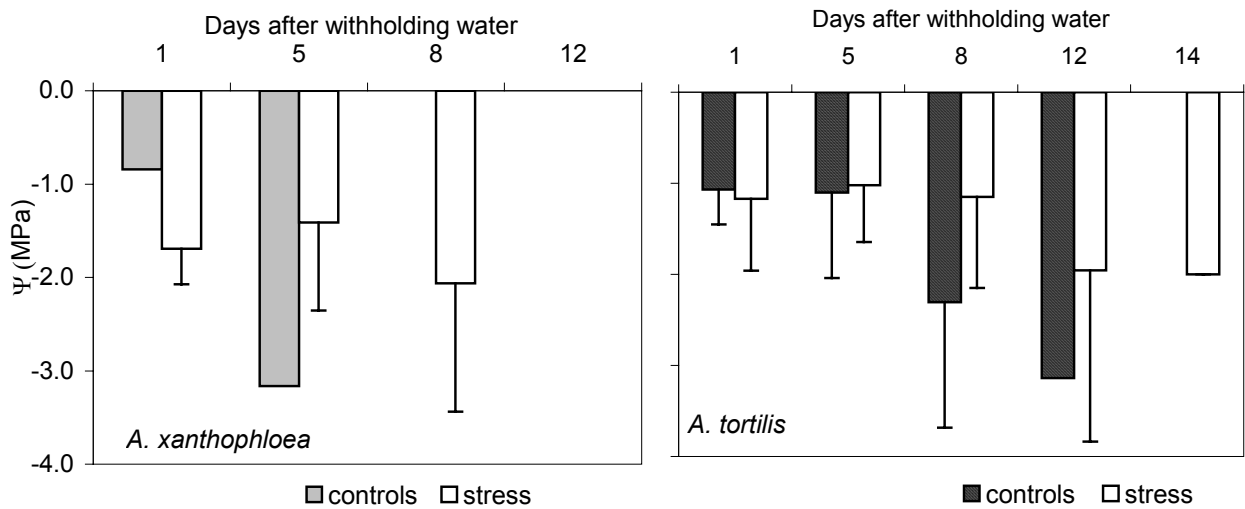
Figure 53. Progressive changes in midday Ψ_L of repeatedly stressed and non-stressed *A. xanthophloea* and *A. tortilis* when plants were subjected to severe water stress. Plants were first watered to container capacity before withholding water until they were wilted overnight. Measurements of Ψ_L were conducted between noon and 2:00 p.m. Data are means of 3 plants per species per treatment. Deviations from the means are shown by error bars.

3.3.7. Leaf transpiration, stomatal conductance and water potential

Results of leaf transpiration (E) and stomatal conductance (gs) for both controls and stressed *A. xanthophloea* (upper panel) and *A. tortilis* (lower panel) trees measured with porometer on day 3 of severe water stress are shown in Figure 51. Maximum E (Figure 51a) and maximum gs (Figure 51b) declined two-fold in control plants of both species. *A. xanthophloea* was, however, more affected, showing a midday depression in gs and E around noon. E and gs peaked in the early part of the day (10.00 am) reaching maximum values of 3 and 1mmol m^{-2} s^{-1} for stressed and controls respectively, but rapidly declined to near zero values before noon and only resumed later in the day. Maximum gs attained by stressed and control plants of *A. xanthophloea* were 250 and 50 mmol m^{-2} s^{-1} respectively.

Compared to *A. xanthophloea*, controls of *A. tortilis* showed higher gs (100 mmol m^{-2} s^{-1}), with no midday depression in E and gs on day 3 of severe stress. Repeatedly stressed plants of *A. tortilis* showed no decline in diurnal maximum rates, maintaining higher E and gs (3 mmol m^{-2} s^{-1} and 220 mmol m^{-2} s^{-1} respectively).
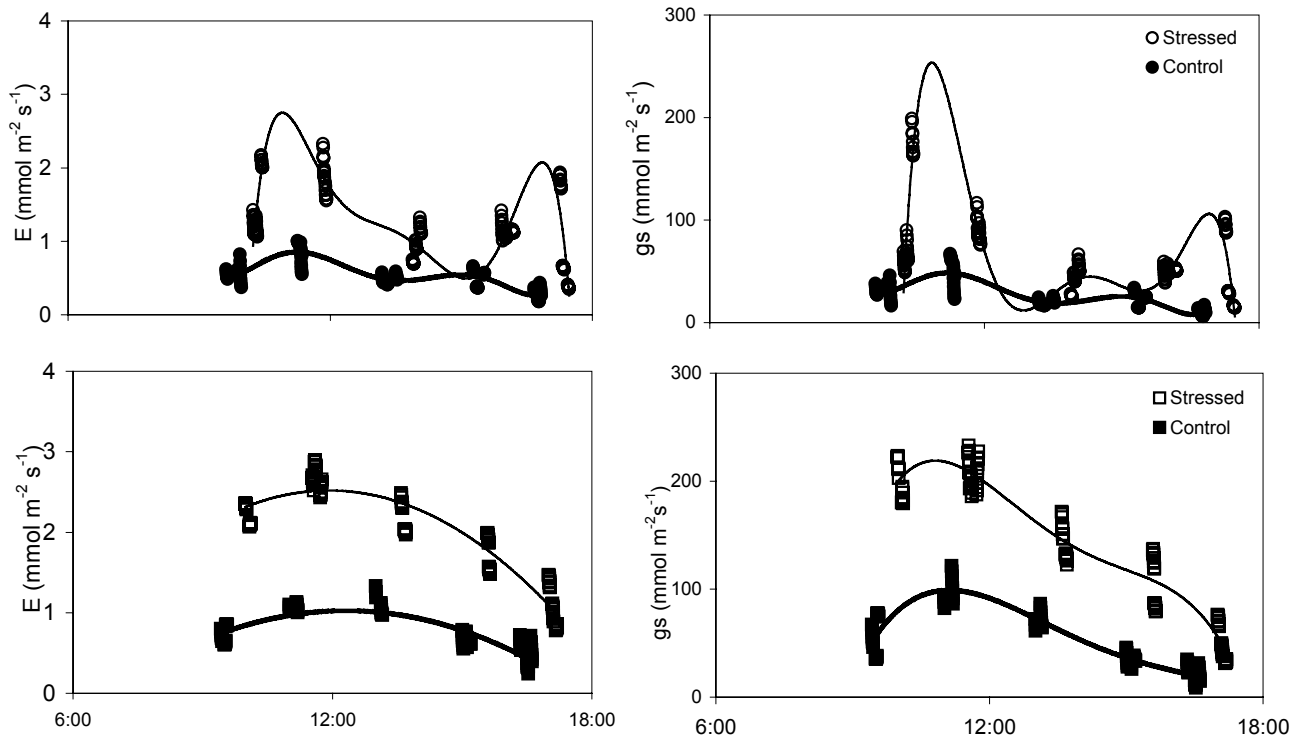
Figure 54. Diurnal courses of leaf transpiration and stomatal conductance of *A. xanthophloea* (upper panel) and *A. tortilis* (lower panel) plants conducted on the third day after withholding water. Seedlings were initially grown either, with full water supply (closed symbols) or 6-day water stress cycles (open symbols) for a period of one year. Before commencing severe water stress, plants were watered to container capacity before completely withholding water.

3.3.8. Post-stress recovery

Plant recovery after severe water stress was associated with leaf initiation, leaf growth and LA development. Since plant transpiration appeared to be directly related to LA, sap flux measurements were used to monitor whole plant LA development. Controls of *A. xanthophloea* trees, however, lost most of their shoots during severe stress except for short stumps a few centimeters above the pot surface. It was, therefore, not possible to measure sap flow with the SHB gauges during recovery. However, new shoots and leaves rapidly developed from these stumps after re-watering. Previously stressed *A. tortilis* retained leaves during severe stress and also after re-watering. Although most of the

leaves looked wilted, they were still transpiring, hence carrying out gaseous exchange as shown in Figure 52. When plants were re-watered after severe stress it was evident that leaf initiation was much slower in previously stressed treatments than the controls (Figure 53a). Leaf initiation was also more rapid in *A. xanthophloea* than in *A. tortilis*. Also affected by previous watering regimes was leaf size with individual leaf area being reduced by about 60 and 80% in previously stressed *A. xanthophloea* and *A. tortilis* respectively (Figure 53b). Recovery of transpiration in previously stressed *A. tortilis* was slower compared to its control and also to *A. xanthophloea*. *A. xanthophloea* showed the fastest recovery irrespective of treatment.
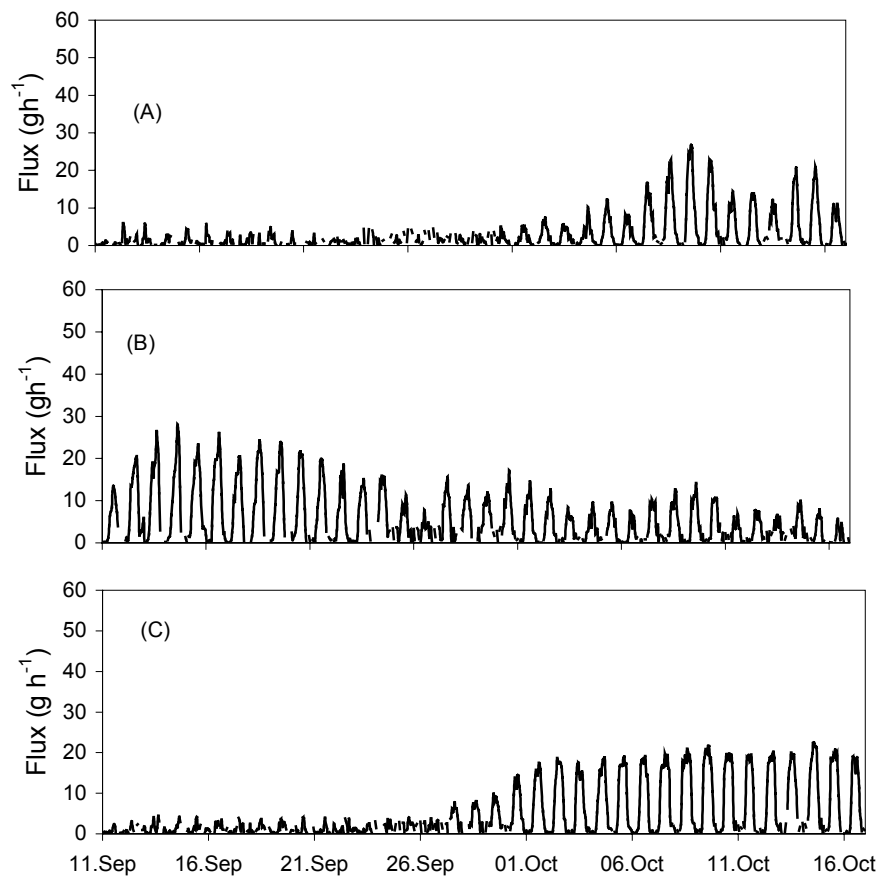
Figure 55 Recovery of transpiration in (A) controls, (B) stressed *A. tortilis* and (C) stressed *A. xanthophloea* after the plants were subjected to severe water stress beyond wilting point and then re-watered to container capacity. Values are means of two plants per treatment. Values for controls of *A. xanthophloea* are missing after the plants lost aerial shoots during severe water stress, hence whole tree transpiration could not be determined. Measurements were done using the stem heat balance method.
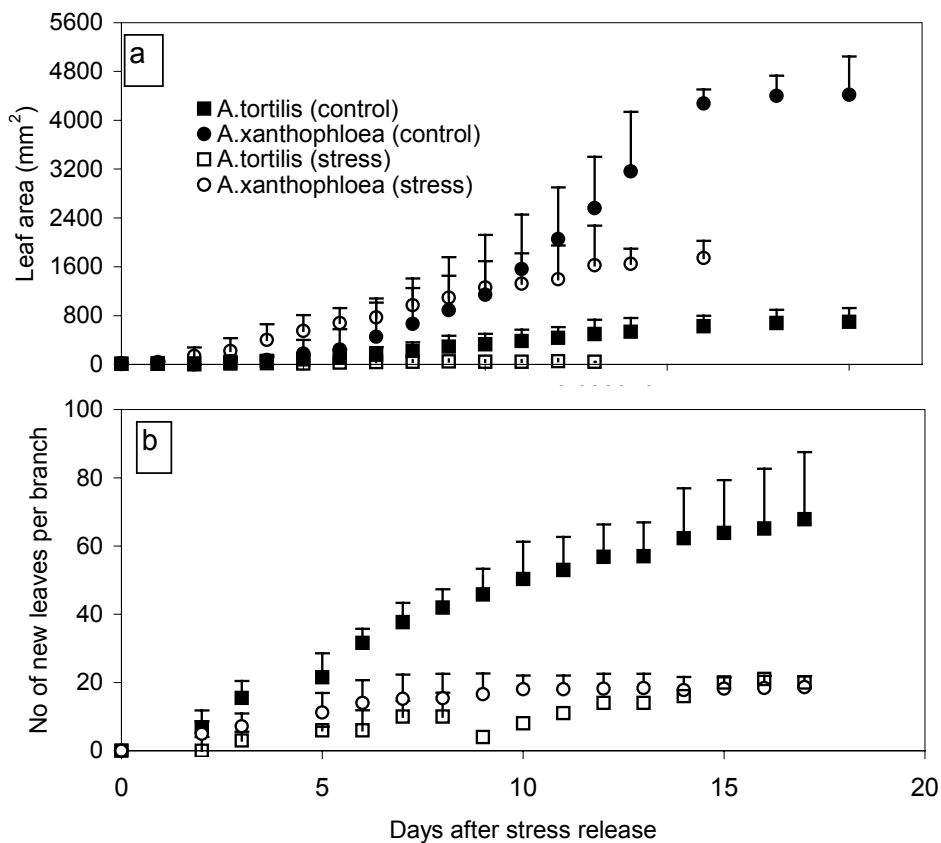
Figure 56. Recovery and development of (a) Leaf area (LA) and (b) leaf initiation of controls (closed) and stressed (open) *A. tortilis* (squares) and *A. xanthophloea* (circles) after they were subjected to severe water stress beyond wilting point and then re-watered to container capacity. Measurements were taken from 4 branches of two trees per treatment. Data for controls of *A. xanthophloea* are missing because plants lost most of the aerial shoots during severe water stress.

3.3.9. Water use efficiency

Table 9 shows results of δ^{13}C analysis conducted on leaves of controls and stressed *A. tortilis* and *A. xanthophloea* plants. Leaves were harvested for analysis the second day after water was withheld. Results indicate that control plants were more conservative than stressed plants, as indicated by less negative δ^{13}C values. There were significant differences between controls and stressed plants of *A. tortilis* (p=0.027) while no significant differences were observed between stressed and non-stressed *A. xanthophloea* (p=0.72). Also *A. xanthophloea* was more conservative than *A. tortilis* in both stressed and non-stressed plants and the difference was significant (p= 0.01), thus according to δ^{13}C results, *A. xanthophloea* exhibited higher water use efficiency compared to *A. tortilis*.

Table 9. Values of $\delta^{13}C$ for controls and stressed *A. xanthophloea* and *A. tortilis* plants. Leaves for analysis were obtained on the second day after imposing water stress.

	$\delta^{13}C$ values	
	Control treatments	Stress treatments
A. tortilis	-28.70 (±0.04)	-29.59 (±0.07)
A. xanthophloea	-26.99 (±0.07)	-27.08 (±0.02)

Chapter Four

Discussion

4.1. Soil water availability, plant water status and water use in the dry savanna ecosystem

The study conducted in the arid savanna of Kenya (*Experiment 1*) attempted to relate changes in morphological and physiological characteristics before and during the development of soil drought to the apparent access that trees have to soil water resources. So far, information is scanty on coordinated morphological and physiological responses of the indigenous east African tree species during the development of drought. For the two *Acacia* species studied, leaf initiation and shoot elongation were associated with water availability and started as soon as soil moisture in the upper rooting zone was favorable. Despite reported slow movement of water down through the soil layers (Radcliffe and Rasmussen 2002), the rapidity and simultaneity with which leaf initiation and growth responded to precipitation was an indication that trees were able to access water very soon after the rainfall, a sign that both species had roots located within the shallow soil layers. Figure 10a showed that SWC at 0-40 cm depth declined rapidly soon after the rains stopped. This decline was attributed to drainage and evapotranspiration, since this is in general the region of greatest root biomass (Ong et al. 1999, Cavander and Bazzaz 2000). If most of the root mass was located within the upper soil layer, it was expected that shoot elongation and leaf growth should stop soon after the rains, since trees would rely primarily on precipitation as their water source. This was the case with *A. xanthophloea* and young *A. tortilis* (Figure 11). Growth in mature *A. tortilis* was, however less affected by soil drying at this depth, suggesting that water supply needed for growth was less interrupted. This was an indication that mature *A. tortilis* had a large root biomass located within the deeper soil horizons with stable water source, while *A. xanthophloea* and the young *A. tortilis* probably had most of their roots residing within the upper soil layers, which dried out quickly and soon after the rainfall.

Seasonal changes in predawn (Ψ_{pd}) and midday leaf water potential (Figures 12a and 13a respectively) were more pronounced in *A. xanthophloea* compared to mature *A. tortilis* while young *A. tortilis* exhibited intermediate responses. Similarly, the relationship between Ψpd and SWC measured at 40 cm soil depth (Figure 12b) demonstrated a stronger coupling between Ψpd and SWC for *A. xanthophloea* as opposed to mature *A. tortilis,* while that of the young *A. tortilis* trees was intermediate. These differences in seasonal patterns of plant water status were probably due to water uptake patterns, given

that predawn water potential is an indicator of Ψs at the rooting zone (Ryel et al. 2003) and that plant tissue water content is in equilibrium with the soil moisture at the ryizosphere during dawn. The results indicate that *A. xanthophloea* was obtaining most of its water from the upper soil layers since its Ψpd was most affected by changes in SWC at 40 cm depth. Thus,Ψpd of *A. xanthophloea* rapidly declined to –2.0 MPa during drought, as a result of rapid water depletion in the upper soil layers. Ψpd of mature *A. tortilis*, however, seamed to be influenced by changes in SWC of the upper soil layers during the rainy season and also by the deeper soil layers during drought as revealed by its relatively high Ψpd (-1.1 MPa) during the time when SWC at 0-40 cm soil layer was relatively low, and the rapid changes in Ψpd during an event of rainfall. Certainly, the high tissue water status experienced by mature *A. tortilis* during drought will not permit substantial water uptake from the shallow soil layers where Ψs is much lower. Young *A. tortilis* may have obtained water from intermediate soil layers or its behavior may reveal a superior quality such as osmotic adjustment or more effective soil water extraction and transport which enabled it to maintain favorable tissue water status compared to *A. xanthophloea,* despite obtaining water at the same soil layers. The results from plant tissue water status strongly supported the proposed water uptake pattern and the sensitivity of growth to soil water availability.

The amount of water lost through transpiration is determined by evaporative demand, soil water availability and plant factors, which include root and xylem characteristics and foliage quality and quantity (Turner 1986). In this locality, the main stress was due to lack of water, resulting from the variability in rainfall. Periods of water stress were associated with high temperatures and evaporative demand (Figure 10), both of which favor increased water loss from trees (Schulze et al. 1987). On a long-term basis, transpiration declined in both young *A. tortilis* and *A. xanthophloea* with increasing soil and atmospheric drought, but not in mature *A. tortilis*, which maintained relatively constant transpiration rates (Figures 14 and 15) despite declining soil water content within the upper soil layers. Under such circumstances, a constant or declining transpiration would mean limited water supply from the roots or restricted water loss at the leaf surface. In response to increasing soil water deficit and atmospheric demand, there was a significant reduction of the transpiring surface by increasing leaf shedding and reducing leaf initiation and growth. This could partially account for the decline in whole tree transpiration. Since shoot elongation and leaf initiation had long stopped, it was also suggested that photosynthetic products originating from the remaining leaves were re-directed to more important areas

such as root growth to improve water uptake which is an important adaptive mechanism to water stress (Kramer 1980; Jones 1992).

Stomatal conductance was also strongly influenced by declining soil water content. The observed relationship between maximum stomatal conductance, Ψ_{pd} and SWC (Figure 17) demonstrated the dependence of stomatal conductance on soil water availability. Differences in response of stomatal conductance to declining SWC between and within species (Figures 16 and 17) could, therefore, serve to demonstrate the differential abilities to access water at different soil layers, root absorption and water transport. Thus, the unrestricted stomatal conductance observed in mature *A. tortilis* suggests unrestricted access to a stable deep soil water source compared to *A. xanthophloea* and the young *A. tortilis* trees (Rambal 1984, Sperry 2000) and explains its continued growth during the time when SWC at 0-40 cm soil layer was low and growth had long stopped in the latter. Changes in stomatal conductance in *A. xanthophloea*, however, followed the pattern of decline in SWC at 0-40 cm soil layer. Assuming that mature *A. tortilis* would behave in a similar manner as young trees of the same species when exposed to greater water stress (As the pattern in Figure 17d predicts), it is likely that both species will respond to seasonal decline in soil water by reducing stomatal conductance. Reduced stomatal conductance therefore partly accounted for the decline in transpiration rates as observed during drought in young *A. tortilis* and mature *A. xanthophloea*.

On a short-term basis, stomatal closure occurred in both species before noon when transpiration rate was increasing due to increasing vapor pressure deficit, thus limiting further decline in Ψ_L (Figure 18). According to Turner (1986), midday stomatal closure may increase water use efficiency by reducing water loss at critical times of the day when VPD are large but allowing photosynthesis to continue early in the morning and late in the afternoon when VPD is low. Stomatal closure has been described as a mechanism employed by trees to cope with both diurnal and seasonal water deficits (Sala and Tenhunen 1994, Jones and Sutherland 1991). Through stomatal closure, trees are able to reduce water loss during the time when water supply from the soil to the leaves cannot cope with transpiration water loss and serve to avoid development of dangerous negative tissue water potentials and hydraulic failure in the soil-leaf continuum (Tyree and Sperry 1989, Kolb and Sperry 1999, Sperry 2000). Trees with deep rooting system, accessing more stable deep soil water deposits however, do not need to cut down

transpiration as rapidly and as drastically as those with limited rooting depths (Larcher 2003). Differences in stomatal behavior between species or individuals as shown in the case of the *Acacia* trees studied are therefore strong indications of their differences in rooting patterns, water uptake and perhaps water transport efficiencies.

The structure of the water-conducting system (vessel size and hydraulic architecture) determines resistance along the water flux pathway and influences water use (Tyree and Sperry 1989, Ni and Pallardy 1990). Changes in tree hydraulic conductance during progressive soil drying as shown in Figure 19 can reveal differences among species (Abril and Hanano 1998) and can be attributed to variation in root to leaf surface area ratio or inherent absorption capacity and root permeability (Reich and Hinkley 1989). These changes suggest a mechanism of adaptation to xeric environments that is important for drought tolerance (Abril and Hanano 1998). Although hydraulic conductance was determined from the time when soil water potential ($\Psi_{pd} = \Psi$s) was -0.7 MPa, it was assumed that no major differences may occur in tree responses at less negative values. A significant drop in K_{plant} in *A. xanthophloea* between Ψs = -0.7 and -1.0 MPa paralleled decreases in maximum stomatal conductance (Figure 17). Similarly, this paralleled significant drop in transpiration in *A. xanthophloea*. It was therefore suggested that the decline in gs as well as E in *A. xanthophloea* was the result of a restricted flux capacity (Reich and Hinkley 1989, Sala and Tenhunen 1994, Sperry 2000). This could also provide an explanation for the anomaly where stomatal closure in *A. xanthophloea* before midday did not result in improved Ψ_L later in the day and also Ψ_L did not fully recover by dusk (Figure 18). A high hydraulic resistance between the soil and the shoot will induce relatively lower Ψ_L in the trees concerned (Turner 1986, Sperry et al. 2002). Resistance to water flow could occur at two points, namely root surfaces (Boyer 1985, Sala and Tenhunen 1994, Jackson 2000) or in the stem and leaves (Turner 1979, Tyree and Sperry 1988, 1989). In this study, there was a marked similarity in responses between young *A. tortilis* and *A. xanthophloea* trees, a fact that was attributed to their limited rooting depth. Young *A. tortilis* also experienced similar decline in K_{plant}, a fact that was attributed to increased resistance at the root surface as a result of rapidly declining SWC. Young *A. tortilis* were, however, able to fully recover their Ψ_L by dusk (not shown), suggesting that failure of recovery in Ψ_L in *A. xanthophloea* was an added effect of inefficient water transport system, which might have resulted in

cavitation (Zimmermann 1983, LoGullo et al. 2003). This may partly explain the observed differences in physiological responses between the two despite, having roots within similar soil layers

Long term monitoring of tree water use efficiency (WUE) through $\delta^{13}C$ analysis (Cowan and Farquhar 1977) showed higher WUE in *A. xanthophloea* than *A. tortilis*. High WUE is associated with increased drought tolerance hence with trees growing in dry areas (Smith and Nowak 1990). However, high WUE would only be of any ecological significance if the conserved soil moisture were available for uptake later in the season (De Lucia and Schlesinger 1990). For mature *A. xanthophloea* trees, which showed signs of shallow rooting system, conserved moisture may not be available due to high moisture depletion from the upper soil layers through evapotranspiration as soon as the rains stop hence no advantage derived from high WUE. Thus, despite its conservative nature *A. xanthophloea* still experienced lower leaf water potentials in a drying soil. The results indicate that the use of $\delta^{13}C$ analysis as a measure of WUE, hence a species ability to cope with water stress, maintaining some level of productivity must be approached with caution, especially when dealing with aridland species.

4.2. Soil water availability, root water uptake and plant water use in the Mediterranean ecosystem

Soil hydration at the study site in Herdade da Mitra, experiencing a mediterranean type of climate (David et al. 2004) was mainly through rainfall. Soil moisture declined significantly (Figure 22) as long it was not raining (Figure 20) and weather conditions favored evapotranspiration (Figure 21), since evapotranspiration accounts for the bulk of soil moisture loss in the Mediterranean regions (Rambal 1984, Bréda et al. 1995). Rapid decline in soil moisture occurred between May and August, coinciding with the vegetation period and increasing air temperatures and VPD. Temporally, depletion of moisture within the upper soil layer (0-0.3 m) commenced much earlier (in May) than in the deeper (1.0 m) horizons (June) (Figure 22). This rapid moisture decline in the upper soil layers between May and June was attributed to increased transpiration rates from trees, herbs and shrubs, increasing atmospheric vapor demand and lack of rainfall. Most of the herbs and grasses dried out by July, after, which there was slow decline in Ψs at the 0-0.3 m depth. Soil moisture content at 1 m depth however, remained favorable for a longer period of time since soils at this depth were not subjected to evaporation and also roots of herbs and shrubs, which accelerated water loss from the upper soil layers could not access moisture at this depth (Le-Roux and Bariac 1995, Bréda et al. 1995, Rodriguez et al. 2004). The

observed seasonal pattern of soil moisture content resembles those previously reported for the Mediterranean regions of southern Europe (Rambal 1984, Sala and Tenhunen 1994, Bre'da et al. 1995).

Changes in Ψs influenced plant water status and decline in both Ψpd and Ψmd (Figure 23) were linked to gradual depletion of soil moisture during increasing summer drought. The rate of decline in Ψpd in response to increasing water stress, however, varied among trees, suggesting significant differences among them. Since trees studied belonged to the same species and were of the same age and size class, no differences in physiological functioning were expected among them (Jones 1992). This was also illustrated in the relationship between Ψpd and rate of sap flux (Figure 27), showing that trees will respond in a similar pattern as long as they are exposed to similar conditions. During this study, trees were subjected to similar atmospheric conditions, strongly suggesting that the observed differences in their seasonal Ψpd was as a result of differences in the soil environment. Soil water uptake by roots, which determines the water status of a plant primarily depends on root density and distribution (Crombie et al. 1988, Le Roux et al. 1995), the existing water potential gradient between the root cells and soil (Tyree and Jarvis 1982) and the soil characteristics, which determine water flow resistance between soil and tree roots (Rambal 1984, Br´eda et al. 1995). During favorable soil moisture conditions resistance in water movement within the soil particles and also between soil and root surfaces is reduced (Radcliffe and Rasmussen 2002) and any observed differences in Ψpd will be the result of resistances to water movement across the root surfaces and also within the plant water conducting pathways (Rambal 1984, LoGullo et al. 2003). Trees acquired similar Ψpd and Ψmd during favorable soil moisture conditions (Figure 23), indicating that trees will experience similar resistances to water movement when exposed to similar soil moisture conditions and also that the soil to root moisture gradient was not different among them. Differences in Ψpd emerging at the onset of drought, therefore, indicated that tree roots were exposed to different soil moisture levels as drought intensified, most likely as a result of differences in rooting depth (Le Roux et al. 1995, Le Roux and Bariac 1995, Rodriguez et al. 2004) or a favorable local situation at the rooting zone, making water more readily available.

Linking plant and soil water status is the most effective way to assess patterns of soil water uptake by the plant roots since predawn leaf water potential is highly reflective of soil moisture conditions within

the rooting zone weighted by the root water uptake efficiency (Sala et al. 1981). Assuming that roots had the same potential of water uptake in the different soil layers, and going by the results shown in Figure 24, the relationship between Ψpd and Ψs at 0.3 m soil depth showed that trees *A11* and *D6* exhibited a different water uptake pattern from trees *A12, A13* and *D22*. Predawn water potentials for both *A11* and *D6* were less affected by changes of Ψs at this depth and remained above -0.5 MPa when Ψs at 0.3 m layer was below wilting point (> -1.5 MPa). This was different in the case of trees *A12, A13* and *D22* whose Ψpd values were much closer to, though less negative than Ψs at 0.3 m depth. This demonstrated an ability to access water in deeper soil layers than 0.3 m by all trees, since deeper soils were expected to remain moister (Rambal 1984, Bréda et al. 1995). Based on this argument, *A11* and *D6* therefore, must have had deeper root systems than *A12, A13* and *D22*. Relating Ψpd to Ψs at 1 m depth showed that trees *A12, A13* and *D22* were obtaining water from soil layers at or close to this depth since there was a near 1:1 relationship between Ψs and Ψpd. Similar relationship however, showed that *D6* was obtaining water from deeper soil layers than 1 m, since its Ψpd was poorly correlated to Ψs and that the drop in Ψpd as a result of drought was less steeper than Ψs at 1 m depth. For example, Ψpd for this tree was -0.5 MPa when Ψs at 1 m depth was approaching -1.5 MPa. A similar relationship demonstrated that decrease in Ψpd were slightly more rapid than Ψs at 1 m depth for tree *A11*, suggesting eventually that rooting depth was just above 1 m soil depth. However, rate of decline decline in Ψs around this tree was unexpectedly slower than for the rest of the trees considered for this study, contributing to its high Ψpd compared to other trees. Possible reasons for this slow rate of decline in Ψs could be due to low competition for soil water resources, since the tree was more isolated compared to the others, or as a result low transpiration water loss due to its small crown size. Although three tree classes can be derived from this study based on rooting depth, horizontal and vertical heterogeneity in soil moisture observed within the study plot may call for caution in the interpretation of the results, since tree roots have a potential to grow far from the main stem (Belsky et al. 1989, Larcher 2003) and could make it difficult to correlate tree moisture status with rooting depth. Relationships between Ψpd and Ψs at the two different soil depths were, however, informative enough to justify the above conclusions. Rapid changes in Ψpd after the September rain events, which was only recorded by soil moisture sensors at 0.3 m depth and not by those at 1 m depth demonstrated the presence of active tree roots within the upper soil layers, which were capable of water uptake soon after

a rain event that follows a long drought period. The ability of tree roots to rapidly recover water uptake after a prolonged drought period is already reported for trees growing in the savanna region of Kenya (Ong et al. 1999) and parklands of Senegal (Deans and Munro 2004). It is considered an important adaptation for growth and success in arid environments, since trees will be able to take advantage of rain pulses that may occur in the middle of a dry season to improve their tissue water status and physiological functioning (Ogle and Reynolds 2003).

Tree water use was significantly affected by drought stress and daily total sap flux reflected changes in tree and soil water status (Figure 25). Although sap flux rates measured at the branches showed a declining trend in June, soon after soil moisture within the upper 0-30 cm started to decline (Figure 26), total daily water use remained unchanged (0.3 kg cm^{-2} d^{-1} - xylem area) until mid June in trees *A12*, *A13* and *D22* and 0.35 kg cm^{-2} d^{-1} until July in *A11* and *D6*, reflecting root distribution patterns and access to soil water reserves. During this time (May-June/July), changes in Ψs were recorded only within the 0- 0.3 m soil depth, suggesting that root water uptake was mainly confined to the upper soil layers. These findings are supported by isotope measurements shown in Figure 38. Similar findings occur in Rambal (1984) and Bréda et al. (1995) for oak species in the mediterranean region of southern France. The observed initial decline in sap flux rates before July was attributed to drying of the upper (0 - 0.3 m) soil layers, which, might have resulted in increased drying of soil near the plant roots, leading to increased soil-to-root surface water flow resistance (Rambal 1984, Sala and Tenhunen 1994, Larcher 2003). Total daily plant water use, however, remained unchanged during this period probably due to increasing leaf area, since growth was observed until July (Figure 29) and also due to increasing day length. Leaf growth in the Mediterranean *Quercus* species is reported to occur until July, when overall growth ceases due to high summer drought (Rambal 1984, Tenhunen et al. 1987a, Nardini et al. 1999, Fialho et al. 2001). The results show the vital role played by shallow roots with regard to daily tree water budget. Rapid decline in Ψs at 1 m depth occurred between mid-June/July to August and this was associated with significant decline in whole tree daily water use. This was attributed to the drying of soil layers between 0.3 and 1 m depth, which seamed to host a large mass of tree roots, thus significantly affecting root water uptake and the whole tree water budget (Le Roux and Bariac 1995). Rambal (1994) found the greatest root accumulation of *Q. coccifera* within the top 1 m soil layer, below which there was gradual decrease with depth. Greatest root density in an oak stand in southern

France was within the top 80 cm soil layer (Bréda et al. 1995). The pattern of seasonal responses observed in this study are similar to those obtained previously for *Quercus* species growing in the Mediterranean regions (Rambal 1984, Bréda et al. 1995, Sala and Tenhunen 1995, Infante et al. 2001, Martinez-Vilalta et al. 2003).

Both trees *A11* and *D6* maintained relatively higher water use and sap flux rates compared to *A12, A13* and *D22*, reflecting their favorable tissue water status, despite increasing drought and supporting the suggestion that they had a stable water source and root water uptake was not interrupted. Results from tree water use in tree *A11* agree with those from the relationship between Ψpd and Ψs and support the initial suggestion that the tree could be obtaining water down to soil layers just above 1 m depth. Although data for whole tree water use were only available from mid June for trees *A11, A12* and A13 due to technical field problems, available results for *D22* and *D6* indicate that water use did not change until mid June and July respectively, when impact of drought was observed. From this information, it was estimated that daily water use in *A11* under favorable soil water conditions was half that of *D6* and about 70 % of *D22* (and probably *A12* and *A13*, since they had relatively similar crown size and structure). Transpiration rates (per unit leaf area) were similar in all trees between May and June (Figure 30a), hence differences in total water use by the different trees was mainly as a result of differences in crown size, supporting the initial argument that small crown size may account for slow decline in soil water around *A11*. However, the conserved water can be available later in the season, as shown in the case of tree *A11*, only if other trees are not competing for it (De Lucia and Schlesinger 1990), supporting the initial suggestion that lack of neighboring trees, hence low competition for soil water further contributed to slow decline in Ψs around tree *A11*. The role of tree density on soil moisture depletion rate in *Q. suber* stand has been previously recognized (Ribeiro et al. 2003, Ribeiro et al. 2004). Maintained high water use during drought as observed for tree *D6* is equally the result of good water supply (Abrams 1990, Larcher 2003, Sperry 2000). Since Ψs at 1 m depth dropped without affecting Ψpd and tree water use, it could only be possible that the tree was drawing water from deeper soil layers, which probably remained moist for a longer time (Rambal 1984, Le-Roux and Bariac 1995, Bréda et al. 1995, Rodriguez et al. 2004). Poor relationship between sap flux rates and Ψs at both depths for this tree further supports the above reasoning. Overall, the results demonstrate the role of root distribution, root depth and tree density on soil water availability and tree water use. Similarly, the

pattern of growth seamed to reflect that of whole tree water use (Figure 29), emphasizing the importance of water availability for tree productivity in this system.

During water stress, plants may increase soil water uptake by increasing solute concentration in the root cells, thus increasing the water potential gradient between the plant cells and soil (Tyree and Jarvis 1982). Increased drought stress was associated with increased cell osmotic potential by a magnitude of 0.7 MPa (Table 6) and was regarded as osmotic adjustment (Tyree and Jarvis 1982). Since these values were derived from osmotic potential at full turgor (Π^{100}), it was definite that the change was an active process and not a passive one, associated with shrinking cell volume during drought (Jones 1992). This active solute accumulation will increase the water potential gradient between the soil and root cell environments, allowing some water uptake from the drying soil, leading to minor decreases in the relative cellular water content and promoting physiological activities and productivity during drought (Larcher 2003). Thus, osmotic adjustment could account for continued growth (Figure 29) observed in the trees a few weeks after Ψs at 1 m had declined below -1.5 MPa, which is usually the threshold for growth in most plants (Jones 1992). The results compare favorably with those of other studies on the same species growing in the mediterranean region. For example, Nardini et al. (1999) obtained an osmotic adjustment of 0.5 MPa between April and July. A lower value of 0.4 MPa was obtained by LoGullo et al. (2003) between May and July. In the current study, a decrease in cell wall elasticity was observed between May and July and bulk modulus of elasticity (ε_{max}) increased by a magnitude of ca. 17 MPa. This was within the range 15 to 18 MPa recorded in other studies for similar period of time (Corcuera et al. 2002, LoGullo et al. 2003). The advantage of a rigid cell wall during drought as reported for *Q. suber* trees would be to allow rapid recovery, especially of the root cells and a resumption of water uptake after drought is alleviated (Corcuera et al. 2002). This could explain why root water uptake resumed soon after a rain event in September that followed a prolonged drought period.

The study demonstrates the importance of soil water availability in plant water budget and growth, however, acquisition of soil water and success of trees in this system may depend more on root distribution and tree density. Although small crown size may be disadvantageous in terms of productivity and growth, it contributed significantly towards restricting water use by trees, prolonging

the period with favorable soil water content as observed in the case of tree *A11*. Thus, a balance between transpiring leaf area and the absorbing root surface could as well play a significant role in survival of trees in the Mediterranean ecosystem. This suggests that a special relationship could be established between effective root surface area and transpiring leaf surface area as a measure of plant success in this system. This was, however, not investigated but could provide new insights into plant interactions in the Mediterranean. Trees showed a capacity to adjust osmotically during the development of drought and this could most likely improve their root water uptake potentials. This demonstrates an inherent adaptation in trees, acquired over long-term and regular exposures to water stress, which has made them well adapted to this system.

4.3. Coordinated responses to water stress in Q. suber trees in the Mediterranean ecosystem

The overall results show that most of the aboveground shoot responses were elicited by changes in soil water status. Trees responded to declining soil water availability and plant water status by reducing stomatal conductance (Figures 30a and 31), leading to reduced transpiration water loss (Figure 30b). Similar results have been reported for *Q. suber* and other related oak species growing in the mediterranean regions of southern Europe (Tenhunen et al. 1987b, LoGullo and Salleo, 1988; Salleo and LoGullo, 1990; Mediavilla and Escudero, 2003). Maximum rates of both transpiration (E_{max}) and stomatal conductance (gs_{max}) recorded during the season compare favorably with other studies on *Q. suber* growing in the mediterranean region (Tenhunen et al. 1987a, Tenhunen et al. 1989, Oliveira et al. 1992, LoGulo et al. 2003) but slightly higher than values reported by Tenhunen et al. (1987b), Nardini et al. (1999), Mediavilla and Escudero (2003). There could be possibility of overestimation of rates of gaseous exchange because one of the porometers used in this study was realized to have problems soon after completion of the field campaigns of 2003. Since this was a comparative study of different trees of *Q. suber*, possible errors applied equally to each tree and therefore do not affect the ensuing argument and overall deductions. Caution is however, called for in the interpretation of absolute values. The relationship between diurnal maximum stomatal conductance (gs_{max}) and predawn leaf water potential (Ψpd) (Figure 31) showed a strong dependence of stomatal regulation of transpiration water loss to plant water status, demonstrating an effective coupling of shoot responses to soil water availability. Thus, depending on the available water at the rooting zone, which determines tree Ψpd (Ryel et al. 2003), different rates of stomatal conductance were recorded for the different trees at

similar times during the season (Figures 30 and 32). This demonstrated a strong co-ordination between soil water availability, root water uptake and stomatal conductance (gs), which determines the rate of transpiration (E), and which has implications for tree productivity and survival.

Seasonal differences in Ψpd occurred among the trees, which was most likely as a result of differences in rooting depths and a favorable local situation such as low tree density or soil quality. These differences were equally reflected in diurnal and seasonal magnitudes of gs as well as E (Figures 30 and 32). Except for differences in diurnal behavior patterns of stomatal conductance observed in June between trees *A11* and *A12*, in which there was midday depression in gs in *A12* that was not observed in *A11*, the general pattern of stomatal response during the season was similar between the two trees. The magnitudes of gs_{max} and E_{max} were, however, significantly different. Results for diurnal patterns of gaseous exchange and leaf water potential (Ψ_L) during early July, when trees were just starting to show signs of water stress indicated that despite having similar Ψpd, gs_{max} was reduced by 40% in tree *A12*, while the reduction was only 20% in *A11* (Figure 32). Also, gs and E remained higher in *A11* throughout the day. At this time, although trees had similar Ψpd, most likely because of the long night hours which could allow equilibration of plant water potential with that of the wettest part of the soil volume accessed by roots, there were already significant differences in whole tree sap flux (*Qtree*), with tree *A12* showing depressed sap flux in mid June but no changes were observed in tree *A11* until after July (Figure 25). Since the trees had similar Ψpd and were exposed to similar atmospheric conditions, differences in gs and E observed among trees could only be attributed to differences in sap flux (delivery of water to the leaves). According to Sperry 2000, and Cruiziat et al. (2002), plant's ability to lose water from leaves is associated with its ability to supply water to the leaves, hence for a species, trees with better leaf water supply will show higher transpiration rates as demonstrated in Figure 32.

Apart from gs, transpiration water loss is also influenced by environmental factors such as VPD (Schulze and Hall 1982, Jones and Sutherland 1991, Oren and Pataki 2001, Cochard et al. 2002). Changes in VPD affects E through altered gradients in atmospheric vapor demand (Schulze and Hall 1982, Saliendra et al. 1995, Oren and Pataki 2001). Increasing VPD during the day will therefore lead to increased E and decline in Ψ_L as long as plants don't reduce stomatal conductance (Schulze et al.

1987, Jones and Sutherland 1991), or increase rate of water supply (hydraulic conductance) to the leaves (Meinzer and Grantz 1990, Jones and Sutherland 1991, Maherali and De Lucia 2001, Sperry 2000). Increasing water supply from the roots to maintain favorable tissue water status has the advantage of allowing continued photosynthesis but leads to rapid exhaustion of soil water reserves. On the contrary, stomatal closure, which limits water loss and ensures extended soil water availability will lead to reduced productivity, hence a balance must be established between the two processes (Bradford and Hsiao 1982, Cochard et al. 2002). Despite varied Ψ_L at which stomatal closure occurred over the season, results showed that stomata remained open as long as VPD did not exceed 30 hPa (Figure 36). Further increase in VPD during the day resulted in progressive stomatal closure, thus, resisting increase in transpiration water loss and protecting against further decline in Ψ_L to preserve hydraulic continuity of the soil to leaf continuum (Cruiziat et al. 2002).

Seasonally, Ks-l significantly declined with increasing soil drought (Figure 34a). Decline in Ks-l during drought has been attributed to increasing resistances along the soil–leaf pathway (Blizzard and Boyer 1980, Jones and Sutherland 1991). Most studies have apportioned bulk of the increasing resistances to the soil root interface as well as in the bulk soil itself (Blizzard and Boyer 1980, Jones and Sutherland 1991, Sala and Tenhunen 1994, Cruiziat et al. 2002). Since loss of conductivity as a result of cavitation of the xylem vessels is irreversible (Tyree and Sperry 1989), and that sap flux resumed to its initial rates soon after rehydration of the 1 m soil layers (data not shown), it is suggested that the major resistance restricting water flow was at the soil to root interface (see also Sala and Tenhunen 1994).

Previous studies have established a strong relationship between changes in gs and Ks-l (Meinzer and Grantz 1990, Saliendra, et al. 1995, Sperry 2000, Meinzer 2002, Schulz 2003), which has been suggested to demonstrate the dependence of gs on Ks-l (Sperry 2000, Meinzer 2002). A curvilinear relationship between gs and Ks-l (anisohydric regulation) as observed in Figure 34b demonstrated a gradually increasing control of stomatal regulation on Ψ_L during the development of drought (Hubbard et al. 2001). The results resemble those presented elsewhere (Meinzer et al. 1995, Andrade et al. 1998, Sperry 2000, Brodribb et al. 2002, Cochard et al. 2002). The advantage of this coupling between gs and Ks-l during the development of drought is shown in Figure 33 in which maximum E was reduced with

increasing soil drought and consequently minimizing the drop in Ψ_L during the day. According to Sperry et al. 2002, anisohydric stomatal regulation provides a broad safety margin for critical Ψ_L at which cavitation occurs (Ψc) and transpiration during conditions when soil water is favorable is only limited by maximum stomatal apertures (Figure 35). The ecological implication of this behavior pattern is the optimization of carbon gain and water resource utilization and is associated with plants adapted to arid conditions (Sperry et al. 2002, Schulz 2003). The establishment of a maximum sap flux capacity as observed when soil water was abundant is as a result of restriction created by the stomatal aperture (Sperry et al. 2002, Ryel et al. 2003). However, restrictions of whole tree sap flux at consecutive lower soil moisture contents, leading to lower maximum sap flux rates could be the result of hydraulic limits imposed on the conducting pathway as atmospheric vapor demand increases, at the absorbing root surfaces when soil water conditions are favorable, but shifting to different locations including stomata as drought intensifies (see also Sperry et al. 2002).

Ideally, stomatal conductance (gs) must be closely linked to Ks-l (Whitehead et al. 1984, Meinzer and Grantz 1990, Jones and Sutherland 1991, Sperry and Pockman 1993). This will ensure maintenance of Ψ_L above a critical point at which cavitation occurs (Wullschlleger et al. 1998, Sperry 2000). For the mediterranean *Quercus* species (*Q. ilex* and *Q. suber*) Ψ_L values of –3 MPa have been established to be equivalent to xylem water potential of –2 MPa at which cavitation occurs, i.e. cavitation threshold, denoted as Ψc (Tyree and Cochard 1996, Cruiziat et al. 2002). During this study, two distinct seasonal levels of diurnal minimum Ψ_L were observed. When soil water was abundant, Ψ_L did not drop below –2.0 MPa. A constant minimum Ψ_L during the day early during the season when soil water was abundant shows a balance between the capacity of plants to take up water from the soil as well as to loose water at the leaf surfaces. This balance is established through a coordinated regulation of root and leaf growth during development and is dictated by the plant's genetic constitution as well as prevailing environmental conditions to which the plant is subjected during its life time (Bradford and Hsiao 1982). Thus, when soil water was abundant, the minimum Ψ_L of –2.0 MPa observed during the day was caused by a limitation imposed on water loss by the available stomatal pores as well as their maximum aperture size. Therefore, despite the fact that the stomata were fully open, there was still an imposed restriction on the maximum rate of water loss and this maximum rate must be related to the rate of

maximum sap flux from the roots in order to safeguard and maintain a constant Ψ_L, otherwise leaves could still loose turgor, leading to wilting despite the abundant soil water availability.

Minimum Ψ_L was however, lowered significantly later in the season as soil water became limiting. The average minimum Ψ_L recorded for the trees was –2.5 MPa and diurnal Ψ_L did not drop below this value despite increasing drought as a result of stomatal closure. This strongly suggested the physiological significance of minimum Ψ_L and that Ψ_L must be related to Ψc in this species. There is always a safety margin between Ψc values and complete xylem embolism (Sperry and Ikeda 1997, Sperry 2000). Given the documented Ψc value, it is definite that the trees operated within safety margins and that no catastrophic xylem embolism occurred. Stomatal control therefore, plays a role in regulating water loss in order not to allow Ψ_L to drop below the threshold values (Cruiziat et al. 2002). The drop in minimum Ψ_L between July and August accompanied significant osmotic adjustment of a magnitude ca 0.7 MPa. Water potential at turgor loss point (Ψtlp) equally dropped significantly in July from -2.0 MPa to ca. -2.4 MPa and most likely continued to drop in August (Table 6). Although the role of solutes in xylem functioning during the development of drought is still unclear (Cochard et al. 2002, Cruiziat et al. 2002), results suggest that solute accumulation may play a role in the establishment of Ψc in trees during drought. It is documented that Ψc is higher in mesic than drought xeric plant species as a result of modifications of the conducting vessels (Tyree and Sperry 1989), Ψc could as well shift with seasons in a species, following build up of solutes, since improved water uptake and the lowering of Ψtlp associated with osmotic adjustment (Tyree and Jarvis 1982) must be accompanied with improved xylem transport competency (Cochard et al. 2002), otherwise it serves no purpose in drought survival when it does not influence xylem Ψ at which cavitation occurs. Thus, responses that are aimed at regulating water loss and tree survival during drought in *Q. suber* appear to be very well coordinated. This close coordination between vapor and liquid phase conductance act to moderate changes in Ψ_L that would otherwise occur during water stress (Whitehead et al. 1984, Meinzer et al. 1992, Sperry 2000) and ensuring survival during drought.

4.4. Hydraulic lift: influence of Q. suber trees on the water status of the rhizosphere

Hydraulic lift is determined by monitoring diurnal fluctuations of the soil water potentials (Caldwell and Richards 1989, Espeleta et al. 2004) as well as through isotope sampling (Dawson1993, Ludwig et al. 2003). Hydraulic lift is the nocturnal movement of water from the more wet deep soil layers through roots to the upper more xeric soil layers (Caldwell et al. 1998). Thus, monitoring of diel changes in Ψs of different soil layers at the rhizosphere of a tree can reveal the occurrence of hydraulic lift. Measurements of Ψs at the 0.3 m soil revealed a decline in soil water potential during the day and an increase during the night in this layer (Figure 37). Hydraulic conductivity of sandy loams is very low, ranging from 10^{-15} to 10^{-13} m/s. This would equally apply to both vertical and lateral migration of water (Radcliffe and Rasmussen 2002) and cannot explain the diel fluctuations and rapid changes in Ψs that were observed in this study. The observed decline in Ψs at the 0.3 m soil layer during the day could only be attributed to uptake of soil water by the tree roots found within the 0.3 m soil layer, to supplement water being absorbed by the deeper roots at a time when transpiration rates were increasing due to increasing light intensity, temperature and VPD. At night, when transpiration water loss had declined, continued water uptake by the deeper roots (declining Ψs at 1 m) led to increased tissue water status of the trees. At some point (around 8:00 p.m. in the case of tree *D6* shown in Figure 37) water potential of the plant tissues including those of the roots found within the 0.3 m soil layers must have exceeded that of the soil medium at the 0.3 m depth, leading to reverse flow of water from the roots back into the soil at this depth. This must have led to the observed increase in Ψs between 8:00 p.m. and 6:00 a.m. as shown in Figure 32.

Isotopic signature of the soil water reflects direct input from precipitation, which can become isotopically enriched in the surface layers by evaporative water loss, and then depleted with depth in the soil profile (Allison et al. 1983). Isotope analysis of soil water during the onset of drought showed isotope enrichment within the first 0.25 m soil depth (Figure 38). $\delta^{18}O$ values of water extracted from soil layers ranging from 0.6 m downwards however, were more depleted and were closer to those of winter rainfall, suggesting that the enrichment process of the upper soil layers was indeed due to evaporation since this layer is more exposed to evaporation processes. Similar conclusions were drawn by Ludwig et al. 2003 in a study conducted on *A. tortilis* growing in the arid savanna of E. Africa. Determination of belowground stable isotopes of δD and $\delta^{18}O$ of water in the xylem and in the soil

provide a powerful tool for determining the sources of water used by plants and thus their rooting depths and patterns (Ehleringer et al. 1991, Dawson 1996). $\delta^{18}O$ of xylem water obtained during June, soon after the long winter and spring precipitation showed values close to winter precipitation. During this time, δD was within the range of winter rainfall values, which showed a very scattered δD. Since the xylem δD values more closer to winter precipitation than to the ground water, the results suggest that most of the water uptake by trees was restricted to the upper soil layers during this time, but probably shifted to deeper soil layers later in the season when water in the upper soil layers were exhausted. Results from isotope analysis, therefore, agree with those of soil water potential shown in Figure 22 (upper panel), which strongly showed that root water uptake was exclusively from the upper soil layers until after mid June, when decline in Ψs was observed at 1 m soil depth of the rhizosphere.

Slightly enriched isotopic signatures (δD) of water from the xylem of the tree twigs during September, a time when intense drought was experienced, compared to June (Figure 22 lower panel) suggested that trees were using water from mixed sources namely shallow and deep soil layers. Initial results based on Ψs and isotope measurements, however, indicated that plant water uptake shifted downwards as drought progressed and that there was minimal net change in Ψs of the shallow soil layers between August and September. This period coincided with the time when diel fluctuations of the Ψs of the 0.3 m soil layers were observed, suggesting that water obtained by deep roots was released back into the shallow soil layers and then reabsorbed in a more enriched form by the shallow roots during the early part of the day resulting into a more enriched xylem water. This supports the argument for hydraulic lift and strongly indicates that hydraulic lift occurred in *Q. suber* trees. However, since the demand for moisture was high as a result of increasing VPD, light and air temperatures, the end result was a net loss of water from the upper soil layers despite significant water being moved into the shallow soil layers by hydraulic lift. Trees with better access to deep-water stores were bound to lose more water since more water will be supplied from the deep root mass as demonstrated by their high transpiration rates. Trees with deep rooting system were equally expected to experience more favourable tissue water status as well as more depleted isotopic signatures of water extracted from their xylem during the day. This was demonstrated by the results shown in Figure 39, further showing the importance of deep rooting in plant water balance during drought. The results equally suggest that water contributed by hydraulic lift is limited and deep rooting is paramount in tree survival in the aridlands. Discrepancies

between the results $\delta^{18}O$ and δD were, however, difficult to explain and calls for caution in the interpretation of some of the results.

4.5. Responses of A. xanthophloea and A. tortilis seedlings to cyclic water stress under controlled greenhouse conditions.

Six-day cyclic water stress resulted in a significant decrease in Tdwt, LA and a shift in carbon allocation. LA was more sensitive to water stress, with significant differences between treatments and also between species appearing much earlier after imposition of stress as compared to other morphological characteristics studied. For instance, drought induced decrease in dry weight was significant after 6 months of growth (Figure 40) while the decrease in LA was significant after only 2 months of water stress cycles (Figure 41). Water stress reduced LA through reduced leaf initiation, size and shedding in the two *Acacia* species.

Water stress caused 45 and 40% decline in Tdwt in *A. xanthophloea* and *A. tortilis* respectively. Major decline in growth in both species, as a result of water stress was mediated through LA reduction (Figure 42) and probably through reduced CO_2 assimilation as well, since rate of stomatal conductance per unit leaf area declined with water stress (Figure 51). Overall, water stress affected Tdwt accumulation in the fast growing *A. xanthophloea* more than in *A. tortilis*, and this was in agreement with the magnitude of decline in LA caused by water stress. The results concur with other studies. For example, a fast growing *Eucalyptus* provenance with higher LA under sufficient water supply exhibited a large proportional decrease in mean leaf size, LA and plant biomass under drought conditions and that reduction in growth was due to reduction in foliage area (Osorio et al. 1998, Pita and Pardos 2001). For *A. xanthophloea*, a 45% reduction in Tdwt due to water stress was therefore in agreement with 41% decline in LA. However, 32% reduction in LA as a result of water stress caused a 40% reduction in Tdwt in *A. tortilis*. This is further shown by differences in the regression coefficients between stressed and controls in the relationship between LA and Tdwt (Figure 42). This discrepancy in *A. tortilis* could be explained in terms of carbon partitioning, with more resources being diverted away from LA development into root growth (Abrams 1994) or for osmotic adjustment (Munns 1988, Blake et al. 1991), either of which contributes to improved soil water uptake but reduces growth (Osunubi and Davis 1978, Abrams 1994). *A. xanthophloea* showed an inherent capacity to grow faster, a fact that was

attributed to its ability to apportion more resources for LA development, maximizing carbon gain when, conditions were favorable. Rapid growth during favorable moisture conditions, however, offers no ecological advantages in an environment where rainfall is erratic and drought periods longer than wet ones, unless it is accompanied with a balanced resource allocation, maximizing on soil water uptake and minimizing on water loss.

Transpiration was closely related to the amount of foliage when water was not limiting and controls of *A. xanthophloea* with higher LA experienced higher water use compared to those of *A. tortilis* (100 and 80 g h^{-1} respectively). On a long-term basis, reduction in transpiration as a result of cyclic water stress was associated with LA reduction. However, mechanisms such as stomatal regulation, leaf folding, root characteristics and root to shoot water transport were involved in controlling the daily plant water budget in order to maintain favorable tissue water status. Under conditions of ample water supply, *A. xanthophloea* experienced higher transpiration rates and also low Ψ_L at the end of the day compared to *A. tortilis* (-2.0 and -1.4 MPa respectively). Results indicated that *A. xanthophloea* plants, which were well supplied with water apportioned more carbon resources to the shoots than to the root biomass (Table 7). Thus, the study demonstrates the significance of a balance between the absorbing root biomass and the total transpiring leaf surface area in determining diurnal minimum Ψ_L experienced by a particular plant species and that plant species vary in this balance. This observed variation among tree species could be a significant determining factor in species ability to withstand water stress as well as their distribution under natural field conditions. Plants however, showed strong regulation of water loss through reduced stomatal conductance during midday when Ψ_L was low, resisting further decline in Ψ_L. For *A. xanthophloea*, the resulting low transpiration rates as a result of stomatal closure were not accompanied with immediate recovery of Ψ_L as observed in *A. tortilis*, suggesting an interrupted supply of water from roots to shoots (Tyree and Sperry 1989, Meinzer and Grantz 1990, Sperry and Pockman 1993, Sperry 2000), which is dependant on characteristics of roots (Boyer 1985) and also those of water transport vessels (Zimmermann 1983, Tyree and Sperry 1989). This further supports the initial argument that a balance between root and shoot biomass during the development of a plant is vital for its success during drought and that this balance is genetically determined, i.e. it varies among plant species.

Hydraulic conductance is associated with water uptake at the root surfaces, root/leaf surface area ratio or inherent absorption capacity and root permeability and also an effective water transport system (Tyree and Sperry 1988, Reich and Hinkley 1989, Ni and Pallardy 1990). Controls of *A. tortilis* exhibited higher hydraulic conductance compared to *A. xanthophloea* (4.73 and 3.48 mmol MP^{-1} m^{-2} s^{-1} respectively). At high soil moisture, the soil resistance is small and any observed differences in hydraulic conductance should be largely attributed to differences in plant resistance (Ni and Pallardy 1990). From these results, it is logical to conclude that *A. tortilis* inherently has a more robust and efficient transport system compared to *A. xanthophloea*. Generally, hydraulic conductance is expected to decline with increasing water stress as a result of increasing resistances along the conducting pathway (Blizzard and Boyer 1980). The decline in Ks-l observed in the repeatedly stressed plants was, however, insignificant, i.e., 0.2 and 0.3 mmol MPa^{-1} m^{-2} s^{-1} for *A. tortilis* and *A. xanthophloea*, respectively. Two reasons proposed for this observation include (1) that there was development of a more competent transport system with imposition of cyclic water stress (Tyree and Sperry 1989) and, (2) that there was an improved balance between the absorbing root and transpiring leaf surface area in the repeatedly stressed plants (Meinzer and Grantz 1990).

According to Tyree and Sperry (1989), there is slower cell expansion during limited water availability while carbohydrate reserves are high, permitting rapid primary and secondary wall growth that leads to formation of cells with smaller pit-membrane pores. Such cells are less vulnerable to cavitation. Thus, it could be suggested that repeated water stress led to the development of better adapted water transporting vessels in the *Acacia* species. Repeatedly stressed plants continually shed off leaves between day 4 and 6 of water stress. This could as well improve or maintain the balance between absorbing root and transpiring leaf surface area (shown as Rdwt/LA), and is likely to improve root-shoot hydraulic conductance (Meinzer and Grantz 1990). Also, there was improved absolute root growth in stressed plants, which could as well improve root water uptake and supply to the shoots. These results, therefore, further support the existence of a strong link between water supply from the soil by roots and the transpiring leaf surface area, and that carbon allocation is one way by which plants determine the effectiveness of water supply from roots to shoots. Increased resource allocation to the roots compared to leaves leads to high hydraulic conductance and this represents a vital adaptation to drought stress (Kramer 1980). This means that for a given rate of transpiration, a species like *A. tortilis,*

which possesses high hydraulic conductance will undergo less reduction in Ψ_L during the day. Naturally, the capacity to dampen the decline in Ψ_L during water stress ensures prolonged photosynthesis during drought periods and has implications for plant survival and productivity in dry environments (Cochard et al. 2002). *A. xanthophloea*, however, exhibited an exaggerated dehydration avoidance mechanism associated with stomatal closure that must be linked to an inherently low Ks-l, and with the disadvantage that Ψ_L declines to levels that may approach thresholds for cavitation (Tyree and Sperry 1989). The fact that pre-conditioned (repeatedly stressed) plants from both species were able to restore Ψ_L when they were re-watered after 6 days of water stress indicates that severe xylem cavitation did not occur during drought and that effective water transport to the shoots was restored. Cavitation however, occurred in non conditioned (previously controls) plants of *A. xanthophloea* during severe water stress leading to lack of resumption of water transport, restoration of Ψ_L and death of aerial shoots in the control plants.

Repeatedly stressed *A. tortilis* showed a significant (p<0.05) reduction in Π^{100} compared to controls. This decrease in Π^{100} may have been largely due to solute accumulation in the cells, since determination of cell osmotic potential at full turgor eliminates increased solute concentration as a result of decreased cell volume (Fan et al. 1994). The observed solute increase, therefore, constitutes osmotic adjustment (Hsiao 1973, Osunubi and Davis 1978, Jones 1992). As a result of the 6-day water stress cycle, *A. tortilis* adjusted osmotically by a magnitude of 0.48 MPa compared to *A. xanthophloea*, which only realized a insignificant adjustment of 0.16 MPa. Osmotic adjustment may constitute an adaptation to drought stress when it causes a decrease in Ψtlp (Morgan 1984, Fan et al. 1994), i.e. maintenance of turgor down to lower values of Ψ_L (Tyree and Jarvis 1982). Osmotic adjustment significantly improves soil-water uptake under dry conditions (Tyree and Jarvis 1982) as well as allowing maintenance of open stomata with larger apertures and higher stomatal conductance and net photosynthesis down to lower values of Ψ_L (Myers and Landsberg 1989). In the current study, *A. tortilis* maintained higher Ψ_L and stomatal conductance rates when soils were drying. Although these observations could be explained by osmotic adjustment, an alternative explanation could relate to the reduced LA. Repeatedly stressed *A. tortilis* had limited LA resulting in lower water loss from the pots. This would mean delayed pot dehydration, hence the plants enjoyed favorable soil water status for a longer time than controls or *A. xanthophloea*. Another explanation might relate to root system

characteristics; *A. tortilis* had a higher root/shoot ratio, roots distributed over the entire soil mass and, possibly, higher water permeability across the root surfaces (as shown by high Ks-l). Thus, repeatedly stressed plants could effectively draw water from a larger soil mass, but were only required to supply a relatively small transpiring leaf surface.

For *A. xanthophloea,* repeated water stress resulted in the reduction of bulk modulus of elasticity (ε) from 18.7 to 9.9 MPa. Elastic modulus reflects the mechanical properties of the cell wall such that a fully turgid tissue is expected to possess the greatest apparent ε (Blake et al. 1991). Bulk modulus of elasticity will, therefore, decline with a decrease in cell water content (Tyree and Jarvis 1982). A more elastic tissue, as denoted by a lower modulus of elasticity, indicates that turgor potential declines less rapidly per unit loss of water (Blake et al. 1991). For a given Π, increased elasticity facilitates turgor maintenance over a greater range of water contents, hence improving water uptake from a drying soil (Tyree and Jarvis 1982). The ability to improve cell wall elasticity during water stress as observed in *A. xanthophloea* could account for its increased water stress tolerance during severe stress and might have contributed to its quick recovery after water stress was alleviated. According to Dale and Scutliffe (1987), and Munns (1988), active solute accumulation shifts photosynthates away from growth towards cell turgor regulation and could have accounted for reduced growth rate and slow recovery in repeatedly stressed *A. tortilis* after re-watering. The ability of preconditioned plants to increase cell wall elasticity in response to stress could, therefore, allow greater carbon utilization in cell repair processes and a more rapid growth after stress (Blake et al. 1991) as observed in *A. xanthophloea*.

4.6. Comparisons between naturally growing trees and seedlings grown under controlled greenhouse conditions

Most responses exhibited by glasshouse plants of the two *Acacia* species during the development of water stress resembled those observed in trees growing under natural field conditions during increasing drought stress. Withholding water for greenhouse plants as well as lack of precipitation for naturally growing trees under field conditions resulted in soil drying and this impacted negatively on plants, leading to decline in tissue water status. Although stress cycles in the greenhouse were limited to six days, compared to several months of drought experienced by plants growing under natural field conditions, it must be realized that roots of potted plants in the greenhouse were confined to a limited

soil volume and the drying cycle for seedlings therefore recapitulates the annual cycle of stress experienced by naturally growing trees. Seedlings of *A. xanthophloea* experienced Ψ_{pd} of ca. -2.5 MPa at the end of 6-day water stress cycle. Similarly, *A. xanthophloea* trees growing under natural field conditions experienced a minimum Ψ_{pd} of –2.4 MPa. This suggests that the levels of water stress experienced by both seedlings and trees were almost similar and that plants were expected to behave in a similar manner during the cycles except for variations due to ontogenetic differences. Similarly, minimum Ψ_{pd} of -1.3 and –1.4 MPa were recorded for plants in greenhouse and young *A. tortilis* trees growing in the field respectively. Mature *A. tortilis* trees, however exhibited higher (-1.1 MPa) tissue water status in June when intense drought was experienced. The results therefore showed that stress levels as a result of drought experienced by naturally growing plants can be attained in the greenhouse, by subjecting plants to repeated water stress through withholding water. For *A. tortilis*, however, ontogenic differences resulted into differences in tissue water status as observed between naturally growing mature trees and young seedlings growing both in the field and greenhouse. These differences were attributed to the development of the root structure and calls for caution when designing such experiments.

Most of the *A. xanthophloea* growing naturally on the experimental field site in the savanna region of Kenya had greater stem diameters and crown sizes compared to *A. tortilis* trees. This was equally observed for the greenhouse plants, where after one year of growth*, A. xanthophloea* seedlings accumulated twice as much total dry weight compared to *A. tortilis,* either with adequate water supply or when subjected to repeated water stress Table 7. Thus, size differences observed between the two species in trees growing naturally in the savanna might have not been the result of age differences but differences in growth rate between the two species, which was an inherent characteristic. Differences in growth rate between the species was attributed to differences in carbon allocation patterns, osmotic adjustment and cell wall properties. The fact that greenhouse plants behaved in a similar manner as those under natural field conditions supports the current objective, and is a crucial starting point in using manipulation experiments in the greenhouse to predict performance under different scenarios since growth patterns will only respond to treatments. The ability of mature *A. tortilis* growing under natural field conditions to retain favorable tissue water status despite increasing drought stress (Figure 12) was attributed to its rooting patterns as well as root activities. Similarly, Ψ of seedlings of *A. tortilis*

remained favorably high on day 6 when maximum stress was attained (Figure 46) compared to *A. xanthophloea*. Close examination of the rooting structure showed a potential in *A. tortilis* plants to develop a strong tap root system during drought (plate 3) as well as apportioning more carbon to the roots than to the shoots (table 7). This supports the initial discussions on naturally growing *Acacia* trees that the observed response patterns were the result of rooting patterns and the soil resource and further shows the similarity between plants in the greenhouse and those in the field.

Physiological functioning of a plant is determined by its tissue water status (Kramer 1980). Changes in gas exchange during the stress cycle reflected the water status of the plants studied both in the field and glasshouse. Control plants under greenhouse conditions experienced maximum conductance as well as transpiration rates compared to those subjected to drought. Lowest values of E and gs were recorded on day 6 when maximum stress was realized and similarly in June for naturally growing trees, a time when lowest soil water status were recorded. Thus, changes in gas exchange were in response to roots' capacity to supply water as well as the capacity of xylem to conduct this water to the shoots and stomatal response was aimed at maintaining xylem integrity (Tyree and Sperry 1989). This was the case for both greenhouse and plants growing naturally in the field. Responses observed in *A. xanthophloea* and *A. tortilis* studied both under natural field and greenhouse conditions are summarized in Table 10.

Table 10. Responses exhibited by mature trees of *A. xanthophloea* and *A. tortilis* growing in a natural field environment and young plants grown in the greenhouse. In both cases, plants experienced varying levels of water stress.

| Response to water stress | *A. xanthophloea* | | *A. tortilis* | |
	Greenhouse	Field	Greenhouse	Field
Tissue Ψ	decreased	decreased	decreased	decreased
Leaf conductance	decreased	decreased	decreased	decreased
Leaf initiation	decreased/ceased	decreased	decreased/ceased	decreased/ceased
Leaf expansion	decreased/ceased	decrease/ceased	decreased/ceased	decreased/ceased
Leaf rolling	occurred	occurred	occurred	occurred
Leaf shedding	occurred	occurred	occurred	occurred
Leaf transpiration	decreased	decreased	decreased	decreased
Total plant growth	decreased	decreased	decreased	decreased
R:S ratio	no change	not investigated	increased	not investigated
Absolute root growth	decreased	not investigated	increased	not investigated
Root distribution	shallow	shallow	deep	deep
Osmotic adjustment	insignificant	not investigated	occurred	not investigated
Cell wall elasticity	increased	not investigated	none	not investigated
Hydraulic conductance	decreased	decreased	decreased	decreased
Xylem cavitation	occurred	occurred	none	none

Results showed that behavior patterns exhibited by mature trees growing under natural field conditions can be effectively qualitatively replicated in young plants growing in the greenhouse through manipulation experiments. Superior qualities in seedlings with respect to drought stress can also be identified in seedlings subjected to cyclic water stress. Plants initially subjected to cyclic water stress maintained favorable Ψ_L and survived longer compared to their control counterparts when both were simultaneously subjected to severe water stress, suggesting that preconditioning could improve ability of seedlings to survive water stress. However, inherent capacity of a species to overcome drought was an overriding factor in water stress resistance as shown by the differences between *A. tortilis* and *A.*

xanthophloea. Also, depending on their inherent characteristics, plants were able to develop stress resistance better, when subjected to slow but regular water stress than when stress was rapid. It can therefore be concluded that responses to water stress exhibited by mature trees under natural field conditions can be effectively replicated in controlled glasshouse experiments. Information acquired from such manipulation experiments is valid enough and can be effectively used to predict field performance under different environmental conditions as well as for screening plants suitable for different field scenarios.

4.7. General discussions

Responses exhibited by trees and their implications for tree productivity and survival during drought have been extensively discussed in the previous sections of this chapter. Table 11 summarizes the responses that were observed during the study as a result of changing soil water status. The overall view is that most of the aboveground shoot responses, as well as whole tree behavior were the result of a modified soil resource base. Long term as well as short term changes associated with the root and shoot systems act to modify plant functioning in order to accommodate changes in the soil environment. The ability of a plant to modify its root system to optimize resource gain from the soil resource base and to maintain a balance between resource acquisition and use ensures its success in a given habitat. The final conclusion, therefore, is that the soil resource base as well as root system development determines productivity, species distribution and the overall behavior of aridland ecosystems.

Table 11. A summary of coordinated responses exhibited by trees subjected to water stress in the savanna region of Kenya, Mediterranean region of Portugal and in controlled greenhouse conditions. A check mark indicates occurrence of the indicated phenomenon.

Experiment/Responses	Savanna (Kenya)	Mediterranean (Portugal)	Greenhouse
Deep rooting	*A. tortilis*	✔	*A. tortilis*
Osmotic adjustment	not examined	✔	*A. tortilis*
Increase in root:shoot ratio	not examined	not examined	*A. tortilis*
Changes in cell wall structure	not examined	decreased elasticity	*A. xanthophloea*
Hydraulic lift	*A. tortilis* -literature	✔	not measured
Leaf shedding	✔	none	✔
Leaf folding	✔	none	✔
Stomatal conductance	decreased	decreased	decreased
Hydraulic conductance	decreased	decreased	decreased
Xylem cavitation	*A. xanthophloea*	none	*A. xanthophloea*

The following discussions make reference to both field and greenhouse results, but with an emphasis on rooting patterns as well as root activities in order to demonstrate the crucial role played by roots in determining individual tree productivity, species distribution as well as dryland ecosystem functioning and balances. The next section emphasizes information both from field and greenhouse experiments that demonstrate the importance of research, particularly ecophysiology, for the management of aridland systems and with special emphasis on the savanna of Kenya. The final section focuses on sustainable management as well as vulnerability of the Kenya savanna in the light of shifting land use systems as well as climate variability.

Mature *A. tortilis* as well as some *Q. suber* trees considered for the current study did not experience water stress throughout the drought period as a result of deep and elaborate rooting system, enabling access to deep and stable water resources (Le Roux and Bariac 1998). Similarly, gas exchange rates remained high and must have been associated with high carbon gain, and somehow avoiding risks associated with desiccation (Jones 1992). Nevertheless, despite favorable tissue water status, mature *A. tortilis* progressively reduced leaf area (LA) as well as stomatal conductance (gs), while *Q. suber* reduced gs in order to regulate transpiration water loss which was likely to increase as a result of increasing VPD and air temperatures. There is an upper limit to the amount of water that can be conducted through the xylem without disturbing the transpiration stream (Sperry et al. 2002), and

transpiration can only exceed root water supply within a limited range if the leaves are to remain alive (Jackson et al. 2000). Stomatal regulation observed in both *Q. suber* and *Acacia* species as well as leaf shedding in *Acacia,* therefore, protect xylem integrity (Tyree and Sperry 1989, Jackson et al 2000). Manipulation experiments conducted to determine the location and magnitude of resistances along the transpiration pathway indicate that resistances to water flow within the xylem are minimal and major changes in hydraulic as well as stomatal conductance can be initiated by root notching (Meinzer and Grantz 1990). Roots, therefore, have the major role in determining the amount of water extracted from the soil volume at any given time as well as the amount of water transported within the transpiration stream, which in turn determines the plant equilibrium maintained by the stomatal mechanism (Sperry et al. 2002). Since this equilibrium set-point determines the rate of carbon assimilation (other factors not withstanding, see Ehleringer 1994), roots, therefore, must be the main determinants of tree productivity in dryland ecosystems. The coordination between root water uptake and transpiration water loss by the stomata will, however, determine the overall plant water status and the conservation of xylem integrity, which in turn determines drought survival by a given species.

Field and greenhouse results showed that *A. tortilis* and *Q. suber* exhibited dimorphic root distribution patterns, while *A. xanthophloea* had roots only located within the upper soil layers. Differences in rooting patterns among species that are found in different plant communities as well as ecosystem types have several ecological implications. Different rooting patterns will reduce competition among plants especially between species, since it is within the soil that competition is most intense (Larcher 2003). The diversity in rooting patterns, therefore, promotes co-existence of different species as a result of niche separation leading to biodiversity. Dimorphic root distribution patterns exhibited by both *A. tortilis* and *Q. suber* allow the uptake of water resources from upper soil layers when soil water is abundant, while conserving water in the deeper layers for use at a time when water in the upper soil layers is depleted. This extends the period of favorable soil water status and has implications for plant productivity and survival during drought, making them competitively superior. Root water uptake however, progressively shifted downwards, as water became scarce in the upper soil layers. Species with dimorphic rooting patterns are able to take advantage of short rainfall pulses that occur in the middle of a drought as well as percolated water resources in the deeper soil layers (Ogle and Reynolds 2004), promoting survival in drylands as was observed for both *A. tortilis* and *Q. suber*. Shallow

rooting patterns exhibited by *A. xanthophloea* will promote rapid reaction to rain events, but subject the plant to the danger of succumbing to drought shortly after the end of rainfall events, unless there are localized mechanisms that improve soil moisture status in the upper soil layers. This type of rooting system could, however, promote success of this species in flood plains and swamps, where oxygen concentrations are limited in the deeper soil layers and where low maintenance respiration of the root system will mean enhanced aboveground production and growth. These two factors probably explain the main distribution patterns for *A. xanthophloea* in the Kenya savanna, where it is associated with floodplains, within the river valleys and near lake shores. Reports of new establishment in dry areas however, cannot be related to these mechanisms (cf. Discussions on savanna of Kenya).

A potential significant ecological contribution of dimorphic rooting system is the possibility of hydraulic lift (Jackson et al. 2000). As explained previously, hydraulic lift is a nocturnal process where water movement from deep and moist soil layers to shallow and drier soil layers as a result of existing water potential gradient is facilitated by dimorphic root systems (Dawson 1996). Measurement of Ψs as well as isotopic labeling established the presence of hydraulic lift among the *Q. suber* trees, while studies conducted in the E. African savanna have established its presence in *A. tortilis* trees (Ludwig et al. 2003). One major importance of hydraulic lift is that water exuded by shallow roots into the top soil layers during the night can then be reabsorbed into the transpiration stream during the day when transpiration rates exceed the capacity that can be supplied by deep roots (see also Dawson 1996). The buffering effect of the shallow root biomass can greatly promote productivity as well as survival during water stress. Reabsorbing water released into the upper soil layers also improves nutrition of the concerned species, since nutrients are more concentrated within the upper soil layers that in the deeper horizons (Larcher 2003). Substantial amount of water is hydraulically lifted to the upper soil layers by plant species (Espeleta et al. 2004). In this study, the amount of water that is hydraulically lifted was not quantified however, there is evidence that substantial amount of water is hydraulically lifted and that this water can be reabsorbed by members of the same or different species, which lack this capability and can significantly increase their productivity and survival during drought (Dawson 1996, Ludwig et al. 2003, Espeleta et al. 2004). In the savanna of E. Africa, it is already established that *A. tortilis* can lift and exude between 75 and 225 litters of water to an area of around 300 m^2 (Ludwig et al. 2003). Analysis of isotopes of xylem water indicated that grasses growing around *A. tortilis* take up

hydraulically lifted water (Ludwig et al. 2003, 2004): Thus, *A. xanthophloea* could equally take advantage of this hydraulically lifted water by trees of *A. tortilis* on the study site, during drought. This could be a contributing factor to the spread of *A. xanthophloea* into drier areas of Kenya also, since it is always found interspersed with other species such as *A. tortilis,* which have already been proven to hydraulically lift water to the surface. Hydraulic lift, therefore, makes a significant contribution to individual trees, to particular species, and to communities as well as to ecosystem functioning, and species that can hydraulically lift will promote dryland species diversity.

Plants are equally able to redistribute water from the upper to the lower soil layers soon after a rain event as shown in the case of *Q. suber* trees in this study. Similar suggestions have been made by Ryel et al. (2003), and Hultine et al. 2004. By redistributing water from the shallow to deeper soil layers, especially in the case of storms that occur in the middle of drought when VPD is high, plants are able to achieve two things. First, they reduce the rate of water loss through evaporation, since water conducted to the deeper soil layers is not accessible to evaporation. Second, they reduce the potential transpiration rate. By redistributing water over a larger soil volume, the soil water potential of the top wet soil layers is effectively reduced and this equally reduces the water potential gradient between soil and the atmosphere, which is actually the driving force for transpiration. Through this mechanism, plants will moderate the rate of water loss and be able to conserve water from rainstorms for a longer period of time than would actually be the case. The current view therefore, is that root processes are the actual conservators of soil water by plants as opposed to the initial ideology where leaves were regarded as the conservators of soil water. Soil water dynamics through roots must, however, be coordinated to balance between water conservation and nutrient uptake and further investigations are recommended along these lines before conclusive deductions can be drawn.

Both experiments 1 and those conducted in the greenhouse were comparative studies of two different species exposed to similar atmospheric conditions as well as equal soil hydration. The revelation that that *A. tortilis* survived better under water stress than *A. xanthophloea* demonstrates the role of inherent characteristics possessed by different species in any given ecosystem. These qualities must have evolved over time to enable such plants to survive under certain environmental conditions (Chaves et al. 2003). An important information derived from these experiments, however, is the need to identify

and select tree species with superior qualities that could enable their establishment and sustainability in dryland conditions, if restoration procedures are to succeed. Research into molecular processes related to stress tolerance could improve our understanding of the competitive abilities of plant species to survive in certain environments. These are currently in progress (Ingram and Bartels 1996, Bohnert and Sheveleva 1998, Chaves et al. 2003). Simple physiological experiments such as the one undertaken in the current study, however, can still provide valuable information with respect to species selection for aridlands. Field experiments such as the one conducted in Kenya and Portugal during this study are however, expensive, time consuming and somewhat unpredictable since one relies on natural weather conditions to realize experimental objectives. The revelation that responses exhibited by mature trees growing under natural field conditions can be replicated in young plants in the glasshouse, enhances the identification and selection procedures because such experiments can be done independent of natural weather conditions and large numbers of samples can be handled, since potted young plants occupy less space and are easier to work with. This will be a saving in terms of time, money as well as propagules and could greatly improve the success of restoration procedures in dryland ecosystems.

Results from greenhouse experiments showed that subjecting seedlings to repeated stress improved their ability to withstand drought by developing both drought avoiding and tolerant strategies. This has practical implications for the restoration processes in drylands, since seedling preconditioning imparts early drought resistance mechanisms in juvenile tissues and could effectively improve plant success during establishment under natural field conditions. Nursery plants are always supplied with adequate water with the objective of attaining rapid growth to have samples for out-planting irrespective of their final destination. In Kenya, the role of preparing seedlings for planting is undertaken by the forestry department and the Kenya Forestry Research Institute (KEFRI). Seedling preparation is, therefore, centralized and care is rarely taken to prepare propagules according to their destined locations. In the past years KEFRI established the importance of provenance as well as the superior quality possessed by indigenous species in establishing and growing in their local sites (Oballa et al. 1997, Hector and Ryan 1997). This discovery has contributed significantly to the success of restoration procedures such as those in the Kibwezi region (Eastern Province). The current findings could be implemented to design appropriate nursery procedures that will adequately prepare seedlings for out-planting in different regions of the country experiencing varying climatic conditions. The results will be an enhanced ability

of seedlings to cope with prevailing local climatic conditions, allowing flexibility and accelerate the restoration processes with reduced costs and improved success.

In the current study, the role of tree density in determining rate of decline in soil water resources in a *Q. suber* plantation was demonstrated. This in turn influenced the rate of decline in tree water status (drought survival index) during drought. The study also showed how reduced tree density enhanced productivity in one of the *Q. suber* trees (tree *A11*) which continued to grow for an extended period during drought. The role of tree density in determining productivity as well as survival of cork oak stands in the Portuguese montado is already recognized (Ribeiro et al. 2003). Tree density is a factor that must, therefore, be seriously considered during transplanting of the seedlings if success in regeneration as well as optimum gains are to be achieved. This calls for prior investigations to establish optimum densities, which depend on the soil resource base, if possible, before restoration procedures commence. The use of simple management tools such simulation models could enhance this process (Ribeiro et al. 2003, 2004).

Restoration of the ASAL without sound management that will ensure sustainability amounts to nothing. The current findings indicate that the major factor underlying sustainability of the dryland ecosystem is the soil resource. The future capability of the dryland ecosystems to provide services such as forest products (timber, cork, anesthetics, fodder etc) is determined by changes in socio-economic characteristics, land use, biodiversity, atmospheric composition and climate that impact on the soil resource. The numerous interactions between ecosystems, competing land uses and global change call for a quantitative integrated assessment of ecosystem responses and ecosystem potentials together with an analysis of the demand for ecosystem services. Thus, there must be continued impact assessments that comprehensively examine the vulnerability of ecosystem services in the context of ongoing environmental as well as land use changes. Only management that considers potential acclimation of the natural system (notably through structural adjustments) to drought, while accounting for gradually changing site and local demand for services, can achieve long term sustainable production from this fragile ecosystem. Some examples of land use systems from the mediterranean region of Portugal and the savanna region of Kenya are provided to support this view.

The montado of Portugal is regarded as a mimic of the tropical savanna (Joffre et al. 1999). Differences, however, arise in their long-term use and management leading to the differences that are observed in their current state (Joffre et al. 1999, Ribeiro et al. 2003). Management of the montado has in the past focused on the soil resource base, and utilization procedures are those that interfere least with the soil structure, such as cork harvesting and grazing by domestic and small wild animals, but with minimal tillage in the forested areas. Tree harvesting is also very selective and legally regulated with sound scientific backing (Ribeiro et al. 2003, 2004). This integrated management has ensured sustainable use of the montado despite its potential vulnerability. On the other hand, the Kenya savanna received little attention in the past; being only governed by natural forces until very recently (GOK 1986). Fortunately, there was no major encroachment or disturbance as a result of loose sanctions, with many areas being regarded as national parks and game reserves. With increasing population pressure, however, encroachment increased and land use systems changed, leading to serious degradation. The increasing population pressure has also led to decline in the area allocated to national parks. Thus, large animals such as elephants, which used to roam large areas, are now being confined behind small fences. This has led to trampling and compaction of soil as well as destruction of 'key' tree species, which are vital for ecosystem functioning, furthering the degradation process (Cumming 1982).

The International Centre for Research in Agroforestry (ICRAF 1997, 1998) has tried to promote agro-forestry in the savanna of Kenya, citing the long-term stable and useful interaction between trees and the herbaceous layer in the savanna. Previous studies have shown that trees improve the nutrient status as well as hydrology of the soil, promoting productivity of the herbaceous layer (Belsky et al. 1989). Improvement of water status of the upper soil layers by savanna trees is most likely the result of hydraulic lift (see Ludwig et al. 2003 and also previous discussions), which operates on the basis of soil water potential gradients and which is promoted by dimorphic root distribution in both the shallow and deep soil layers. Field studies conducted both in the Kenya savanna and Portuguese Mediterranean ecosystems showed the importance of shallow roots in taking advantage of short rains that occur during drought. This is vital for tree productivity as well as survival during drought. Thus, non-interference with the below ground environment has been the reason for the success and stability of the tree-herbaceous layer interaction in the savanna. Introduction of activities such as farming which break the soil structure leads to elimination of the shallow tree roots, hence the lifted water can no longer be

released back into the top soils to be reabsorbed by other plants such as crops. The end result is loss of trees, since the role of shallow roots is usurped, as well as death of shallow rooted plants, which depend on hydraulically lifted water during drought. This is probably one reason why agroforestry has not realized much success. The point is that aridland systems such as savanna must be managed *in toto* and introduction of practices such as agriculture or intense grazing pressure, which interfere with the soil structure as well as species composition, will only lead to further degradation.

The study recognizes the impact of water stress on plant functioning. During manipulation experiments in the greenhouse, drought was amplified by confining roots within a limited soil volume, and plants died during severe water stress, showing that increased pressure on the soil resource base under natural conditions, probably due to shifts in climatic factors or anthropogenic interventions will result in tree mortality and species loss, hence ecosystem shifts as already reported for the S. African savanna (Rutherford et al. 1999). The importance of the soil environment, which includes both soil structure and below ground plant structure, in determining productivity and stability of the ASAL must be emphasized. The role of biodiversity, particularly the importance of key tree species such as those that conduct hydraulic lift in determining ecosystem functioning is clear. Management of the ASAL must, therefore, take account for all of these interactions as well as consider scenarios related to increased demands in order to achieve long term sustainability.

4.8. Conclusions

In summary, data obtained via monitoring of soil water potentials, tree water use and leaf responses identify the soil environment and root system as key control points, that either allow a programmed behavior of stomata dependent on atmospheric conditions (with built in limits on extraction of water from the soil store), or more strongly regulate stomatal behavior in a direct manner as the soil water store is depleted and transport from the soil to the leaves becomes critical. Given that the root system is more vulnerable to cavitation than the shoot system (Jackson et al. 2000), this type of control system oriented to the soil environment and a coordinated function of shoots controlled by the root system should not be unexpected. Simply said, this means that the determination of canopy conductance, probably both over the short-term and long-term with adjustments in plant development and growth, is strongly dependent on a "plant-based assessment" of soil environmental conditions. Through root

activity such as hydraulic redistribution/lift, plants also have a capacity to modify the soil environment, allowing them to operate with some degree of independence, from the existing natural soil moisture and nutrient conditions.

Chapter Five

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APPENDIX 1

Calculation of sap flux from branch sensors (Stem Heat Balance-SHB)

$Pin = Qr+Qv+Qf$ ....………………………………………......... (1)

Where:

Pin = Electrical power to the heater (W). = V^2/R .... (2)

V = Voltage on heater (V)

Radial heat loss, Qr (W) is calculated as

$Qr = Ksh \, \Delta Tr$. ....(3)

Ksh. = Effective heat conductivity constant of the sheath (W . K^{-1}). Ksh is calculated under conditions of no sap flow.

ΔTr. = radial temperature difference between the surface of the heater and the outer side of the heat flux meter. (K)

Heat loss by conduction in the wood (cellulose etc), Qv is calculated as:

$Qv = A.Kst \, (\Delta Tb - \Delta Ta)/ \Delta Z$. ....(4)

A = Mean x-section of the heated stem area. (m^2)

Kst. = Thermal conductivity of the wood structure. (W $m^{-1}K^{-1}$)

ΔZ = Distance between the ΔTa. and ΔTb. Thermocouple junctions (m)

Kst is taken to be 0.42 for many wood species and 0.54 Wm-1K-1for many herbaceous species.

$Qf = Pin – (Qv +Qr)$

Flow (F) = $Qf/Cp\Delta Tsap$ ($Jg^{-1}K^{-1}$) ....(5)

$\Delta Tsap$ = Temperature difference of the sap below and above the heater.

$\Delta T \, sap = (\Delta Ta + \Delta Tb)/2$...(6)

(Adopted from F.P. Weibel and J.A. de Vos, 1994)

APPENDIX 2:

Mean daily max. branch sap flux/leaf area.

t-Test: Two-Sample Assuming Unequal Variances

S=significat   ns=not significant

	Variable 1(Tree A11)	Variable 2(Tree A12)
Mean	0.831	0.56
Variance	0.077	0.113
Observations	95	95
Hypothesized Mean Difference	0	
df	181	
t Stat	6.051	
P(T<=t) one-tail	4.047	s
t Critical one-tail	1.653	
P(T<=t) two-tail	8.093	s
t Critical two-tail	1.973	

	Variable 1(Tree A11)	Variable 2(Tree D22)
Mean	0.831	0.617
Variance	0.077	0.067
Observations	95	95
Hypothesized Mean Difference	0	
df	187	
t Stat	5.502	
P(T<=t) one-tail	6.12095E-08	s
t Critical one-tail	1.653	

	Variable 1(Tree A13)	Variable 2(Tree A12)
Mean	0.66	0.56
Variance	0.128	0.113
Observations	95	95
Hypothesized Mean Difference	0	
df	187	
t Stat	1.994	
P(T<=t) one-tail	0.024	ns
t Critical one-tail	1.653	
P(T<=t) two-tail	0.048	ns
t Critical two-tail	1.973	

	Variable 1(Tree D6)	Variable 2(Tree A12)
Mean	1.156	0.66
Variance	0.097	0.127
Observations	95	95
Hypothesized Mean Difference	0	
df	185	
t Stat	10.222	
P(T<=t) one-tail	4.974	s
t Critical one-tail	1.653	
P(T<=t) two-tail	9.947	s
t Critical two-tail	1.973	

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1(Tree D6)	Variable 2(Tree A13)
Mean	1.156	0.66
Variance	0.097	0.127
Observations	95	95
Hypothesized Mean Difference	0	
df	185	
t Stat	10.222	
P(T<=t) one-tail	4.974	s
t Critical one-tail	1.653	
P(T<=t) two-tail	9.947	s
t Critical two-tail	1.973	

	Variable 1(A11)	Variable 2(A13)
Mean	0.831	0.66
Variance	0.077	0.127
Observations	95	95
Hypothesized Mean Difference	0	
df	177	
t Stat	3.683	
P(T<=t) one-tail	0.00015	ns
t Critical one-tail	1.654	
P(T<=t) two-tail	0.00031	ns
t Critical two-tail	1.973	

	Variable 1(Tree D6)	Variable 2(Tree A11)
Mean	1.156	0.831
Variance	0.097	0.077
Observations	95	95
Hypothesized Mean Difference	0	
df	186	
t Stat	7.608	
P(T<=t) one-tail	6.720	s
t Critical one-tail	1.653	
P(T<=t) two-tail	1.344	ns
t Critical two-tail	1.973	

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1(Tree A12)	Variable 2(Tree D22)
Mean	0.660	0.616
Variance	0.127	0.067
Observations	95	95
Hypothesized Mean Difference	0	
df	172	
t Stat	0.968	
P(T<=t) one-tail	0.167	ns
t Critical one-tail	1.654	
P(T<=t) two-tail	0.334	ns
t Critical two-tail	1.974	

	Variable 1(Tree A13)	Variable 2(Tree D22)
Mean	0.660	0.616
Variance	0.127	0.067
Observations	95	95
Hypothesized Mean Difference	0	
df	172	
t Stat	0.968	
P(T<=t) one-tail	0.167	ns
t Critical one-tail	1.654	
P(T<=t) two-tail	0.334	ns
t Critical two-tail	1.974	

	Variable 1(Tree D6)	Variable 2 (Tree D22)
Mean	1.1555	0.616
Variance	0.097	0.067
Observations	95	95
Hypothesized Mean Difference	0	
df	182	
t Stat	12.993	
P(T<=t) one-tail	4.923	
t Critical one-tail	1.653	s
P(T<=t) two-tail	9.846	
t Critical two-tail	1.973	s