Stomatal conductance in a North-west European coniferous forest

The effect of environmental factors

By: Leon Mossink
MSc thesis Climate Studies, June 2012
Wageningen University and Research centre, Wageningen, The Netherlands

Supervision by:
Dr. Bart Kruijt
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Table of contents

Abstract

Using mainly open system (leaf scale) measurements, eddy covariance data and sap flow data, the impact of water stress related environmental factors on different forest layers and the total ecosystem is calculated for the Loobos area, Veluwe. Next to this, the contribution of dominant forest layer species to total ecosystem fluxes is measured and compared to ecosystem data. Trends have been observed suggesting that dominant tree -*Pinus sylvestris*- and bush -*Prunus serotina*- species show little response to water stress factors during measured daily cycles. Dominant undergrowth species *Deschampsia flexuosa* however shows a decreasing trend of stomatal conductance with increasing Vapour Pressure Deficit.

Analysis of eddy covariance (ecosystem) data and sap flow (tree) data using the Penman-Monteith equation showed no real decreasing trend of *Pinus sylvestris* on increasing VPD, while the ecosystem did show a decreasing trend, presumably caused by *Deschampsia flexuosa* and evaporation from soil or litter layer. Besides, when up scaling stomatal conductance, photosynthetic rate and transpiration rate using Leaf Area Index and the Lambert-Beer equation (light extinction through canopy), derived from leaf scale measurements; *Deschampsia flexuosa* during diurnal cycles showed a decrease in the afternoon of stomatal conductance and photosynthetic rate on sunny days, while other species remained a stable level throughout the day. Ecosystem measurements here showed a slight decreasing trend, with GPP levels in the afternoon structurally around 50% lower than up scaled leaf measurements. During cloudy days *Deschampsia flexuosa* showed high photosynthesis values compared to other species. The results of this study suggest that a single forest layer can have considerable influence on ecosystem response to water stress or other environmental factors. This can be an important issue in for instance further development of land-atmosphere coupled modeling.

Preface

This thesis research is performed as part of the master Climate Studies at Wageningen University (specialization ESS). I would like to thank my supervisors Bart Kruijt and Frank Sterck for giving me the opportunity to carry out this interesting research and for giving me inspiration, clear advises and ideas throughout the research process. I learned lots about the practical, analytical and technical sides of examining species and ecosystem in relation to their environment. Besides my supervisors, I would like to thank Wilma Jans, Eef Velthorst and Leo Goudzwaard for supporting me with knowledge needed for field work, and providing technical and analytical necessities. Furthermore I would like to thank Jan Elbers, Eddy Moors and Marleen Vermeulen for sharing their knowledge about Loobos with me, and helping me with providing background information needed for interpreting data and up scaling of data (here also special thanks to Bart Kruijt, Wilma Jans and Jan Elbers)

In conclusion, I very much enjoyed working in Loobos, and I hope that this report can be used as input for further research on Loobos or other (comparable) sites.

List of tables

List of figures

measurement days of *Pinus sylvestris* 1st and 2nd year leaves (E), and *Deschampsia flexuosa* and *Prunus serotina* (F).

Fig. 6: gs of all species during three cloudy days (A), and three sunny days (B) plotted against Ψ

Fig. 7: gs plotted against VPD, on 3 cloudy (A,B) and 3 sunny days (C,D)

Fig. 8: Photosynthetic rate of *Pinus sylvestris* (A), and *Deschampsia flexuosa* and *Prunus serotina* (B) after up scaling

Fig 9: GPP graph from eddy covariance dataset

Fig 10: Transpiration rate of *Pinus sylvestris* (A), and *Deschampsia flexuosa* and *Prunus serotina* (B) after up scaling

Fig. 11: Transpiration of ecosystem during three daily cycles (A), and transpiration rate of ecosystem vs. sum of transpiration rate from leaf measurements (B)

Fig. 12: A: Stomatal conductance *Pinus sylvestris* (A), and *Deschampsia flexuosa* and *Prunus serotina* (B)

Fig. 13: Stomatal conductance of ecosystem during three daily cycles (A), and stomatal conductance of ecosystem vs. sum of stomatal conductance from leaf measurements.

Fig 14: gs vs. VPD ecosystem (A) gs vs. VPD *Pinus sylvestris* (B)

Fig. 15: Example of calculating amount of leaves that represent total leaf chamber coverage. In this example, 10 pine leaves have a surface of 3.50 cm^2 which means 6.25/3.50 * 10 leaves would totally cover the leaf chamber surface.

Fig. 16: Correction of A for shading, values expressed in m^2/m^2

1. Introduction

Forests play an important role in the functioning of the hydrological, carbon and oxygen cycle. Forests extract large amounts of water from the soil, while at the same time carbon dioxide (CO2) is taken from the atmosphere for the process of photosynthesis. Oxygen and water are released into the atmosphere as a result of this process. A large part of the world's forests are coniferous forests and are situated on the northern hemisphere. The influence of forests on global climate is significant, as during summer because of photosynthesis on the northern hemisphere CO_2 levels in the atmosphere are reduced with 6 ppm on a total of 390 ppm (NOAA, 2012). During winter CO_2 levels increase by +/- 8 ppm because of low photosynthesis levels, respiration and the attribution of CO_2 to the atmosphere through anthropogenic activity (Fig. 1).
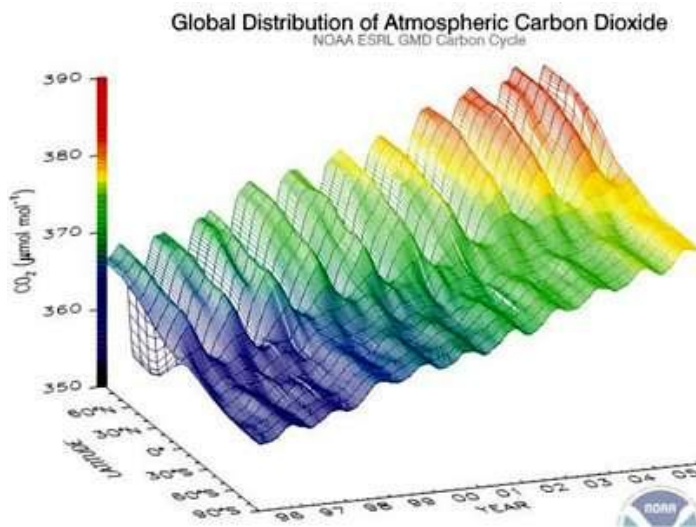
Fig. 1: NOAA Earth System Laboratory, Global Monitoring Division: Global distribution of atmospheric CO_2

The yearly net increase of measured CO_2 in the atmosphere is predicted to cause a variety of changes in climate. Thousands of scientists and dozens of institutes have over the last decades made predictions of the degree of climate change and their impacts. According to IPCC 4ar 2007 for instance, global irradiative forcing and temperature will rise and periods with droughts will occur more in future summers (IPCC, 2007). For the Netherlands, the KNMI predicts that per $1°C$ increase in temperature, summer precipitation will decrease with about 10%. Next to this, precipitation deficit in 2100 has risen in every scenario (5-30%) and extreme droughts will occur more frequently (1.5-5 times more frequent than present) (KNMI, 2011). It is unclear what impact such climate change has on trees and forests, and on fluxes of CO_2 and H_2O.

Changing climate can change the way forests respond in terms of ecosystem exchange and production (Vennetier et al., 2003). Because drought periods will occur more often and will become increasingly severe, research is needed to look at the effects of drought stress related factors on forests ecosystems. Permanent measurements on different types of ecosystems already exist, and provide information on how whole ecosystems respond to changes in climate. However, forest ecosystems consist of various layers of vegetation (f.c. tree, bush and undergrowth).

The different vegetation layers are subject to different circumstances regarding environmental stress factors. For instance; radiation is high in the canopy and through absorption and scattering of vegetation low near the soil. The various plant types and species present in a forest have developed their own strategy in dealing with (changing) climatic conditions and other environmental factors like (soil) hydrology in daily and seasonal cycles (Kosakivska, 2008). There are for example huge differences in plant physiology like rooting system or leaf thickness, which determine how a species actively responds to (sudden) changes in environmental factors. Plants respond to changes in environment mainly through opening and closing of their stomata, which allows gases to enter and leave the plant (Arve et al., 2009). The degree of opening of stomata is expressed in stomatal resistance, or its inverse; stomatal conductance. The changes in stomatal conductance, transpiration and photosynthetic rate together with environmental factors during the day can be used as a measure for determining different plant strategies in relation to (water)stress.

Because climate change will lead to increased precipitation deficit and more frequent and extreme periods of drought, more research is needed on how different layers of vegetation within forests respond to drought (through soil and air). This response can be examined on seasonal- and daily scale, where it can be assumed that species that react strong on drought during a daily cycle are more susceptible to long term drought (Pers. Comm B. Kruijt). Understanding how different vegetation layers respond to environmental factors, and especially water availability, is important for understanding the functioning and the contribution of each species to the total ecosystem exchange. When for instance a certain layer or dominant species responds significantly more on drought than other layers or species, ecosystems species composition- (by competition with other, more drought resistant species) and fluxes (other balance in carbon and water cycle) can change. Therefore the next questions are formulated:

- How do different forest layers respond to water stress factors in terms of stomatal conductance, photosynthesis and transpiration?
- How does this differ between different forest layers, the ecosystem, and the main forest species during daily cycles? And can ecosystem fluxes within daily cycles be predicted with up scaling of ecosystem main species data?

Hypotheses

I expect that grasses and bushes are more subject to water stress because of their relative thin leaves and shallow rooting systems, which make stomata respond fast to changes in water availability. Grasses encounter because of this even more stress than bushes. Trees are more likely to be limited by factors like radiation than water stress factors. First because of lack of shading, latter because they have good access to soil water even in periods of drought. During periods with abundant soil water supply the most important water stress factor for all species is air humidity deficit, because species can respond to dry air with closing their stomata even when enough soil water is present. Water stress from the soil can be the most important factor for bushes and especially grasses in periods of sustaining precipitation deficit, as it gets more difficult for roots to take up enough water to meet the water supply demand for transpiration caused by vapour pressure deficit.

There will be little difference between the response of the ecosystem and the main forest species when the leaf area of the forest consists mainly of the main species leaf area. Ecosystem fluxes can be predicted through usage of information on leaf scale, when estimations are made for contributions to total flux that are unknown (not every species can be measured, just as is the case for ecosystem respiration).

2. System and measurement techniques

Biological and environmental characteristics of forest layers

Forests characteristically consist of a tree, bush and under growth layer. Trees typically invest a large amount of energy in tissue development, which make them grow slow but steady. Because of this they dominate the forest canopy and large rooting systems help them to reach relatively deep into the soil to obtain soil water (although there are exceptions). Leaves within the canopy means exposure to high radiation and temperature relative to the other vegetation layers. This brings benefits and drawbacks, as more light means more available Photosynthetically Active Radiation (PAR), but on the other hand more risk of damage by high radiation and the need for protection mechanisms like hairs and wax (which also decrease leaf temperature). This causes leaves to become less efficient. Deep rooting systems help trees to access soil water, even when the water table is deep below the soil. To have such a rooting system requires investment in tissue building but means resilience to periods of drought.

In the under growth, grasses, mosses and herbs are dominant. They typically do not invest (much) energy in building of 'expensive' tissues like wood cells, which makes them able to grow and reproduce fast under favourable conditions (Kosakivska, 2008). Most under growth species are adapted to grow optimal in the shade of trees and bushes, which prevents them from being exposed to large quantities of radiation, high temperatures- and wind velocity. Drawbacks can be the shallow rooting system that much species have, which causes problems with accessing soil water during times of persistent precipitation deficit (Kramer, 1983), and lower available PAR which causes relatively low photosynthetic rate.

In between of these layers, bush vegetation is dominant. There are bush species that need gaps in the canopy to be able to grow, others are adapted to grow in the shade. The strategy in investment of tissue differs largely from species to species, but in contrast with undergrowth species, bush species can invest in wood vessels and can grow meters in height. This also means that many species can grow rooting systems relatively deep in comparison to undergrowth species, which makes them relatively drought tolerant.

Water flow and gas exchange through plants

Plants regulate water flow mainly by opening and closing of stomata. Most of the stomata are situated on the lower side of the plants leaves. Stomata are protected by guard cells, which have a mechanism to regulate the amount of gas entering and leaving the plant. Water is taken up by roots and is transported through xylem towards the evaporating surfaces (leaves). Evaporation creates negative pressure inside the plant which is the driving force for upwards water movement (Kramer, water relations of plants, 1983). Water is mainly used for turgid pressure which keeps the leaves (and- or other plant parts) firm. A small amount of water is used for photosynthesis. Eventually water leaves the plant through transpiration from the plants stomata. The regulation of water flow is based on mechanisms which respond to environmental factors. In this way, plants usually minimize water loss and maximize CO_2 gain. Under stress conditions the guard cells are triggered to close the pore opening of stomata.

However, there is still much uncertainty about the functioning of processes controlling gs. Research on this topic is needed to better understand these processes.

Stress factors

Factors that cause stress can have for instance environmental, biological, anthropogenic origins, but this report focuses on (water) stress due to soil moisture and air humidity levels (where low air humidity increases the demand for water as transpiration rates increase which can result in plant response, and low soil moisture means lower supply of water which can also result in stomatal response). The influence of the two other most important stress factors radiation and temperature are analysed as well, to determine whether species response is related to water stress only or due to (a combination of) other factors. Radiation is measured as photon flux density in $\mu mol/m^2/s$. Temperature is expressed in °C, soil moisture stress is measured as Leaf water Potential and expressed in MPa, stress due to low air humidity levels is expressed as Vapour Pressure Deficit (VPD) in kPa.

For all these factors, there is an optimal range that differs per plant species. When for instance temperature increases to a value that exceeds the optimum range, it causes gs to decrease. A decrease in gs leads to lower gas exchange and therefore lower H_2O and CO_2 fluxes between vegetation and atmosphere. When all factors are within their optimum range, gs will be maximal, the more there is a deviation from the optimum range for every factor, the more gs will decrease towards its minimum value.

Measuring ecosystem and forest layer performance

How plants respond to their environment can be examined by looking at stomatal resistance, transpiration rate and photosynthetic rate. Stomatal resistance is the degree in which gas exchange between plants and atmosphere through the plants stomata is possible, relative to the maximum exchange possible. In this report; stomatal conductance (the inverse of stomatal resistance, and from now on to be called gs) will be used as a means to determine response to climatic circumstances.

The way plants within different forest layers respond to their environment can be measured using a leaf chamber, which measures gas exchange of individual leaves. At the same time environmental factors like temperature, radiation and air humidity are measured. Gs can be calculated from the amount of transpiration, air humidity and boundary layer resistance of the plants leaf. Besides making use of a leaf chamber, water stress from the soil can be examined by measuring leaf water potential (expressed as Ψ) using a pressure chamber.

Leaf chamber

Within a leaf chamber, gas exchange is measured between the leaf and its environment. An IRGA (infrared gas analyser) system determines variations over time in CO2 level in the air before and after it passes the leaf chamber. Humidity sensors are installed to measure difference in H2O levels. Because a constant chamber size and air speed passing through the chamber is maintained, automatic calculations of values for A,E and gs are possible. Values are expressed typically in mol (or mmol, μmol)/m^2/s. Because the leaf chamber surface is not 1 m^2 but much smaller, gas exchange amounts extrapolated to 1 m^2 of leaf surface using the next equation: gas exchange * 100 cm^2/leaf chamber surface in cm^2. Logically, the amount of air flowing through the leaf chamber per unit of time is considered when

determining A,E and gs rates, because difference in the amount of air flow per unit of time influences the difference in gas concentration measured per unit of time after air leaves the leaf chamber.

Transpiration rate (E) is a measure for the amount of water in gaseous form leaving the plants leaf surface per unit of time. It is typically expressed in $mmol/m^2/s$ and is calculated by first multiplying differential in water vapour concentration between plant and environment by mass flow per square meter of leaf area, and then dividing the outcome by current atmospheric pressure (LC Pro+ user guide).

Photosynthetic rate (A) is a measure for the amount of photosynthesis by the plants leaf surface per unit of time. It is typically expressed in $\mu mol/m^2/s$ and calculated from the difference in CO2 concentration entering and leaving the leaf chamber multiplied by the mass flow of air per m2 of leaf area (LC Pro+ user guide).

Gs is the inverse of stomatal resistance, whichcan be calculated using a set of parameters such as saturated water vapour concentration at leaf temperature, differential in water vapour concentration (in and out leaf chamber) and boundary layer resistance of the plants leaf to water vapour. Gs is expressed in $mol/m^2/s$. The equation used for calculating gs can be found in the LC Pro+ user guide.

Pressure chamber

Leaf water potential can be used as a measure to determine the amount of water stress from the soil a plant experiences. A common way to measure Ψ is making use of a pressure chamber which is a chamber that can be filled with a non-explosive gas like nitrogen. Inside this chamber, a plant sample (branch with some leaves) is fixed, with only the cut-end of the branch emerging from the chamber. Then pressure is increased inside the chamber until water emerges from the cut-end of the branch. With this technique, the negative hydrostatic pressure (or tension) that exists in the plant sample is measured, which ranges from 0 MPa when there is absolutely no water stress to up to -6.0 MPa measured in some desert plants (Jones, 1992). It is assumed that the pressure is about the same for the whole branch. The pressure needed therefore equals the tension that existed in the sample before it was cut. (Raven et al., 2003) Ψ is typically expressed in MPa.

Ecosystem exchange and sap flow

At many sites around the world, ecosystem exchange is measured making use of the eddy covariance technique. Ecosystem exchange with this technique is measured per unit of land area, whereas measurements on leaf scale measures exchange per unit of leaf area. To measure meteorology and ecosystem exchange, equipment is installed on top of a tower on-site (which preferably extends several metres above the canopy). Such a system can for instance permanently measure wind speed in all directions, CO_2 level and concentration, air humidity and -temperature. From this information fluxes can be calculated, as a flux is for example the covariance between wind and CO_2, which can be expressed in $mol/m^2/s$ (Baldocchi, 2001). This information is therefore largely comparable to measurements on leaf scale.

Sapflow measurements are performed with a technique making use of a heater and thermocouple that are placed in the trees sapwood. The registered temperature will be constant as long as the tree with installed sap flow measurement technique does not transpire. When transpiration takes place, sap flow is initiated, leading to declining registered temperature as the sap passes the heater. This change in registered temperature is used to calculate the total amount of sap flowing through the tree's stem, which can be used as a measure for the amount of transpiration by the tree (Smith and Allen, 1996; Raven et al., 2003).

With this technique, daily cycles of sap flow are monitored throughout the year. Besides, with this information transpiration rate and gs during the day can be calculated for a single species within a forest (Granier, 1986). However an estimation of the average sapwood area within a tree stand is needed to calculate this. To compare transpiration rate and gs derived from sap flow data, it must be taken into account that sap begins to increase its velocity in the morning first in the twigs, and then in the trunk (Raven et al., 2003). Because sapflow is measured in the trunk, there is a time lag that must be considered when comparing sapflow data with data derived from other instruments. Sap flow can be expressed in both Liters/30 minutes, and watt/m^2.

3. Study object and research strategy

Study object

To examine water stress in different layers of vegetation within a forest ecosystem with, it has been chosen to use the Loobos area as research object. Loobos is a North-West European coniferous forest and research site. It is located on the Veluwe, near Kootwijk at 52.09'59.34" N and 5.44'36.79" E. The predominantly Scots Pine (89% coverage) forest was planted around 1900 on a sandy soil, with a water table of 3.5 to 6.5 metres deep depending on the surface fluctuation. Some 3.5% of the forest consists of open area dominated by grasses, mainly *Deschampsia flexuosa*. A closed cover of this species also forms the undergrowth of the forest. (Climateexchange.nl, 2012) Main bush species is *Prunus serotina*.

For years, information is gathered regarding the functioning of this forest as an ecosystem using permanent measurement systems such as eddy covariance. There has also been some research on for instance soil and under storey plant respiration and carbon fluxes. However, little is known about the individual vegetation layers and their dominant species in relation to their production and response to environmental (water stress related) factors on a daily basis.

Ecosystem and dominant plant species

In this ecosystem there are three distinct vegetation layers, namely: tree, bush and undergrowth. The canopy arises 15-25 metres above the soil, bushes are approximately between 1-3 metres in height and undergrowth vegetation is mostly <15 cm above the soil.

The three typical layers of vegetation are all dominated by a single species. The tree layer consist mostly (>90%) of *Pinus sylvestris* (Scotts Pine). This species has a shallow root system (1-2 metres), with deeper tap roots in deep soils (Čermák, 2007). The average root depth maximum in Loobos is 3.9m (Moors, 2012). The leaves are adapted to high radiation frequencies and dominate the canopy.

Prunus serotina (Wild Cherry) is the dominant bush species. It has a spreading root system which is mostly located in the upper 60 cm of the soil, and occasional sinker roots of about 1m deep. The leaves are broad and thin. It is an opportunistic (and exotic) species which is becoming increasingly dominant within Loobos. Main undergrowth species is *Deschampsia flexuosa*, which with 90% of the root system found in the first 30 cm of the sandy soil in Loobos (Moors, 2012) is excellent in quickly retrieving (and storing, in the upper few centimetres) of water. However, during periods with excessive drought this characteristic makes this species vulnerable to water stress, as the top soil layers are depleted from water firstly. Grasses like *Deschampsia flexuosa* can grow fast under under favourable conditions because they do not need to invest much in long lasting tissue like for instance trees. The drawback is fragility and low production under less favourable conditions. Besides these main species there are small amounts of other trees, bushes and grasses growing in Loobos (Annex 1, LAI). Between the grasses grow mosses with approximately the same amount of biomass as *Deschampsia flexuosa* (Annex 1, LAI)

Field work

Leaf types and measuring strategy

To answer the research questions, field work is performed during summer and existing datasets are used. Field work is done to obtain information on how different forest layers respond to (water) stress factors by measuring at leaf scale, while eddy covariance- and sap flow datasets are used to calculate ecosystem and the forests main species response on water stress factors. Next to this, diurnal cycles of gs, A and E can be reproduced using field work and permanent measurement data sets. Field work is performed on sunlit leaves of the three dominant species of each forest layer in Loobos; *Deschampsia flexuosa*, *Prunus serotina* and *Pinus sylvestris*. *Pine* trees that have been measured are individuals standing in canopy gaps, rather than individuals which are part of the upper canopy. Because *pine* trees during summer have sunlit leaves with different ages (leaves of current and previous year, from now on to be called 1st and 2nd year leaves), both have been measured during field work.

Gs, A, E, and ψ measurements during daily cycles are performed in ambient conditions (which means without controlling of environmental factors except air flow through the leaf chamber). This has been decided partly because total ecosystem measurements are performed without controlling environmental factors, which makes leaf scale- and ecosystem data comparable.
Measuring throughout the day in ambient conditions can give good insight in how plants react on different stress factors, which is needed to answer one of the research questions. Besides, to reconstruct ecosystem behaviour from leaf scale information requires a Leaf Area Indexation (LAI) to determine the contribution of each species to the total ecosystem fluxes.

Daily cycles and routine

To obtain information on how the dominant plant species in Loobos perform during the day it has been chosen to measure each species during at least ten daily cycles. The measuring cycle starts every day from the moment the sun shines on at least two (predetermined) individuals of each *Pinus sylvestris* and *Prunus serotina*, and when the condensed water on their leaves has evaporated (which is approximately between 9:30 and 10:30 AM). Latter is needed to prevent large measurement errors, which occur when condensed water enters the leaf chamber, resulting in malicious air humidity, evaporation and gs rates. This also means that measurements could only take place during days without precipitation. In the afternoon, the measurement cycle is ended on the moment that there is no direct sunlight shining on at least two individuals of each species anymore (approximately around 16:45 PM). Measurements on leaf scale are consistently performed on sunlit leaf type and - when possible - in direct sunlight, which means without diffusion of incoming radiation by leaves, branches or clouds.

For every species, and leaf age (*Pine*), four measurement plots have been selected. This was for *Pinus sylvestris* the maximum amount of individuals in full sunlight during field work days. Plots of *Pinus sylvestris* consist of one individual, with at least two branches situated in full sunlight during the daily measurement cycles. It has been chosen to measure individuals standing in canopy gaps rather than in the upper canopy. Not only was this more practically executable because more individual measurements can be performed throughout the day, these were also the only individuals nearby that could be

measured in full sunlight during the whole daily cycle. To make sure ψ values are comparable throughout the day, and other daily cycles, it has been chosen to consistently measure leaves at the end of branches that originate from the tree at 2 metres above the soil. Every of the four individuals is measured once per two hours (the duration of a measuring cycle) at both 1st and 2nd year leaves. Also, each of the four individuals had 2 predetermined (sunlit) branches, of which the leaves are always measured alternately. This means that for each leaf age every day a maximum of around (4 individuals * 2 branches * 4 measurement cycles=) 32 recordings were possible.

The same routine has been performed for *Prunus serotina*, only the height at which the measured branches originate is around 1 meter, and two different leaves have been measured at each individual. Selecting from branches originating at 1 meter height is done so because average height of *Prunus serotina* in Loobos is around 2-3 meters. Plot sites of *Deschampsia flexuosa* are selected by first determining which surface areas are in full sunlight during a daily cycle, and then randomly selecting four plot sites (consisting of approximately 20 cm^2 grassland) from this larger area. It was not possible to measure eight different sets of exactly the same leaves at the four individual plot sites with every measurement round, because risk of leaf damage during multiple measurements on the same leaves. Therefore every day alternative plot sites were used near the originally used plot sites.

Conditions

In this report, measurements are used that have been collected during six full days. During 3 of these days the sky was completely clear, so these days are from this point on referred to as 'sunny' measurement days. During the other three days the sky was partly cloudy, so these days are in this report called 'cloudy' days. Cloudy days typically had a VPD between 1 and 2.5 kPa, a relatively low temperature and large fluctuations in PAR. On cloudless days PAR was abundant, and VPD variable. The most important specifications of these two groups of days are expressed in table 1.

Parameter	Cloudy	Cloudless
Temperature leaf chamber	22-27 °C	+/- 33 °C
Temperature top of flux tower	+/- 20 °C	25-27 °C
Wind speed	2-4 m/s	0.5-2.5 m/s
PAR	50-1800 μmol/m2/s	1200 -1800 μmol/m2/s
VPD	1-2 kPa	1-4 kPa

Table 1: Environmental circumstances measurement days

LAI

To be able to answer research question 2, a Leaf Area Index has been determined. This is to determine the contribution of each species to total ecosystem fluxes and gs, the leaf surface in m^2/m^2 for each species must be known. Leaf Area Index has been performed for dominant bush species *Prunus serotina* and dominant undergrowth species *Deschampsia flexuosa*. This research can be found in Annex 1. Values are expressed in m^2/m^2 (which means m2 of leaf area per m2 of ground surface), and are 0.1 m^2/m^2 for *Prunus serotina*, and 0.46 m^2/m^2 for *Deschampsia flexuosa*. LA of *Pinus sylvestris* is approximately 2.8 m^2/m^2 (Alterra, 2008).

Besides this, leaf chamber surface coverage is corrected for *Deschampsia flexuosa* and *Pinus sylvestris*, because 100% leaf chamber coverage could not be realized for these species, while for *Prunus serotina* this was possible (Annex 2).

Permanent measurements

During field work days, the eddy covariance system and sap flow measurements (latter on *pine* trees only) were performed automatically, providing diurnal courses with measurements every half hour. The eddy covariance system in Loobos has a footprint of 1 km in all wind directions (Pers. Comm. W. Jans), and a maximum flux contribution distance of 300 meter (Alterra, 2011), which means that under every weather condition possible, gas exchange of the system can be measured with a maximum of 300 meter from the flux tower. With eddy covariance measurements ecosystem, A and E are measured and at the same time temperature, air humidity, wind velocity, aerodynamic friction and soil moisture. Gs can be calculated from this information. This information is needed to examine the ecosystems fluxes and to compare reaction of ecosystem, dominant forest species and *Pinus sylvestris*'s on (water) stress factors during the day.

Sap flow measurement data exists of information gathered from six trees and is expressed in both Liters/30 minutes, and watt/m^2. A study has been performed to estimate the total sapwood area per ha for Loobos (Soudant, 2009). The amount of sapflow calculated using sapflow measurement equipment (expressed in sapflow per cm^2) is multiplied with the estimated amount of sapwood per ha, which on its turn is derived from sapwood area in cm^2 multiplied by measured diameter at breast height (DBH) of *Pinus sylvestris* in Loobos (Pers. Comm. J. Elbers). This sapwood area is multiplied by the calculated amount of sapflow, resulting in a *pine* tree stand level transpiration rate. This transpiration rate is expressed in watt/m^2, and is then time lag corrected (2.5 hours).

Calculating VPD and gs

Vapour Pressure Deficit (VPD) is one of the two water stress factors which is examined in this research. VPD is caused by the difference between air humidity and saturated humidity- (an estimation of leaf humidity) at air temperature, and can be calculated from air temperature and humidity (Annex 4).

gs is not measured permanently with eddy covariance and sap flow measurements, but can be calculated using information provided by these systems, which are used as input for the inverse Penman-Monteith equation (Annexx 4). Using this equation makes comparing between gs on species and ecosystem scale possible.

4. Results leaf scale measurements:

The results displayed and described in this chapter are all based on datasets containing data of the three species and four leaf types described under the heading "Leaf types and measuring strategy" in the previous chapter. This data is gathered during six days of field work, separated in two categories (sunny and cloudy). First the environmental factors during sunny fieldwork days are analysed, then the responses of examined species and leaf types. After this, results gathered during the three cloudy field work days are described.

Environmental circumstances sunny days

As is explained in chapter 2 there are four environmental factors that vary on a daily cycle which are mainly responsible for regulating stomatal conductance, and therefore photosynthetic- and evaporation rates. To examine whether there is stress due to low soil moisture level, ψ is measured. This is not an environmental factor itself, but can be used as an indicator of water stress due to low soil moisture availability. In this section, variability throughout the daily cycle in environmental factors and gs, A, E is shown of 28, 30 September and 1 October. All measurements have been performed using a leaf chamber, which means that measured values are specific for the leaf or leaves in the leaf chamber at the specific time and location of each particular measurement.
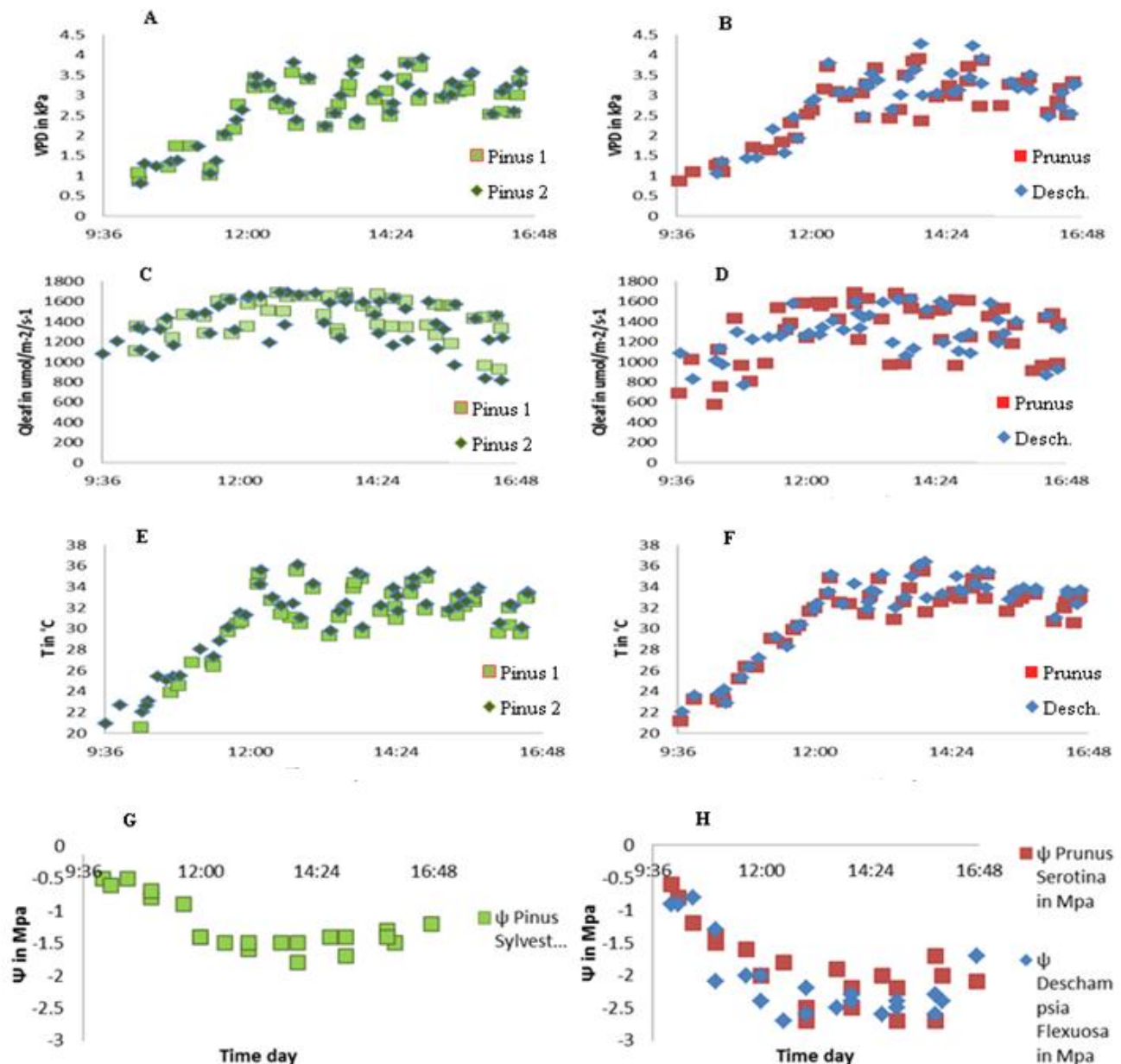
Fig. 2: A, B: Diurnal courses of vapour pressure deficit measured for all leaf types C, D: Diurnal courses of incoming radiation measured at all leaf types E, F: Diurnal courses in temperature in leaf chamber at all leaf types G, H: Diurnal courses in Leaf water potential of all leaf types except *Pinus sylvestis* 2nd year leaves.

As can be seen in fig 2; A,B and E,F; Diurnal courses in leaf chamber temperature (from now on to be called Tch) and VPD are quite similar for every leaf type during these sunny days. There is a small difference in incoming radiation C,D; between *Pinus sylvestris* and the other leaf types, mainly because of plot site positioning. This is because at the beginning and end of each daily cycle it was more easy to

measure leaves of selected individuals of *Pinus sylvestris* in direct sunlight than leaves of other individuals.

There is a large difference visible in Ψ; fig x G,H; between *Pinus sylvestris*, *Deschampsia flexuosa* and *Prunus serotina*. Whereas branches of *Pinus sylvestris* during the day slowly reach a value of about -1.5-
-2 MPa, *Deschampsia flexuosa* has reached -2.5 already at noon. *Prunus serotina* shows about the same curve and maximum values as *Deschampsia flexuosa*.

Responses

In the previous section the changes in environmental factors during the daily cycles were shown. Next, the responses in gs, E and A for all measured leaf types will be analysed. All values are measured on leaf scale, and are expressed in m^2 of leaf area (so not per m^2 of land surface area as with sap flow and eddy covariance data). In chapter 5 the outcomes of the measurement results in this chapter are used for scaling up to stand level.

Fig. 3: Diurnal courses of stomatal conductance during 3 sunny measurement days of *Pinus sylvestris* 1st and 2nd year leaves (A), and *Deschampsia flexuosa* and *Prunus serotina* (B). Diurnal courses of photosynthetic rate during 3 measurement days of *Pinus sylvestris* 1st and 2nd year leaves (C), and *Deschampsia flexuosa* and *Prunus serotina* (D). Diurnal courses of transpiration rate during 3 measurement days of *Pinus sylvestris* 1st and 2nd year leaves (E), and *Deschampsia flexuosa* and *Prunus serotina* (F).

As can be seen in Fig 3; (A) gs of *Pinus sylvestris* remained about constant throughout the measurement days. The same holds for *Prunus serotina* (B), while *Deschampsia flexuosa* shows a significant decrease in gs during the day. A follows the same trends as gs (C,D), while E shows the same pattern as VPD, with an increase during the morning and more or less stable values in the afternoon (E,F).

Environmental circumstances cloudy days

In annex 3, fig. 4 A,B; an increase in average VPD is seen during the morning. During the afternoon, there is a small decrease again. During these days VPD maximum values are significantly lower than during sunny days. B,C show that there is huge scatter in radiation, this is caused by clouds blocking direct sunlight. D,E show that the temperature during cloudy measurement days remains lower than during sunny days, but also here a large scatter can be seen.

Responses:

As expected from the large scatter in photosynthetically active radiation incident (from now on to be called Q), there is a large variation in A for all species during cloudy days Fig. 5: C,D. E is relatively low for all species except *Deschampsia flexuosa*, which also has relative high gs levels compared to the other species. gs and E levels in general are quite stable, which can be explained because of the slow response time of stomata to changing levels of radiation, and the fact that the other environmental factors are quite stable throughout the day.

From leaf scale measurements during sunny and cloudy days can be concluded that *Deschampsia flexuosa* during cloudy days performs better than the other species. On sunny days however, during the afternoon *Deschampsia flexuosa* seems stressed, which leads to stomatal closure. *Pinus sylvestris* and *Prunus serotina* do not show signs of stress, as gs and A remain stable throughout the day and E rises in the morning to stable levels in the afternoon.
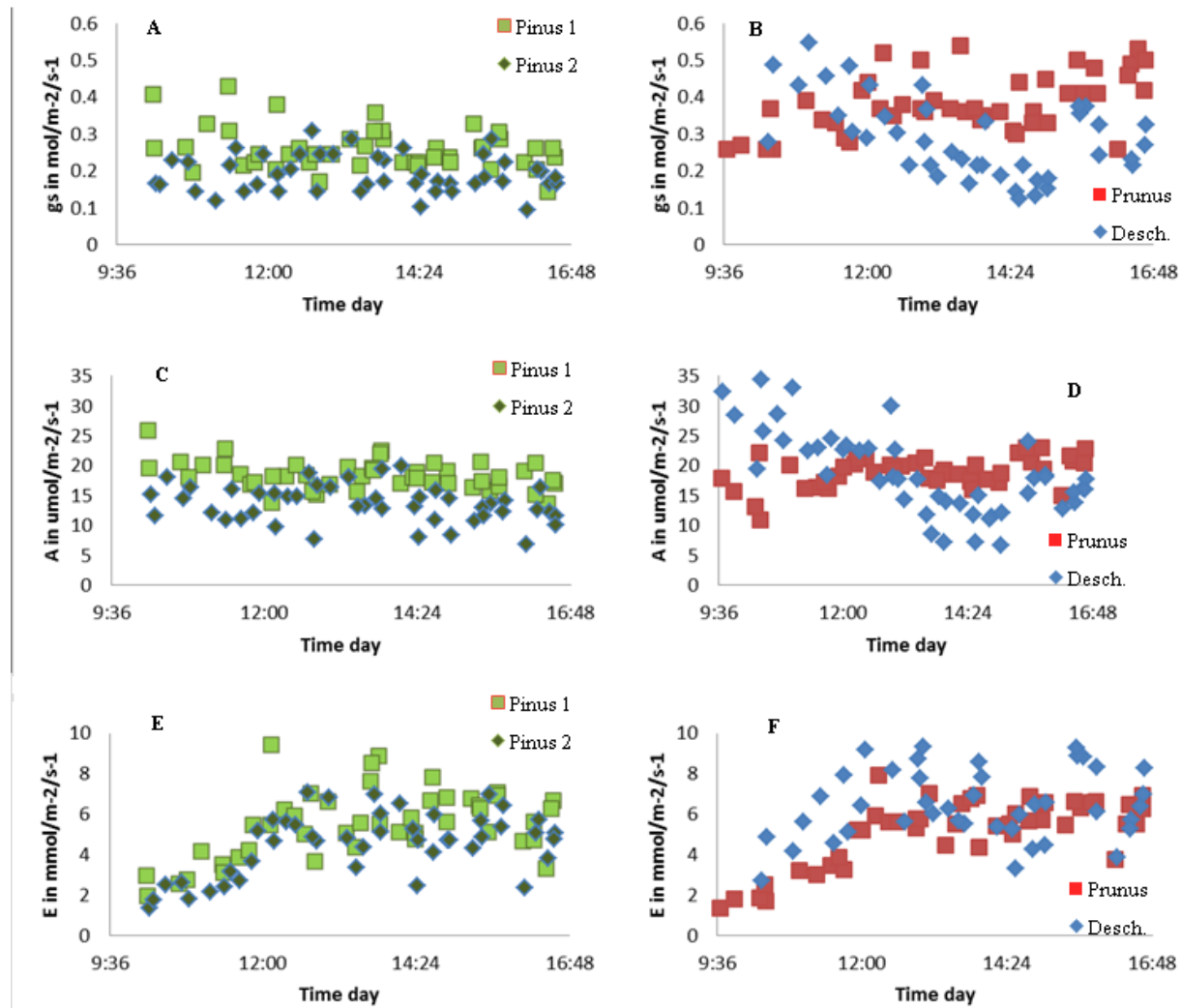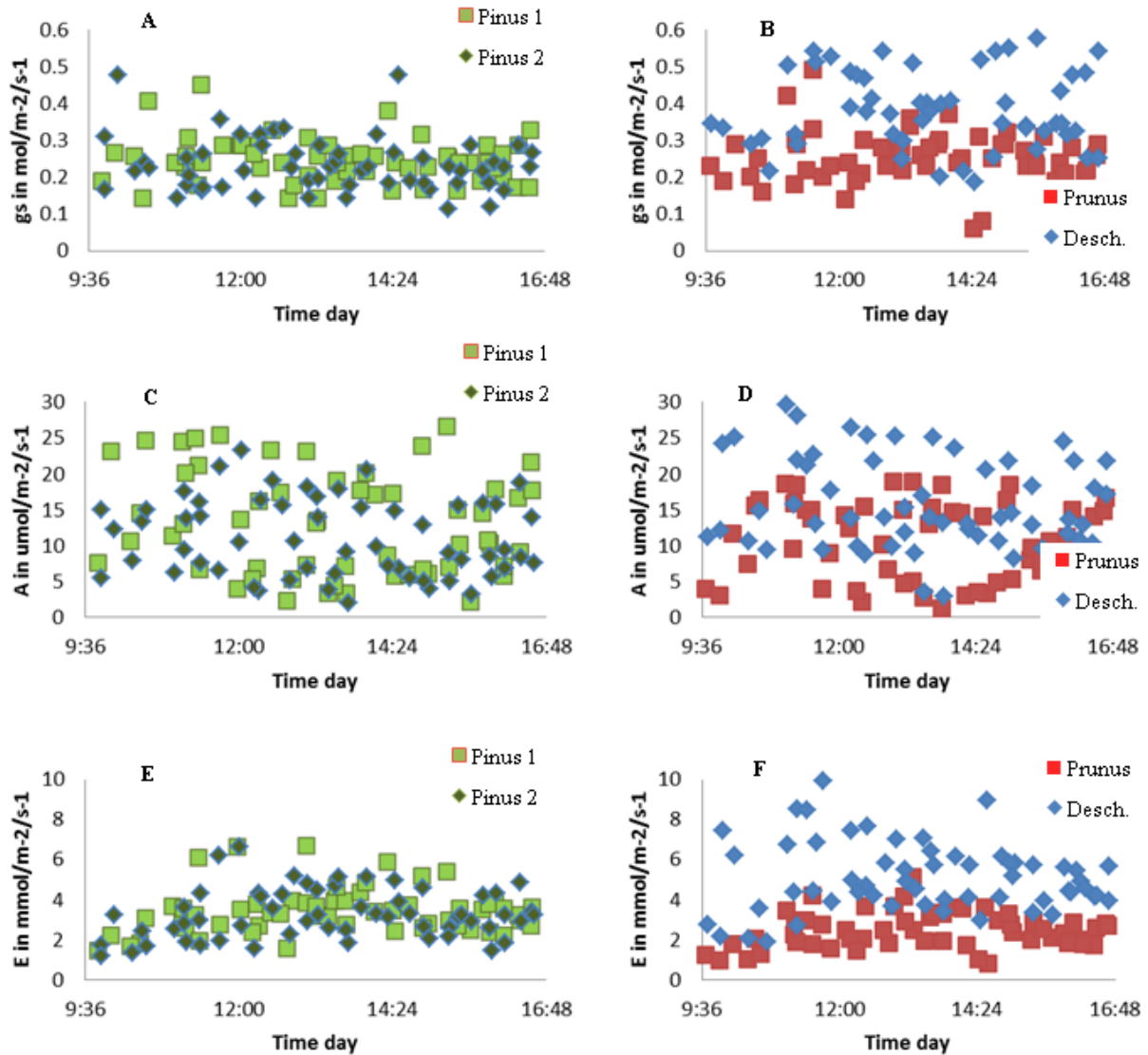
Fig. 5: Diurnal courses of stomatal conductance during 3 cloudy measurement days of *Pinus sylvestris* 1st and 2nd year leaves (A), and *Deschampsia flexuosa* and *Prunus serotina* (B). Diurnal courses of photosynthetic rate during 3 measurement days of *Pinus sylvestris* 1st and 2nd year leaves (C), and *Deschampsia flexuosa* and *Prunus serotina* (D). Diurnal courses of transpiration rate during 3 measurement days of *Pinus sylvestris* 1st and 2nd year leaves (E), and *Deschampsia flexuosa* and *Prunus serotina* (F).

Relations responses to environmental factors

Now that daily trends of environmental factors and gs, E and A are displayed, the next step is to examine what are the causes for these trends. In other words: What are the controlling factors in regulating gs, and therefore A and E? To find an answer for this question, gs for every leaf type has been plotted against the four main controlling environmental stress factors: Q, Tch, LWP and VPD in the leaf scale measurement data sets. It has been chosen to show only examples of these plotted responses of gs to water stress factors in this report, all other graphs can be found in the datasets considering 6 days of leaf scale measurements in Loobos.

Radiation

From data analysis, all species show stable gs levels when Q is >200 µmol/m^2/s. No evidence of gs decrease due to high Q values has been found.

Temperature

For *Pinus sylvestris* 1st year leaves and *Prunus serotina*, gs remains stable with increasing Tch. *Pinus sylvestris* 2nd year leaves show a small decrease with temperatures >32 °C. The significance is however unclear because of the small amount of measurements (<10) that this trend consists of. *Deschampsia flexuosa* shows a decrease in gs when leaf chamber temperature is >28°C, plotting leaf chamber temperature against VPD however shows a linear increase in VPD when temperature increases. This means that with all measurements performed during high VPD, leaf chamber temperature was high as well. During days with stable VPD levels, plotting gs against temperature did not show a decreasing or increase in trend.

Leaf water potential (Ψ)

Pinus sylvestris 1st and 2nd year leaves show a stable gs with increasing Ψ. gs of *Prunus serotina* increases when LWP increases. This implies that gs of these three leaf types is not decreasing with increasing Ψ. *Deschampsia flexuosa* seems to show a decrease in average gs when LWP increases, there is however a large scatter in LWP measurements of *Deschampsia flexuosa*, with also high values of gs at high Ψ level. Therefore it is difficult to suggest an observable downwards trend in gs with increasing Ψ (Fig. 6 A,B).
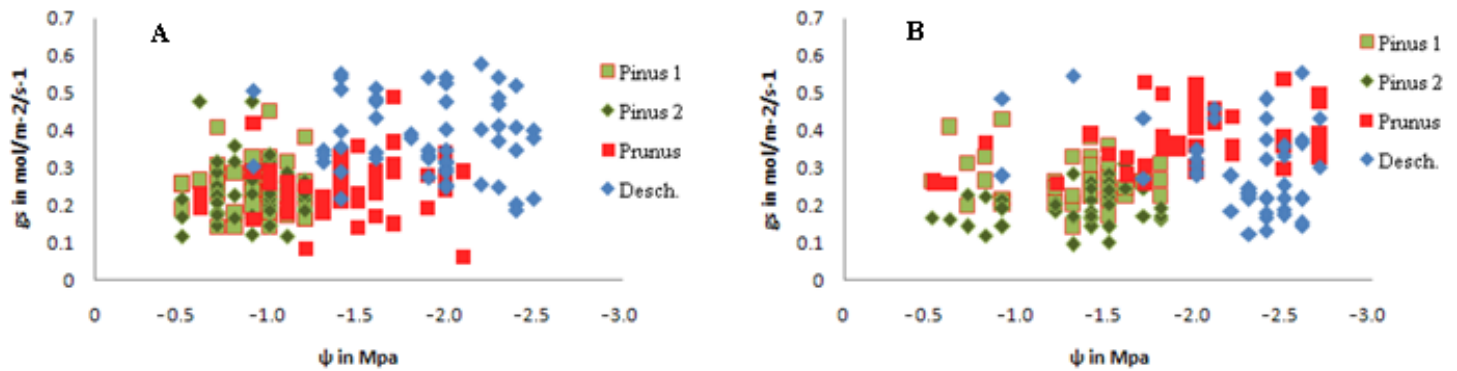
Fig. 6: gs of all species during three cloudy days (A), and three sunny days (B) plotted against ψ.
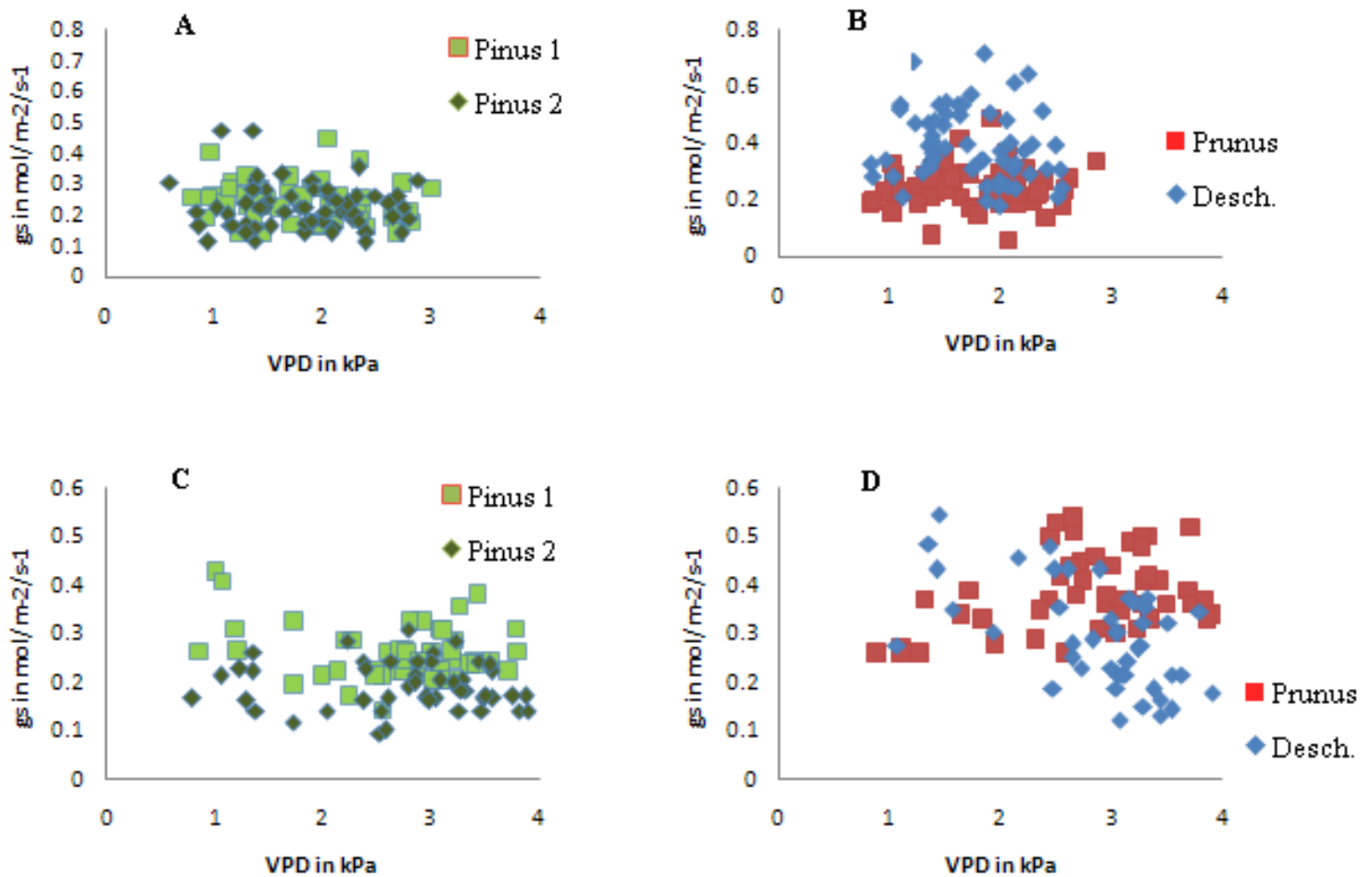
Fig. 7: gs plotted against VPD, on 3 cloudy (A,B) and 3 sunny days (C,D).

Vapour Pressure Deficit

Fig. 7 shows that when VPD increases gs of *Pinus sylvestris* remains stable (A,C), during both sunny and cloudy measurement days. This accounts for *Prunus serotina* as well (B,D). The only species that shows a clear trend is *Deschampsia flexuosa*, which shows a decrease in gs with a VPD of >2.5 kPa (D).

From the displayed data in this chapter can be concluded that there are no observable trends for all species assuming water stress, except for *Deschampsia flexuosa*. This species shows a decreasing trend in gs with increasing VPD. It is also possible that ψ influences gs of *Deschampsia flexuosa*, this however cannot be proven with interpretation of available data.

5. Results Up scaling leaf scale to stand level, and water stress response on stand level

Introduction

In this chapter, with up scaling measurements on leaf scale are used to make predictions about ecosystem gs, A and E. The leaf-scale responses of gs on water stress factor VPD can also be compared with ecosystem-scale response derived from eddy covariance data, and the response of *Pinus sylvestris*, derived from sap flow data using 'the big leaf' approach (Annex 4). This approach considers an ecosystem or forest layer to consist of one big leaf layer which responds to its environment uniformly. With this method canopy VPD and ecosystem gs can be calculated from eddy covariance data. Sap flow data recalculated to transpiration rate (Annex 4) of *Pinus sylvestris* is used to calculate gs of *Pinus sylvestris* as a separate species, while VPD values derived from eddy covariance data is used for *Pinus sylvestris* as well as for ecosystem response. This is done because in this way a comparison between the ecosystem and *Pinus sylvestris* in terms of response in gs on changing VPD is possible. It has been chosen to make a comparison on only gs versus VPD for ecosystem and *pine* tree data because no signs of stress caused by exceeding temperatures, shortage of sunlight or low soil moisture levels have been observed.

For up scaling from leaf scale to ecosystem scale, a LAI research has been performed to determine Leaf Area (LA) for *Prunus serotina* (0.1 m^2/m^2) and *Deschampsia flexuosa* (0.46 m^2/m^2) (Annex 1). LA of *Pinus sylvestris* is 2.8 m^2/m^2 (average LA from yearly LAI measurements in Loobos, Alterra, 2008), consisting of approximately 70% 1st year and 30% 2nd year leaves.

In this research the dominant species from each layer in the forest is examined, this means that there are species contributing to ecosystem flux (for instance mosses) that are not included in this research. Total ecosystem respiration is estimated (WUR, 2012), and GPP is calculated by considering day and night observations of NEE separately, and extrapolating night time respiration to day time values using air temperature as controlling factor (Pers comm. J. Elbers; Rocha, 2010).

Up scaled leaf measurement data compared to ecosystem scale data

The specific LA of each of the four leaf types, and influence of shading of leaves on gs, A and E is applied on leaf scale measurement data using the Lambert-Beer equation (Annex 4). The results of applying these features on data of 3 sunny measurement days (28, 30 September, 1 October) are displayed in Fig. 8.
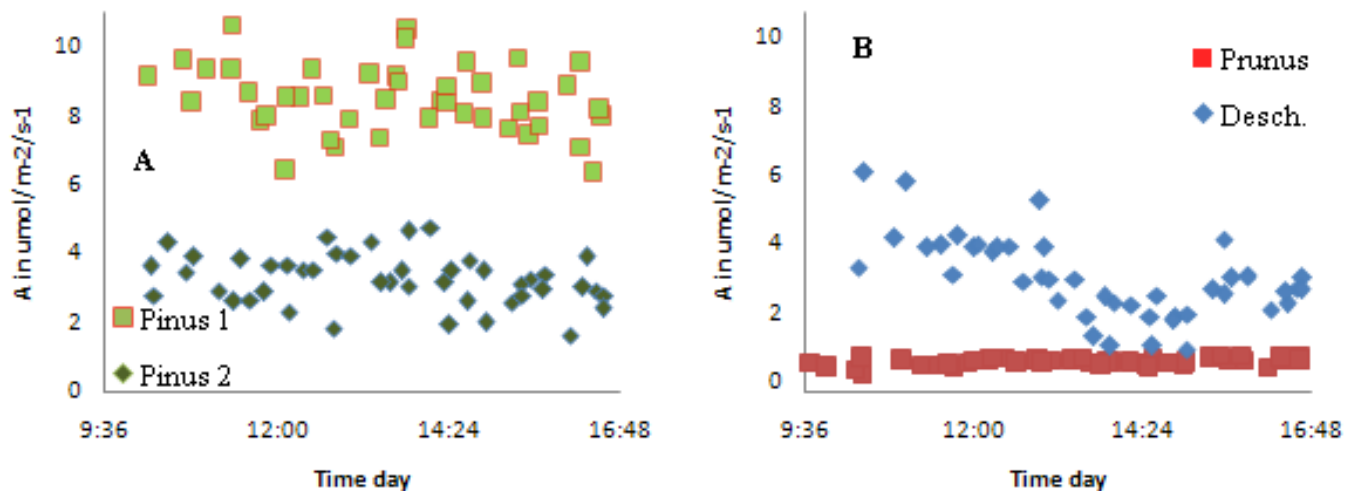
Fig. 8: Photosynthetic rate of *Pinus sylvestris* (A), and *Deschampsia flexuosa* and *Prunus serotina* (B) after up scaling.

Fig 8 shows that Photosynthetic rate is dominated by *Pinus sylvestris* (A), which during the first half of the daily cycle has an A of around 9 + 3.5 = 12.5 µmol/m^2/s. This however is an estimation, as actually adding up of these data points is not possible because all measurements took place on different moments in time, unlike sap flow and eddy correlation data of which data points are created every 30 minutes. Later in the afternoon A values decrease to around 8 + 3 = 11 µmol/m^2/s. *Deschampsia flexuosa* (blue) declines from between 4-6 µmol/m^2/s in the morning to between 1-3 µmol/m^2/s in the afternoon. *Prunus serotina* (B) remains stable around 1 µmol/m^2/s throughout the day (Fig 8 B). When adding these trends up there is a total A (or GPP) of around 18 µmol/m^2/s during the morning and 14 µmol/m^2/s during the afternoon.

In Fig. 9 (A) ecosystem GPP is visualized. GPP varies largely during the morning at levels between 4-14 µmol/m^2/s, in the afternoon GPP levels are around 8-10 µmol/m^2/s . This means that there is an overestimation (Fig. 9 B)of flux totals derived from leaf scale measurements in comparison to ecosystem data, but the overall decreasing trend in GPP during the afternoon is visible when comparing Fig. 9 (A) with Fig. 8. The reason for the higher GPP values in the morning with leaf scale measurements can be a methodology aspect; while measurements on leaf scale were performed in full sunlight throughout the day, the ecosystem receives less radiation during the morning. Hereafter incoming radiation level peaks around 1-2 PM and decreases again in the late afternoon.
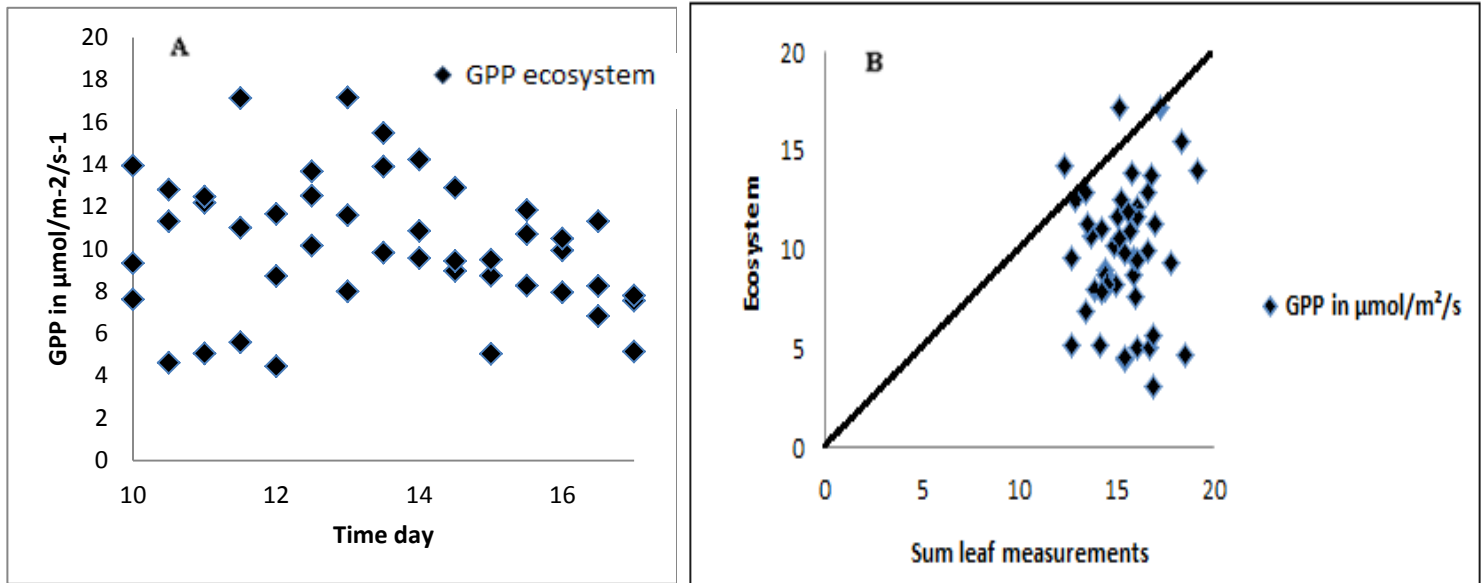
Fig 9: GPP of ecosystem during three daily cycles (A), and GPP of ecosystem vs. sum of GPP from leaf measurements (B).
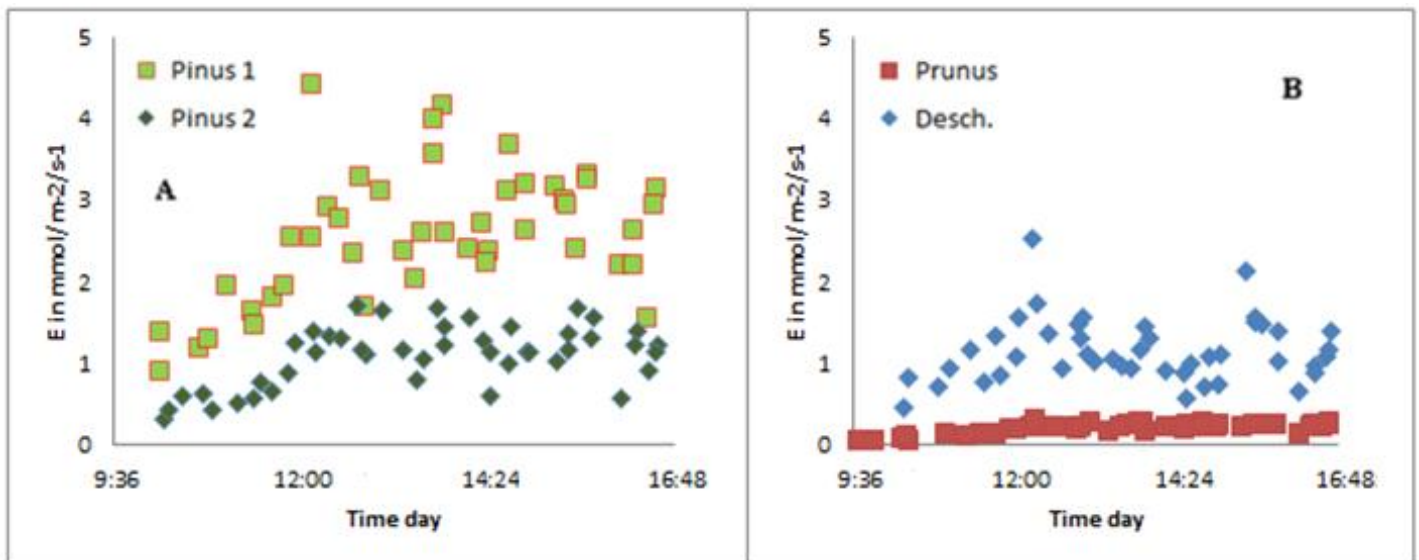
Fig 10: Transpiration rate of *Pinus sylvestris* (A), and *Deschampsia flexuosa* and *Prunus serotina* (B) after up scaling.

Observed up scaled leaf measurement transpiration rates show an increasing trend in the morning for all species (Fig. 10). Maximum E of the four species combined is around 6 mmol/m^2/s, in the late afternoon this decreases to around 4-5 mmol/m^2/s. Ecosystem and tree transpiration rates (Fig. 11) do not show a large increase during the observed morning hours, the transpiration rates are already relatively high around 10:00. Maximum values of ecosystem transpiration are around a third or half as low as up scaled transpiration values of all species combined (Fig. 11 B). The same accounts *pine* transpiration rates derived from sap flow compared combined transpiration rates of first and second year leaves of *Pinus sylvestris*. Next to this, a clear decline in transpiration during the afternoon can be in sap flow measurement data, which is less visible in the other graphs.
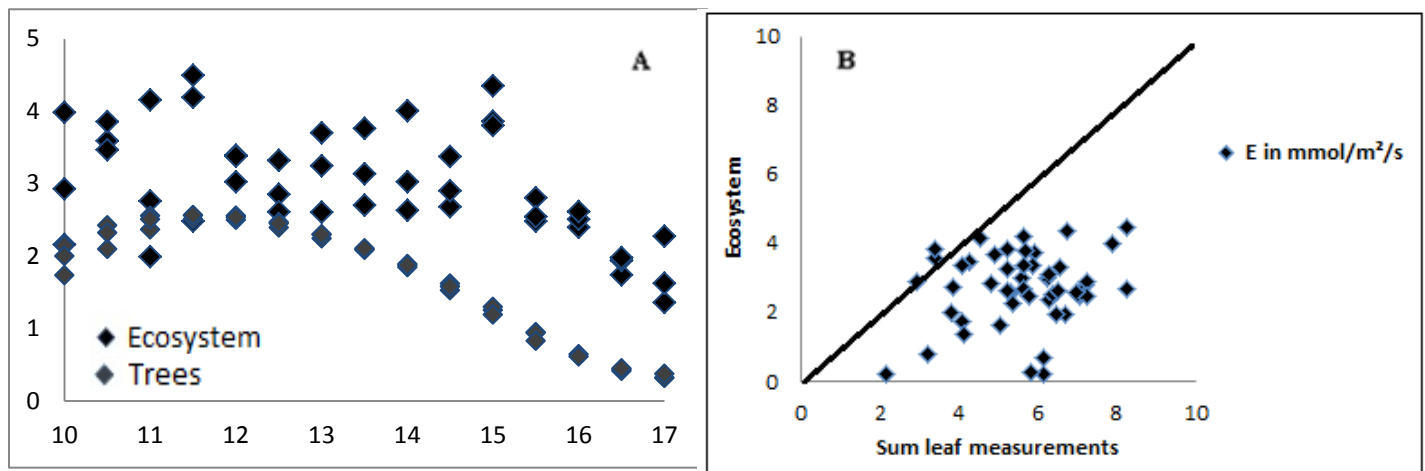
Fig. 11: Transpiration of ecosystem during three daily cycles (A), and transpiration rate of ecosystem vs. sum of transpiration rate from leaf measurements (B).

Transpiration of ecosystem and *pine* tree on stand level, as can be seen in Fig. 11, is high in the morning and decreases in the afternoon, where pine tree transpiration decreases earlier than ecosystem transpiration. The (strong) increasing trend of transpiration in the morning found with up scaling of leaf scale measurements cannot be seen in Fig. 11, as well as the early decline in transpiration rate of *pine* trees on stand level. Also, total values are relatively low for ecosystem and *pine* tree on stand level compared to up scaled leaf measurement data.
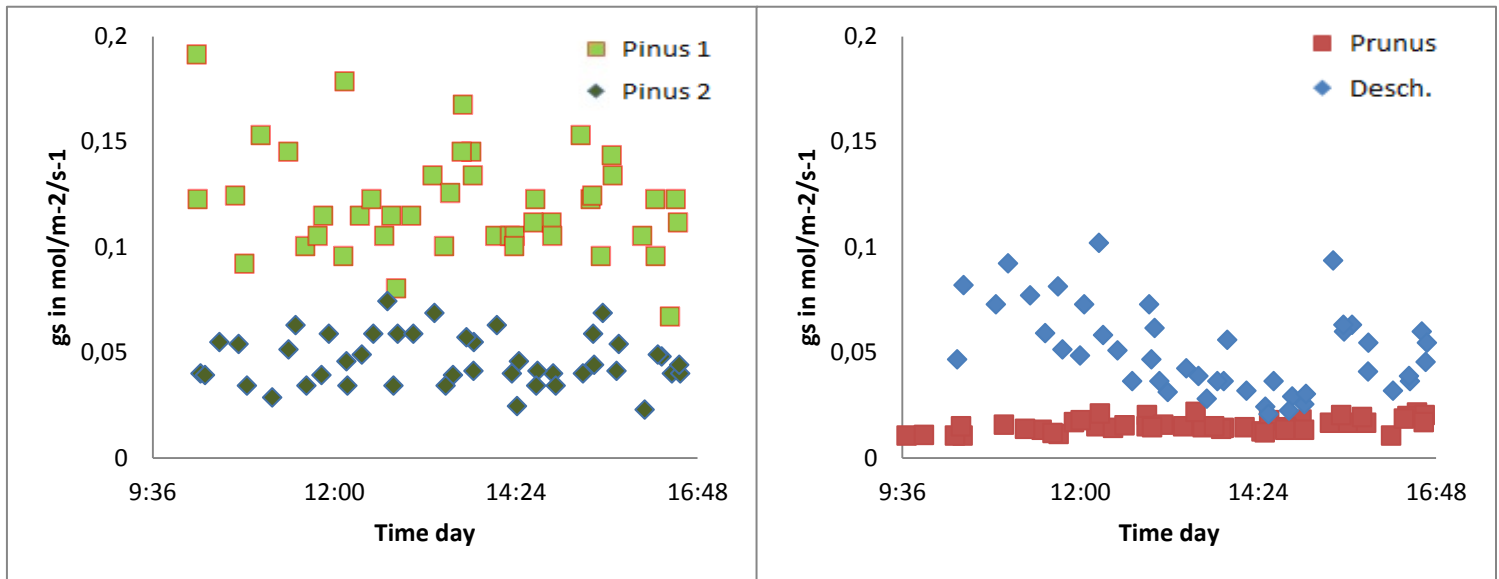
Fig. 12: A: Stomatal conductance *Pinus sylvestris* (A), and *Deschampsia flexuosa* and *Prunus serotina* (B).

Fig. 12 shows stomatal conductance calculated from leaf scale measurements during the 3 sunny measurement days. For gs the same trends can be seen as for A. gs of *Pinus sylvestris* remains stable at about 0.15-0.20 mol/m^2/s, while *Deschampsia flexuosa* has values of gs between 0.05 and 0.1 during the morning and between 0.02 and 0.06 in the afternoon. Adding these figures up gives an ecosystem gs from leaf scale data of around 0.3 in the morning and between 0.2 and 0.3 mol/m^2/s in the afternoon (Fig. 13).
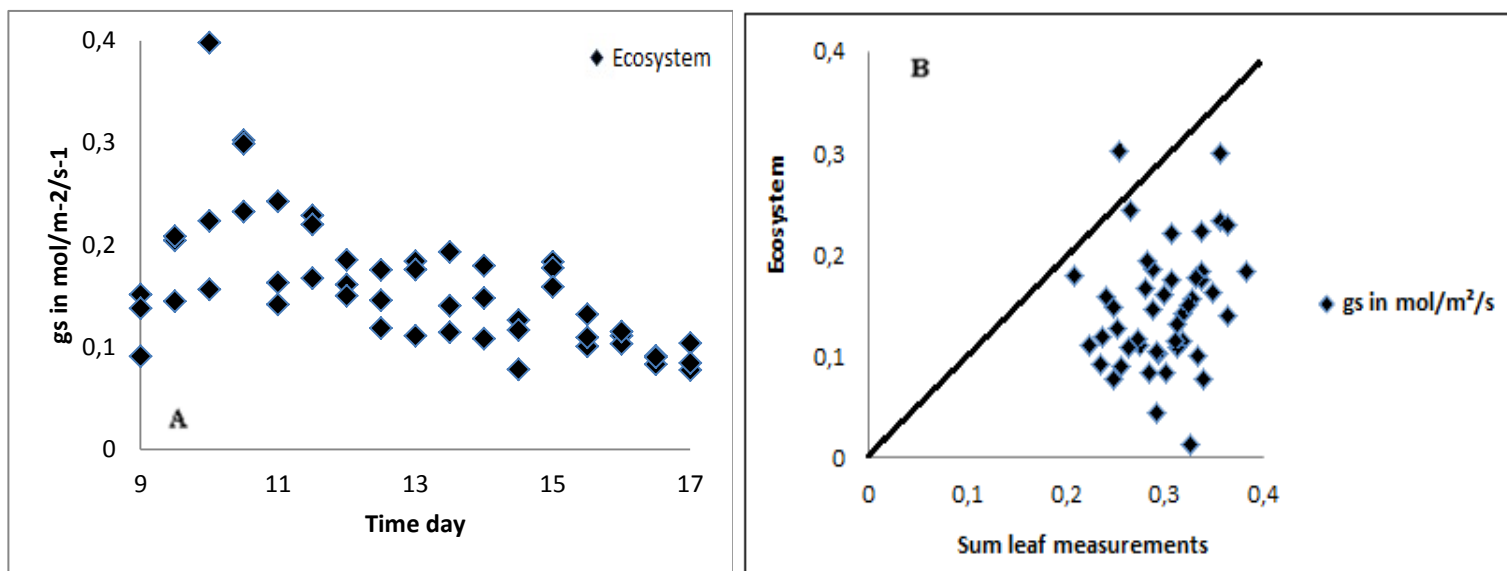
Fig. 13: Stomatal conductance of ecosystem during three daily cycles (A), and stomatal conductance of ecosystem vs. sum of stomatal conductance from leaf measurements.

Stomatal conductance of the ecosystem is calculated from eddy covariance data, using the Penman-Monteith equation (Annex 4). Values of gs during the day vary between 0.15 and 0.25 in the morning to between 0.10 and 0.15 in the afternoon. Fig. 13 shows the same trend as the four measured leaf types combined; a decrease of gs during the afternoon. Absolute values are somewhat lower than expected for ecosystem gs compared to leaf scale data, just as leaf scale measured A seemed relatively high compared to GPP from eddy covariance data.

Ecosystem/*Pinus sylvestris* and water stress

From data analysis, it showed that the four different leaf types showed no water stress from air humidity and soil water availability, except for *Deschampsia flexuosa*. To see whether there is a difference in response of gs to water stress (increasing VPD) between the ecosystem and *Pinus sylvestris* as a species, a comparison has been made based on eddy covariance and sap flow data. This comparison is displayed in Fig. 14.
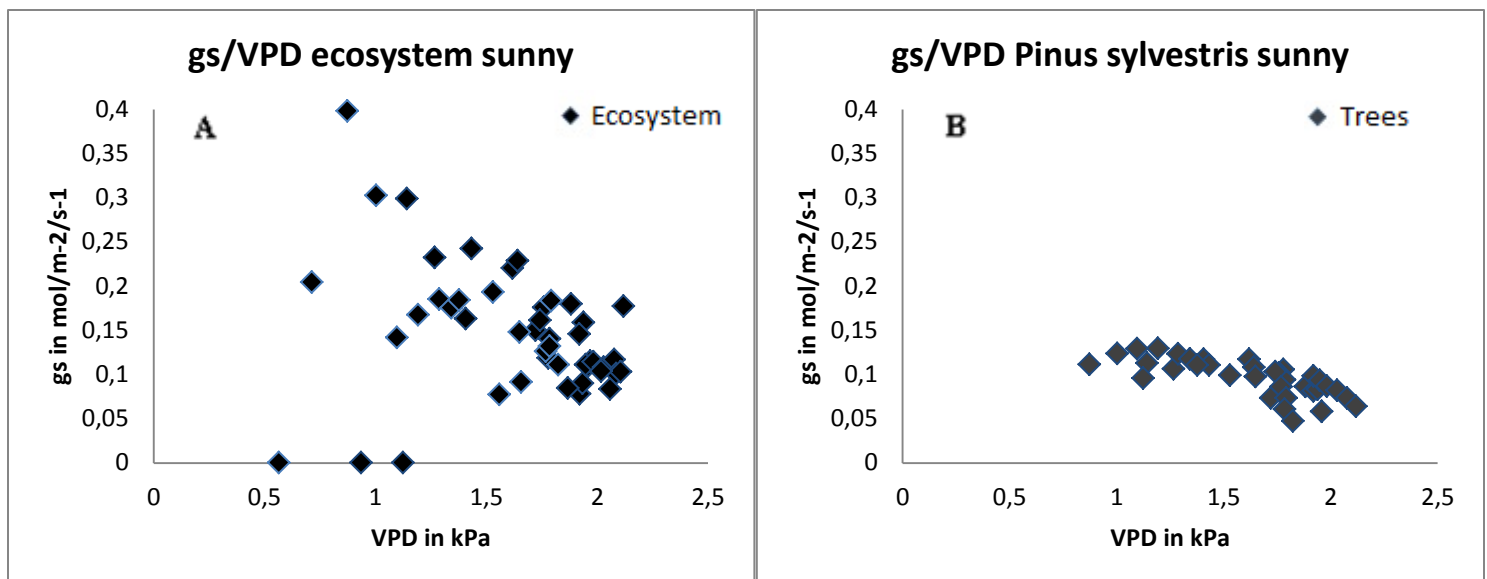
Fig 14: gs vs. VPD ecosystem (A) gs vs. VPD *Pinus sylvestris* (B).

From fig 14 can be concluded that both ecosystem and *Pinus sylvestris* encounter the same VPD during the 3 measured sunny daily cycles (all 9AM-5PM). While *Pinus sylvestris* shows lower maximum gs levels compared to the ecosystem (which seems logical as the fraction of *Pinus sylvestris* compared to total ecosystem transpiration is about 60%), also the upper values of gs are decreasing slower with increasing VPD as compared to the ecosystem. This means that the total ecosystem seems to respond stronger on increasing VPD and therefore increasing water stress from the air than *Pinus sylvestris* does. It must be stressed here that contribution to gs values for the ecosystem consist of transpiration from the top soil is around an estimated 10-20%, which gives little overestimation of ecosystem gs values.

In general, trends in daily cycles are comparable between ecosystem and added up leaf measurements after up scaling. Absolute values however are somewhat lower with ecosystem and sap flow data compared to leaf scale data. The cause for this can lie in measuring methodology (measuring in full sunlight throughout the day) and overestimation due to too high values of k, or too low values of leaf area per m^2 of land area in the Lambert-Beer equation.

6. Discussion

In this chapter, the research methodology is discussed firstly, followed by a discussion of the main questions, hypotheses and associated conclusions.

Methodology

Field work and up scaling methodology has been designed in such way that all data is gathered in a consistent way, and that field work data can be compared with permanent measurement data. Results from earlier research stated that it is difficult to correlate stomatal conductance with particular environmental variables, as gs can for instance be simultaneously affected by an increase in VPD and temperature (Jarvis, 1976). However, during the days of field work no excessive temperatures, radiation amounts and soil water deficit (although uncertain for top soil layer) was observed.

During field work always some uncertainties or methodology errors can remain; hereunder follows a discussion of these issues:

Leaf chamber Recording

Measurements are executed according descriptions given in the manual of ADC Lc-Pro; where it is stressed that stable Ci must be maintained during leaf measurement (LC Pro+ user guide). However: gs stabilizes difficultly during fast changes in irradiation, even when Ci is stable. This causes uncertainty in gs values measured during field work days with abrupt changes in radiation level due to cloud cover. Next to this, during leaf measurements little heating of leaf chamber due to technical (internal) composition occurs; this problem is addressed in the manual as well. The impact on measurements is however thought to be small as there is a chance of slight overestimation of E during leaf measurements, but the few degrees increase in temperature are thought to have negligible influence on gs and A values.

There is an presumed uncertainty of up to 20% for all leaf types except *Prunus serotina* in calculated absolute values for A,E, gs because of the method for extrapolating actual leaves in the chamber to 100% leaf chamber surface coverage (see annex 2). The leaves are not always situated in the leaf chamber perfectly straight (without an angle) and flat, and leaves can be larger or smaller than average. To decrease this level of uncertainty, perhaps a more sophisticated methodology than counting leaves and extrapolating to 100% leaf chamber coverage can be used.

The gs measured for each leaf type is expected to differ around 20% (lower) from stable conditions. This is because the leaf should be inside the leaf chamber for about 5-10 minutes until gs is totally stable. This was however not possible during this research because this time range is too long to adequately measure A and E. For further research on only gs it is recommended to take more time for each measurement.

Types of leaves measured

Measured leaves where thought to be always of the 'sunlit' leaf type. However, small underestimations of for instance measured A values can be caused when incidentally individual leaves of the 'shaded' leaf type have been measured.

Individuals and individual measured leaves are thought to be representative for the species in the ecosystem. However, pine trees in canopy gaps are measured instead of trees in the upper canopy. Stating that their performance in terms of gs, A, E and reaction on water stress during the day is exactly the same can be considered doubtful (because larger individuals for instance can have better access to soil water), but the actual difference is unknown.

Leaf water potential

Ψ is measured based on visual observation, which gives a small uncertainty in results, and possibly a large uncertainty for values of *Deschampsia flexuosa*. Latter because this species has leaves with a relatively very small diameter, which increases difficulty to determine the moment when a colour change occurs at the leaves end (this indicates that pressure exceeds hydrostatic tension inside the leaf) Besides, branches can be slightly damaged which can result in erroneous values. Other (natural) variations can give slightly false numbers as well. The time between cutting and measuring a branch is not the same for every Ψ measurement, causing disturbance of hydrostatic tension and resulting in slight differences between actual and measured Ψ.

LAI: methodology

Within the plot site of 1ha around the eddy covariance tower random selecting of *Prunus serotina* has taken place. 15 individuals have been examined for LAI. Although this is a normal way of estimating LA, it can lead to a bias in measured versus actual values of gs, A, E as the examined individuals can never be totally representative for the 'average' individual within the plot.

For LAI of *Deschampsia flexuosa*, approximately 25% of biomass taken from the plot site is lost while separating *Deschampsia flexuosa* from other material like mosses. Also, gathered grass samples are dried, after which samples of dry grass are compared to 'wet' grass (Annex 2) to extrapolate dry grass LA to normal grass LA, which can result in under- or overestimation of LA. The average amount of *Pinus sylvestris* leaves covering the leaf chamber 100% is estimated (Annex 2), the estimated number however has an uncertainty of around 20%.

Up scaling of leaf measurements

Up scaling of gs, A, E of each leaf type is done using LA as well as the Lambert-Beer equation. The latter is needed because all leaf measurements are performed in full sunlight while actual radiation is less for each forest layer (where the lowest layer receives the least radiation), therefore knowledge about the distribution of radiation top-down through the canopy is required (Kinerson, 1973). While grass and bush vegetation is thought to be almost entirely in the shaded area under the canopy, the place (sun/shaded) of Pine leaves are more difficult to predict even during completely sunny days. This is because of clumping of pine leaves, the rotating movement of the sun above the canopy, different heights of trees causing shading of others, etc. Light response curves have been made to estimate the amount of gs, A and E for each species calculated from leaf scale measurements under species specific

LA. Light response of gs showed to be insignificant except for *Prunus serotina* when recalculating Q values to 'shaded ecosystem values' using the Lambert-Beer equation. A and E values however did show a decline with decreasing Q. This estimation of A levels with forest layer specific shading using light response curves can however result in an under- or overestimation of actual gs, A and E levels, because of the scatter in y values with a certain x value involved in the response curve (see Fig. 16).

Up scaling of evaporation seemed to be even more difficult than up scaling of A and gs Two main reasons for this are firstly the unknown amount of soil evaporation during the day, causing overestimation of ecosystem transpiration during the morning as top soil moisture gradually evaporates during the first hours the sun shines on the ecosystem. This soil evaporation is not included in the up scaled leaf and sap flow measurements, causing lower values for up scaled leaf scale- and sap flow transpiration levels. Contradictory to transpiration during the morning, in the afternoon the observed trend in transpiration rates of leaf scale measurements are higher than evaporation derived from ecosystem and sap flow data. This can be a result of the significantly higher VPD and temperature values in the leaf chamber compared to the forest canopy.

The Big leaf approach
The Penman-Monteith equation is often used to estimate surface conductance of a forest from measured factors like transpiration, radiation, roughness of forest etc. (Stewart, 1987). While it is an effective way of estimating surface conductance on stand level, it has some drawbacks like a bulk-surface resistance (which normally is higher near the soil than at the top of the canopy) and boundary layer mixing (Baldocchi, 2010).

Up scaling of sap flow
Poyatos et. al. in 2005 described several reasons for uncertainty with up scaling of sap flow data to stand level, such as difference in light availability per individual tree (and thus evaporation potential) and non-uniformity of sap flow velocity in tree stems. Monitoring sap flow at six trees as is done in Loobos however is a reasonable amount to outrule large over- or underestimations caused by differences in individuals.

Responses forest layers and up scaling
In this research, differences in reaction on water stress has been examined for different forest layers. It was expected that grasses and bushes would be more sensitive to water stress than trees. This was found to be not entirely true, because only grasses showed a reaction to water stress factor(s). Also it was predicted that ecosystem and dominant species would show little differences in response to water stress. Research results however showed that there is a response in gs of the ecosystem to VPD, while the forests dominant species did not show a significant response. These results are in line with a recent study, in which was concluded that there is a large variation in understory evaporation during drought periods, while pine trees in Loobos did not seem to respond in terms of decline in transpiration (Moors, 2012). The found responses assume a water preserving strategy for undergrowth specie *Deschampsia flexuosa* during sunny days with low air humidity. This species however can reach high maximum gs and A rates on days with high air humidity. For tree and bush species this was the other way around (high gs

on sunny days, low on cloudy days). Earlier research showed that grass species can act as a 'balancing factor' in total ecosystem transpiration (Roberts, 1983) in the sense that they respond to humidity deficit more or less in the opposite way compared to the other forest layer species. Trees in Loobos are presumably not forced to respond on drought as they have access to sufficient levels of soil water at any time (at least during the days of research), which explains the steady gs levels during the sunny measurement days. *Prunus serotina* manifests itself as a drought tolerant bush species, as with high LWP and VPD levels, gs reaches maximum levels.

The results and conclusions, which imply 'decreasing' or 'stable' values of gs with changing (water) stress related factors, cannot be seen as statistical proof. However, observed trends suggest the influence of each stress factor on each species, and the influence of VPD on the ecosystem and the forests dominant species *Pinus sylvestris*.

Up scaling of results showed an overestimation (0-50% for gs, A, up to >50-100% for E) of recalculated measurement values on leaf scale compared to actual fluxes on ecosystem scale. Such overestimation after up scaling is also observed during similar research (Revollo, 2010). The difference between observed and up scaled gs, A and E levels can be caused by uncertainty in overestimations leaf area estimations, estimations in received radiation by vegetation layers, differences in sample locations, representativeness of measured leaves and equipment accuracy.

7. Conclusions

In this study, dominant plant species of three different forest layers in Loobos, a Scots Pine forest in the Netherlands, have been investigated in terms of gs, A, E and responses to water stress factors ψ and VPD. This has been done by gathering leaf scale data during multiple days and comparing this with data retrieved from permanent measurement systems (eddy covariance and sap flow).

The research questions were formulated as follows:

- How do different forest layers respond to water stress factors in terms of stomatal conductance, photosynthesis and transpiration?
- How does this differ between different forest layers, the ecosystem, and the main forest species during daily cycles? And can ecosystem fluxes within daily cycles be predicted with up scaling of ecosystem main species data

These can be answered as follows:

During sunny days, as can be seen in Fig. 3; gs of *Pinus sylvestris* remained about constant throughout the sunny measurement days. The same accounts for *Prunus serotina*, while *Deschampsia flexuosa* shows a significant decrease in gs during the day. A follows the same trends as gs, while E shows the same pattern as VPD, with an increase during the morning and more or less stable values in the afternoon. During cloudy days, as expected from the large scatter in Q, there is a large variation in A for all species during cloudy days Fig. 5: C,D. E is relatively low for all species except *Deschampsia flexuosa*, which also has relative high gs levels. gs levels in general are quite stable, which can be explained because of the slow response time of stomata to changing radiation conditions, and the fact that the other environmental factors are quite stable throughout the day. The ecosystem shows a downward trend in gs and A during the afternoon, which seems logical considering the more or less stable values for gs observed on leaf scale for all species except *Deschampsia flexuosa* (of which latter accounts for about 20% of ecosystem LA). Found responses of the forest layers are in line with outcomes of earlier research (Moors, 2012; Roberts, 1983)

The most important water stress factors is found to be VPD, and possibly also Ψ for *Deschampsia flexuosa*. Latter can however not be proven because there is a large scatter in gs values plotted against Ψ. VPD had most influence on ecosystem gs during the measured daily cycles. For all species except *Deschampsia flexuosa* (including *Pinus sylvestris*, sap flow data) no observed water stress trends were found after analysis of data used for this research.

In general, trends in daily cycles are comparable between ecosystem and added up leaf measurements after up scaling. Ecosystem fluxes could however not be reconstructed fully, amongst others because of uncertainties in for instance actual LA of each vegetation layer and conversion of measured to actual PAR within the vegetation layers using the Lambert-Beer equation. Overestimations of up scaled leaf

measurements compared to ecosystem and sap flow data are mostly within a range of 0-50%. For evaporation, overestimation ranges up to 100%. Such overestimation has also occurred in similar research (for instance with the research of Revollo on up scaling of tree transpiration, 2010). One of the causes for this are much higher VPD levels measured on leaf scale (up to around 3.5 kPa) compared to VPD levels on ecosystem and tree scale (up to around 2.2 kPa).

Besides the overestimation of ecosystem GPP, E and gs with up scaled leaf measurements, the relatively low values in gs during the morning found in ecosystem gs are not present. This can be caused by measuring methodology (leaf chamber measurements in full sunlight, radiation in canopy is less during the morning). The analysed trends on leaf scale, ecosystem scale and gs trend derived from sap flow data (except gs during the morning) are however quite similar.

Both the trends in daily cycles and responses to water stress of individual dominant species of each forest layer and the ecosystem show that a forest with multiple vegetation layers like the Scotts Pine forest in Loobos should not be seen as a in this case 'Scotts Pine dominated forest'. Because when analysing forest layers compared to the ecosystem during this research, VPD sensitivity during the day was negligible for all species but *Deschampsia flexuosa*. This forest layer species nearest to the soil is also likely to cause most of the ecosystems sensitivity to VPD, while the tree layer dominated by *Pinus sylvestris* showed minimal sensitivity to VPD when analysing leaf scale- and sap flow data. The relative contribution of each forest layer to the ecosystem fluxes can in this way due to strategy in terms of response to water stress factors also substantially be influenced by differences in climatic conditions (on daily and perhaps seasonal scale).

Further research

Because of the suggested response to water stress and impact on ecosystem fluxes and of *Deschampsia flexuosa*, more research is needed to determine the actual influence of understory species on ecosystem response and performance (fluxes). Besides, an interesting question would be what cause understory species to respond relatively strong on water stress, while species in other vegetation layers show almost no response.

Research is needed to determine whether water stress during daily cycles can be used to predict species and ecosystem responses during seasonal cycles, which is particularly interesting because periods of drought are expected to occur prolonged and more frequent during the coming decades. Another question that arises is whether prolonged drought periods cause understory species only to be less productive like observed during the sunny measurement days, or that drought periods also lead to significant biomass loss and a shorter growing season.

Next to this, because the circumstances for drought intolerant species will become less favorable in future, it is possible that other (more drought tolerant) species will gradually replace less drought tolerant species with a changing ecosystem as result. Research can be done in Loobos and other ecosystems to predict influence on changing of ecosystem species composition due to increase in periods of water stress.

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Other

- Alterra spreadsheet LAI Loobos 2002-2008

9. Annex

1. Leaf Area Index of dominant bush and undergrowth species at Loobos site

In this LAI research, the LAI of the dominant brush (*Prunus serotina*) and undergrowth (*Deschampsia flexuosa*) species at the Loobos site will be investigated. An accurate specification of the LAI of the dominant tree species (*Pinus sylvestris*) is already available.
The LAI research has been performed on an area of 1 ha, with the Loobos flux tower situated in the exact middle. The research can be summarized as follows:

Prunus serotina
- Counting # of trees and bushes (higher than 0.5m) in plotted area
- Random selecting of 15 individuals of *Prunus serotina*
- Determination of # of shoots per selected individual
- Collecting of 6 shoots per individual
- Measuring leaf area of shoots per individual bush
- Calculating LAI of *Prunus serotina* within 1 ha plot site

Deschampsia flexuosa
- Random selecting of 15 plots of undergrowth (0.5m^2)
- Extracting all green material from undergrowth plots
- Seperating *Deschampsia flexuosa* from any other material
- Measuring dry weight of *Deschampsia flexuosa* per plot site
- Measuring leaf area of *Deschampsia flexuosa* per plot site
- Calculating LAI of *Deschampsia flexuosa* within 1 ha plot site

Prunus serotina

Counting # of trees and bushes in 1 ha plot
To calculate the LAI of *Prunus serotina* in a 1 ha plot, the total amount of individuals must be determined. Therefore an indication is made of the total amount of individuals of all trees and bushes in the 1 ha plot site. This is done by first dividing the total 1 ha area into four strips of 25 by 100 meters. After this, for each strip the amount of individuals taller than 0.5 meter of each specie was noted (table 2).

Specie	# Plot 1	# Plot 2	# Plot 3	# Plot 4	# Total 1 ha
Pinus sylvestris	98	111	91	111	411
Prunus serotina	26	79	80	112	297
Rhamnus frangula	36	87	88	72	283
Sorbus aucuparia	3	3	1	1	8
Pinus sylvestris (small)	18	4	2	34	58
Betula lenta	0	0	1	0	1
Quercus robur	3	1	1	0	4
Ilex aquifolium	1	1	0	0	2

Table 2: # trees and bushes within 1 ha plot site at Loobos flux tower

Note here that the amount of individual *Rhamus frangula* is relatively large but is not seen as a dominant specie. This is because *Rhamus frangula* often is situated in groups of dozens of individuals on a few square meters surface, resulting in a relative small leaf area per individual.

Selecting individuals and shoots

Now that the number of individuals is determined, the total amount of shoots and leaves must be estimated to calculate LAI of *Prunus serotina*. This is done by randomly selecting 15 individuals within the plot site, and counting the amount of shoots per individual (table 3). After writing down the amount of shoots, six shoots of each individual are sampled for lab research.

Sample nr.	# Shoots	Sample nr.	# Shoots	Sample nr.	# Shoots
1	45	**6**	72	**11**	190
2	52	**7**	24	**12**	48
3	105	**8**	409	**13**	210
4	76	**9**	322	**14**	21
5	126	**10**	72	**15**	167

Table 3: Amount of shoots per selected individual of *Prunus serotina*

Random selecting
The random selecting of individual bushes is performed by rotating around own axis a couple of times with closed eyes walking 20 steps straight ahead, the nearest bush is selected. When the border of the plot area is reached, 40 steps will be taken towards the centre of the plot. Shoots are selected by estimating the number of larger and smaller shoots, and taking for instance two large and four smaller shoots as samples according to the estimated large/small ratio.

Measuring leaf area of selected individuals

All leaves of the shoots are now taken from the branches and stored in 15 different compartments to dry. After the leaves have dried, they are taken to a leaf area measurement device. Here, the total leaf area is measured for each individual selected bush, by measuring and adding the surface of every separate leaf in cm^2. The total leaf area of the sample shoots can be seen in table 4. Before all leaves of every sample are processed in the leaf area measurement device, an example surface of exact 50 cm^2 is

placed into the device to check whether there is any anomaly that has to be corrected for after the actual measurements. Ultimately, the estimated leaf area per individual bush (as to be seen in table 4) is calculated by the following equation: (((Total leaf surface*(50/Reference))/(Sample shoots/Total shoots)/10.000

Sample nr.	Reference (50 cm^2 = actual ?cm^2)	Total leaf surface cm^2	# Sample shoots / Total Shoots (6/x)	Estimated leaf surface individual in M^2
1	49.45	1.636	0.133	1.24
2	49.56	1.238	0.115	1.09
3	49.48	1.813	0.057	3.21
4	49.52	1.529	0.079	1.95
5	49.50	1.950	0.048	4.10
6	49.59	1.686	0.083	2.05
7	49.49	1.399	0.250	0.57
8	49.53	1.465	0.047	3.15
9	49.52	1.576	0.019	8.38
10	49.57	1.535	0.083	1.87
11	49.67	1.548	0.032	4.87
12	49.63	1.700	0.125	1.37
13	49.60	1.377	0.029	4.79
14	49.63	2.359	0.286	0.83
15	49.50	2.987	0.036	8.38

Table 4: Leaf surface per sampled individual of *Prunus serotina*

Total LAI of *Prunus serotina* in 1 ha plot

Now that the leaf surface of 15 individuals is calculated, an estimation can be done of the total leaf area index of *Prunus serotina* within the 1 ha Loobos plot. The 15 estimated individual leaf surfaces are added up and divided by 15, and this number is multiplied by the total counted amount of individuals within the 1 ha plot. This gives (47.85/15)*297= 947 M^2 per ha, which is an LAI of 947/10.000= 0.094 M^2/M^2 or 0.1 M^2/M^2.

Deschampsia flexuosa

Next, the LAI of *Deschampsia flexuosa* will be determined for the 1 ha lot on the Loobos site. This is done by randomly selecting plot sites, gathering and weighing samples and ultimately measuring and upscaling of leaf area.

Selecting and sampling plot sites

The random selecting of plots is done in the same way as with selecting individuals of *Prunus serotina*. When arriving at the random selected location, a plot is set of half a square meter. Within this plot, all green material is extracted and taken to the laboratory in sample bags.

Drying, weighing of samples and measuring leaf area

First, the material from the sample bags is carefully separated in such way that only green material is taken out to dry, divided in two categories (The grass specie *Deschampsia flexuosa*, and Moss, which consists of all other green material besides *Deschampsia flexuosa*). This is done for all 15 sample plots, and is left to dry in an oven at 70 degrees centigrade.

After a few days (minimally 48 hours) the dry plant material is weighed. This is done by weighing an empty container (zero weight) and then the container with added plant material. All results are displayed in table 5.

Plot #	Zero weight (g)	Zero + Moss (g)	Moss (g)	Zero +Grass (g)	Grass (g)
1	252.10	269.42	17.32	263.90	11.80
2	252.12	271.22	19.10	296.27	44.15
3	252.10	274.15	22.05	271.91	19.81
4	252.13	270.00	17.87	279.18	27.05
5	252.09	269.23	17.14	275.35	23.26
6	252.13	274.51	22.38	277.20	25.07
7	252.10	259.63	7.53	280.12	28.02
8	252.08	270.31	18.23	275.46	23.38
9	252.10	278.42	26.32	278.52	26.42
10	252.16	257.19	5.03	268.16	16.00
11	252.04	262.99	10.95	276.07	24.03
12	252.00	257.64	5.64	262.48	10.48
13	252.30	276.18	24.34	266.13	13.83
14	252.01	269.52	17.51	266.74	14.73
15	252.02	273.10	21.08	262.22	10.20

Table 5: Dry weight Grass and moss

From all the 15 *Deschampsia flexuosa* samples, a small subsample is taken for leaf area measurements. With the leaf area of the subsamples, the total leaf area of the dried grass samples can be estimated by dividing the total sample weight by the subsample weight and multiplying this with the leaf area of the subsample, this number has to be multiplied by 50/49.5, which corrects for the difference in real surface and measured surface. (Table 6)

Plot # (0.5-0.5m)	Total sample (g)	Subsample (g)	Leaf Area Subsample cm^2	Leaf Area total cm^2 per plot*	Leaf area cm^2/M^2
1	11.80	0.95	27.5	342	1.380
2	44.15	1.01	25.6	1.130	4.521
3	19.81	1.22	32.2	528	2.113
4	27.05	1.42	52.2	1.004	4.018
5	23.26	1.86	60.0	758	3.032
6	25.07	1.22	34.2	710	2.840
7	28.02	1.20	31.8	750	3.000

8	23.38	1.32	38.1	682	2.729
9	26.42	1.17	40.3	919	3.677
10	16.00	1.43	46.6	527	2.107
11	24.03	1.80	57.6	777	3.107
12	10.48	1.29	36.9	303	1.211
13	13.83	1.51	48.6	450	1.798
14	14.73	1.01	24.6	362	1.450
15	10.20	0.92	25.6	287	1.147

Table 6: Corrected* Leaf area dry grass samples

Now that the leaf area of the dried grass samples is known in cm^2 per M^2, the leaf area of normal grass must be estimated. This is done by measuring the difference in leaf area between 20 dried leaves and 20 normal leaves of each 2.5 cm long, which is performed 15 times. The average ratio between the results is used as a multiplier to calculate actual LAI of *Deschampsia flexuosa* per M^2 plot.

Sample nr.	Surface 20 dried leaves of 2.5 cm	Surface 20 normal leaves of 2.5 cm	Multiplier (Average 1.463)	Leaf area dried leaves cm^2/M^2	Leaf area normal leaves M^2/M^2
1	1.63	2.82	1.73	1.380	0.20
2	1.75	2.68	1.53	4.521	0.66
3	2.11	2.91	1.37	2.113	0.31
4	1.76	2.68	1.63	4.018	0.59
5	2.10	3.26	1.55	3.032	0.44
6	2.17	2.99	1.38	2.840	0.41
7	1.87	3.25	1.74	3.000	0.44
8	2.05	2.92	1.42	2.729	0.40
9	1.69	2.77	1.63	3.677	0.54
10	1.93	2.68	1.39	2.107	0.31
11	2.25	2.81	1.25	3.107	0.45
12	2.34	2.90	1.24	1.211	0.18
13	2.11	3.02	1.43	1.798	0.26
14	1.98	2.79	1.41	1.450	0.21
15	2.28	2.83	1.24	1.147	0.17
Average			**1.46275**		**0.37**

Table 7: LA Deschampsia flexuosa per M^2

In table 7, estimated average LA in M^2/M^2 of *Deschampsia flexuosa* is displayed. This number must be multiplied by 1.25 because an estimated 25% of organic matter wast lost (and thus not measured) during the process of separating grass and moss. After multiplying 0.37 with 1.25 the estimated LA of *Deschampsia flexuosa* is 0.46 M^2/M^2.

2. Leaf chamber coverage multiplier

Leaf chamber coverage is measured to determine with what number each specific measurement on *Pinus sylvestris* and *Deschamsia flexuosa* must be multiplied to get a value for every specie 'as if they had full leaf chamber coverage with every measurement'. The leaf chamber of ADC LC-Pro has a surface of 6.25 cm^2, which means that leaf scale measurements must be recalculated to coverage of 6.25 cm^2

For creating a multiplier which can be used to recalculate measured values to values in m^2/m^2, average leaves needed for fully covering the leaf chamber are calculated for *Deschampsia flexuosa* and *Pinus sylvestris* (Table 8)

Sample nr.	Surface in cm^2		Surface in cm^2
1	3.64	9	4.11
2	4.95	10	4.45
3	4.14	11	3.89
4	4.20	12	4.69
5	4.44	13	4.21
6	4.38	14	4.50
7	5.05	15	4.88
8	4.25	**Average**	**4.38**

Table 8: Leaf surface 10 pine leaves of 2.5 cm (length and width leaf chamber)

The total leaf chamber surface of 6.25 cm^2 is covered when 6.25/4.38*10 leaves = 14.3 leaves are fitted in leaf chamber.
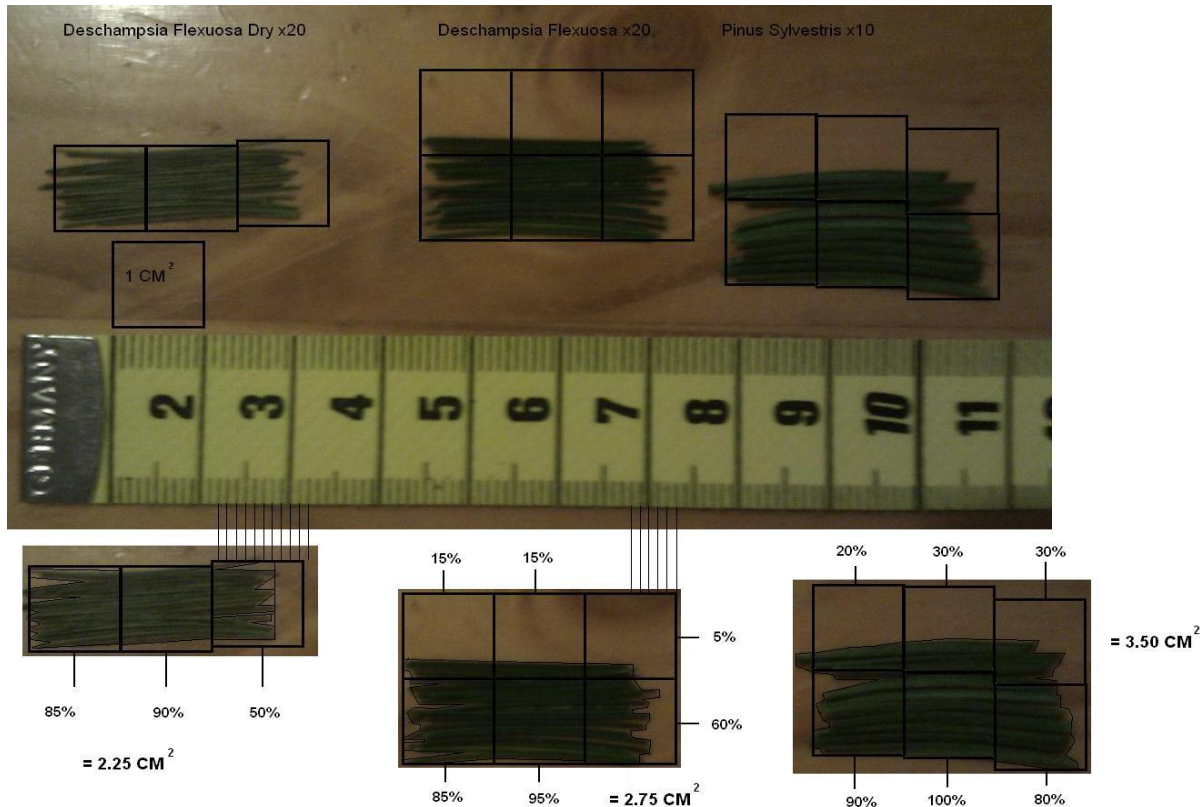
Fig. 15: Example of calculating amount of leaves that represent total leaf chamber coverage. In this example, 10 pine leaves have a surface of 3.50 cm^2 which means 6.25/3.50 * 10 leaves would totally cover the leaf chamber surface.

Calculating actual value gs, A,E, can be done by multiplying old value of for instance A 8.05*(14.3/7)= an A of 16.45 with full leaf chamber coverage

Sample nr.	Surface in cm^2		Surface in cm^2
1	2.82	9	2.77
2	2.68	10	2.68
3	2.91	11	2.81
4	2.68	12	2.90
5	3.26	13	3.02
6	2.99	14	2.79
7	3.25	15	2.83
8	2.92	Total	2.89

Table Leaf surface 20 grass leaves 2.5 cm

6.25/2.89 * 20 = 43.25
(43.25/17) * old A 7.23 = 18.40 new A

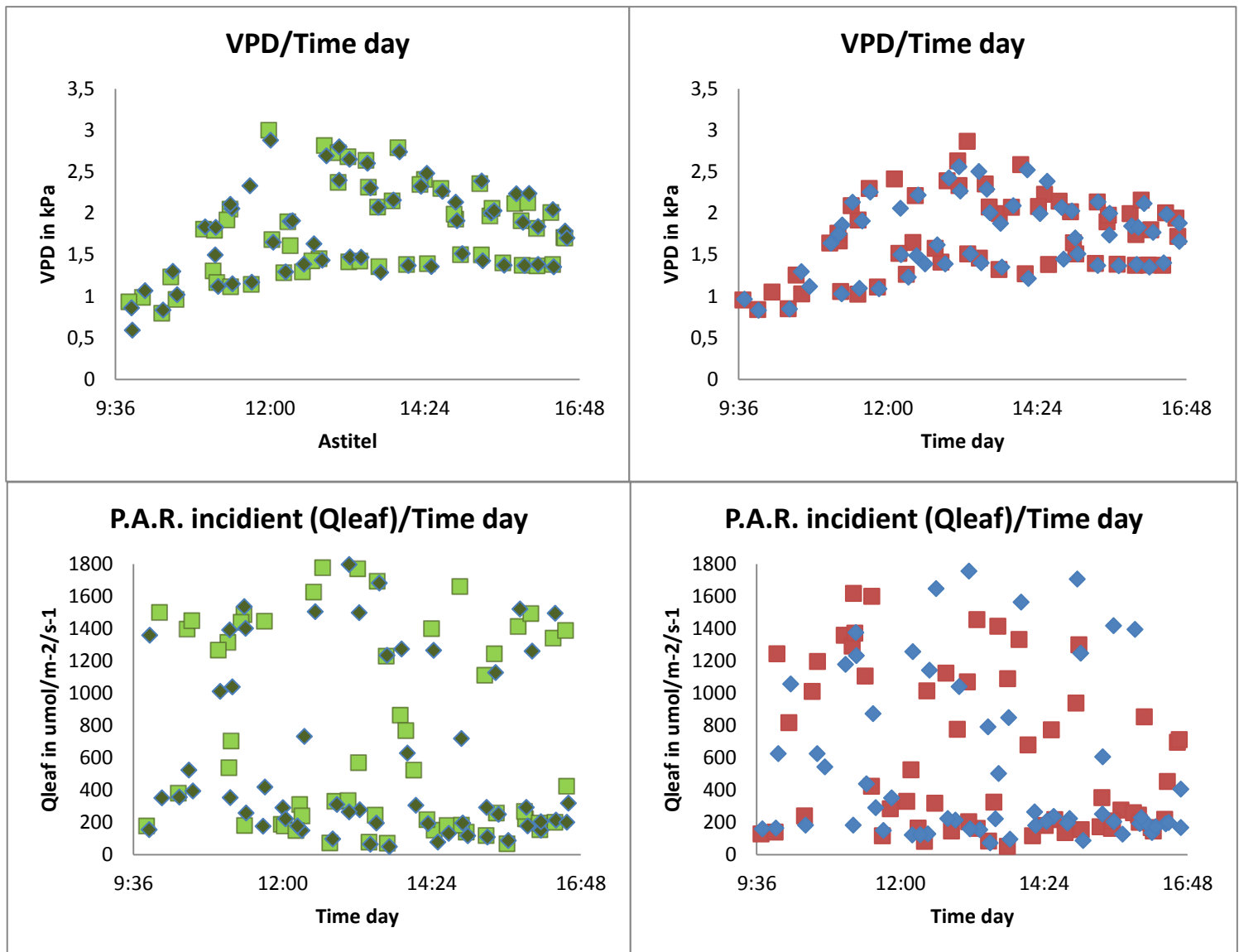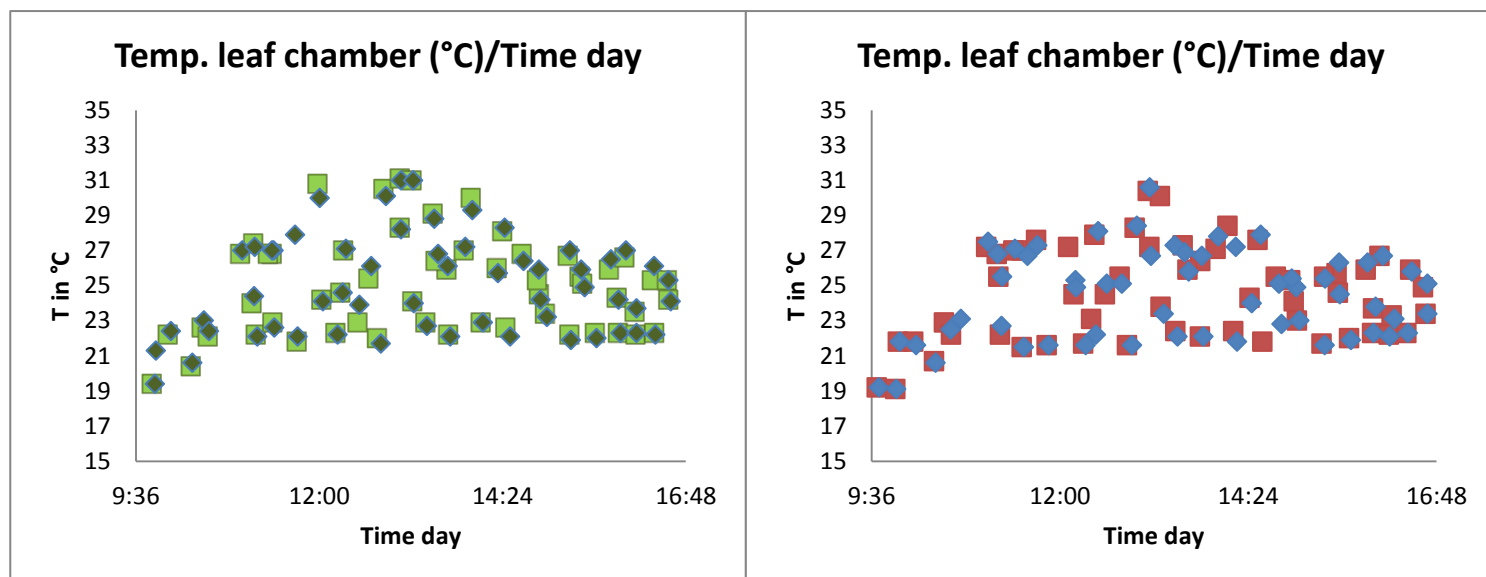## 3. Environmental factors, gs and fluxes cloudy days

Fig .4 (A,B) VPD during three cloudy measurement days. (C,D): P.A.R incident during three cloudy measurement days. (E,F): Temperature in leaf chamber during three cloudy measurement days.

Temp. leaf chamber (°C)/Time day

Temp. leaf chamber (°C)/Time day

4. Calculations data sets

All output data used in this report originates from spread sheets that consist of measurements with ADC-LC-Pro, eddy covariance and sap flow measurement system. With these spread sheets, the next calculations have been performed:

Leaf scale conversion to flux/m²

For two of the three leaf types measured with ADC LC-Pro during field work, calculations are performed to determine actual A,E and gs rates per m^2 of leaf area. This is because 100% cover of the leaf chamber surface was only possible with leaves of *Prunus serotina*, but not with the other species.

For every measurement the amount of leaves in the chamber was noted, and for every leaf type the amount of leaves needed to cover the leaf chamber surface 100% was estimated. For *Pinus sylvestris* leaves this number is estimated to be 14,3, For *Deschampsia flexuosa* this number is 43,25.

The result is a <u>multiplier</u> used to determine actual A,E, and gs rates for every measurement for every leaf type.

Pinus sylvestris: Actual A,E or gs = 14,3/x * measured A,E or gs
Deschampsia flexuosa : Actual A,E or gs = 43,25/x * measured A,E or gs
(Where x is the amount of leaves in leaf chamber)

Vapour pressure deficit

Vapour pressure deficit expressed in kilo Pascal (kPa) is calculated using:

- Saturation partial pressure of water vapour (or saturation vapour pressure, es(T) es(T)= 613.75 * EXP((17.502*Tch)/(240.97+Tch)) Where Tch is the temperature measured within the leaf chamber. (Jones, 1992)
- And water vapour pressure (Wvp) expressed in Pascal (Pa): Wvp= (eref in mBar)*100 Where eref is partial pressure of H2O in air. (LC Pro+ user guide)

Vapour pressure deficit = saturation vapour pressure – water vapour pressure; or es(T)-Wvp
VPD in kPa= ((613.75 * EXP((17.502*Tch)/(240.97+Tch)))-(Wvp))/1000
Where Tch is the temperature measured within the leaf chamber and Wvp is the calculated water vapour pressure.

The big leaf approach

Ecosystem and *pine* gs is calculated from eddy covariance data and sap flow data using 'the big leaf approach'. This is done using the inverse Penman-Monteith equation (Moors, 2012):

$$g_s = \frac{1}{r_s} = \frac{\gamma \lambda E}{r_a \Delta_e A + \rho c_p e_D - r_a (\Delta_e + \gamma) \lambda E}$$

To calculate gs (in mol/m^2/s) from eddy covariance data the following input is needed:

γ = Psychrometer constant = Pcp/0.622 lambda= 66.1Pa K at 20 degrees Celcius (Jones, 1992)
e_D = VPD = Vapour Pressure Deficit (hPa)
Δ_e = Delta = slope of saturation vapour pressure curve expressed in Hector Pascal per degrees Celsius (HPa) (Jones, 1992)
r_a= Aerodynamic friction expressed in meters per second =(Ustar*Ustar/windspeed) in m/s
A= Flux Tsonic (sensible heat flux in W/m2)+ Flux op H2O (latent heat flux in W/m2) (eddy covariance dataset)
λE= Flux op H2O is latent heat flux in W/m2 (eddy covariance dataset)
degrees Celsius Jones mm/s to mol/m2/s (Jones, p357)
ρ = weight of one M^3 of air (gram)
C_p= specific heat capacity of air in KJ/Kg/K, which is 1.01 (Jones, 1992)
g/g= factor converting conductance in units of mm/s to mmol/m2/s, which is a factor of 41 at 20°C
Density of air = 1.204 kg/m^3 (Jones, 1992)

The inverse Penman-Monteith equation is applied to eddy covariance- and sapflow data sets in four parts:

1st part= (γ/100)*Flux op H2O
2nd part = (1/(ga))*(Delta/100)*(Flux Tsonic+Flux op H2O)
3rd part = (1204*Cp)*(VPD*10)
4th part = (1/ga)*((Delta/100)+(Psychrometer constant/100))*Flux op H2O

Total inverse Penman-Monteith

=(1st part)/(2nd part +3rd part -4th part)* g/g

Lambert-Beer law

All leaf scale data is recorded in direct sunlight, while in reality different forest layers in Loobos are on most places subjected to different levels of incoming sunlight and shading. Therefore leaf scale measured values for benefits of up scaling must be recalculated to values that represent realistic levels of for instance A for different layers of vegetation within the research-site's ecosystem. This can be done by combining the Beer-Lambert law, which considers extinction of light through the canopy and vegetation layers, the forest layers (or leaf type) specific LA, and species specific response in terms of gs, A, E to diminishing radiation levels. For each leaf type/age (or forest layer), the inverse of the extinction rate is multiplied with LA and the fraction of gs, A, E derived from response curves of each of these parameters plotted against Q.

Measured A and E and calculated gs on leaf scale can be recalculated to for lobos realistic values by applying these mentioned steps, using the next equation (using parameter A as example): Actual A = measured A *(1-EXP(-k*(LA))) * F, where LA is the species specific leaf area in m^2/m^2 as determined in the LAI research and the Alterra spreadsheet, and where F is A at Q calculated from measured Q * EXP(-k*LAL) where LAL is the leaf area in m^2/m^2 covering the specific forest layers leaf type/age. k is the species specific extinction coefficient, a value between 0 and 1, where a value near 1 means that the leaf type is almost completely horizontal with respect to incoming radiation, and near 0 means that leaves are situated in such vertical way that most radiation bypasses the leaf surface without being absorbed. k = estimated to be 0.5 for *Pinus sylvestris* 1st and 2nd year leaves, 0.45 for *Deschampsia flexuosa*, 0.7 for *Prunus serotina*. F in the equation is flux fraction correction, which resembles the fraction of A with the calculated amount of light extinction within the specific forest layer, derived from light response curves (of which Fig. 16 is an example).
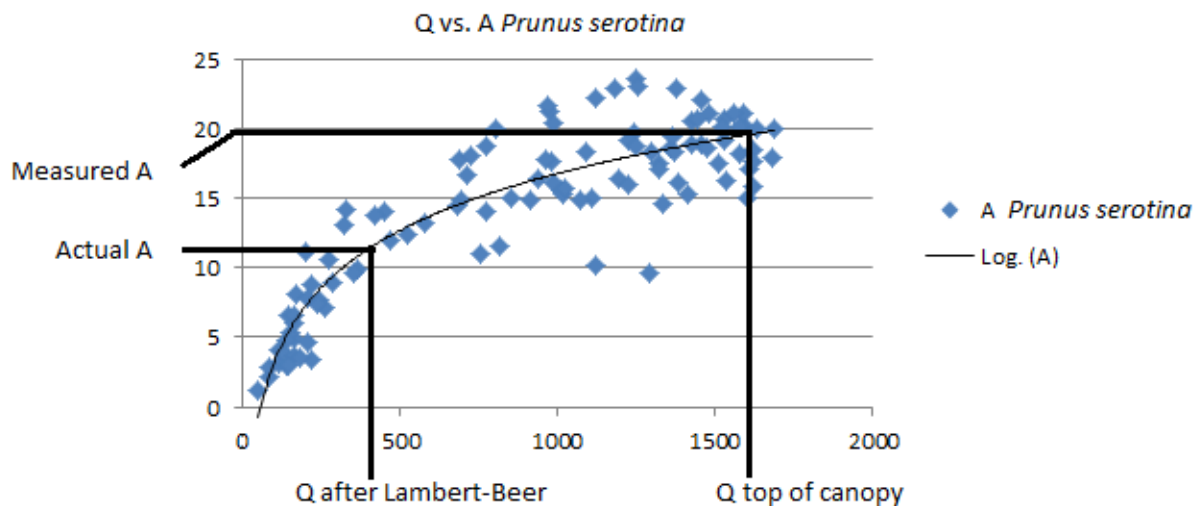

Fig. 16: Correction of A for shading, values expressed in m^2/m^2

For instance: when for a particular species an A of 20 µmol/m^2/s is measured in full sunlight (top of canopy), but the measured species normally lives near the forest soil with a LA of 2.9 m^2/m^2 of the forest layer above, and its extinction coefficient is 0.7, then actual A level would be corrected for LA by

applying the following equation: 20*1-(exp(-0.7*0.1))= 1.35, the outcome must hereafter be multiplied with a figure between 0 and 1 that can be read from a light response curve made of the particular species A is measured for, to correct for shading through LA from the leaf layer(s) above. When the value of A=20 is measured in full sunlight (1800 μmol/m^2/s^1, this can be at radiation of 1650*exp(-0.5*2.9))= 380 μmol/m^2/s^1. Note that here k is set to 0.5 because the leaf layer situated above the leaf layer in this example has an extinction coefficient of 0.5. When A is about 0.6 times the value with radiation of 380 μmol/m^2/s^1 compared to with 1650 μmol/m^2/s^1, 1.35 should be multiplied with 0.6, resulting in an actual (calculated) A on stand level for this particular species of 0.90 μmol/m^2/s.