University of São Paulo
"Luiz de Queiroz" College of Agriculture

Water use and wood productivity of *Eucalyptus grandis* plantations: effects of management, water supply and soil texture

José Carlos de Deus Júnior

Thesis presented to obtain the degree of Doctor in Science. Area: Forest Resources. Option in: Silviculture and Forest Management

Piracicaba
2019

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Bachelor in Forest Engineer

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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2019

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Deus Junior, José Carlos

Water use and wood productivity of *Eucalyptus grandis* plantations: effects of management, water supply and soil texture / José Carlos de Deus Junior - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2019.

69 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura "Luiz de Queiroz".

1. Uso da água 2. Eficiência do uso da água 3. Fotossíntese 4. Plantações de eucalipto 5.Condutância estomática 6. Condutância estomática I. Título

ACKNOWLEDMENTS

I would like to thankfull the peolpe who were with me on my doctorate period. All people that accepted to went to Itatinga Experimental Station. People that help me to improve my skills and habilities. I am deeply grateful to my family, all people from UFSCar, UNESP, ESALQ/USP and CIRAD/FR.

To my family: my mom Ivana, thank you for being my mom. Thank you for providing me a shelter, for giving me education and to stay with me in all moments. I would like to thanks my grandfather (Sebastião), grandmother (Ivana), my uncles Marco, Francisco, Adriano and Evandro.

To Luciana, my girlfriend. I'm really thankfull by her dedication. Thank you so much to motivate myself in many dificult times in my life, to help me finish my measurement in the field in each month, help me to take important decision about my life, to stay with me and to support me many time. Thank you for being such a great partner, my love, Luciana Arruda G.

Thank you so much professor Jose Leonardo M. Gonçalves, who accepted to be my supervisor, to be a friend that help me many times, to give me the oportunity to learn more about Silviculture an to participate of PTSM/IPEF.

To Jean-Paul Laclau, who was my master supervisor and give me the first opportunity to work with research with CIRAD/FR. Thank you for some tips, moments and collaboration in all.

To Joannes Guillemot, who arrived in Brazil at dificult time of my doctorate. Thank you to make some effort to solve the problems about the equipments. I also thankfull about the explanations and formulate my project on deadline.

To Timóteo Herculano, who taugh me how to use and calibrate the equipment of Licor 6400. He also presented to me people which studied similar subject in Biossystem department at ESALQ.

To Toninho, my greats friends, who talked to me in difficult moments, for the happiness times at the laboratory and to share with me reflections and laughters.

I would also thankfull my collegues Amanda, Ivanka and Alvaro, who frequently motivate myself to finish the doctorate.

I'm also thankfull from professor Pedro Bancalion, who help me and support me in another moment.

Thank you all the stuff and Alexandre Ferraz, executive coordinator, of the program of IPEF/PTSM for all the structure and the finnancial supports.

To the member of ESALQ, Sabino, Natanael, Rildo Moreira, Elaine, Amarildo, Lorival and Toninho, thank you to give me support during my passage in the station at Itatinga.

To CAPES- Coordenação de Aperfeiçoamento de Pessoal de Nível de Superior – for the scholarship to development this research.

To University of São Paulo /ESALQ that support me there during these 4th years of my doctorate period.

SUMMARY

RESUMO

Uso da água e a produtividade de plantações de *Eucalyptus grandis*: efeito do manejo e das características do solo

A alta produtividade das florestas de *Eucalyptus* sp está diretamente associada aos recursos disponíveis no ambiente como água, nutrientes e luz, como também pelo uso eficiente dos recursos por diferentes genótipos. Diversos trabalhos foram realizados sobre o uso da água das florestas de eucalipto. Pesquisadores e gestores florestais sempre são questionados sobre o uso da água e a eficiência do uso da água pelas florestas de eucalipto. Diversos trabalhos mostraram que o uso da água em florestas de eucalipto podem alcançar 1100 mm a 1300 mm. No entanto, essas estimativas podem variar em função das condições edafoclimáticas e de manejo da floresta O objetivo principal dos estudos atuais é entender a dinâmica do ciclo da água em diferentes escalas da floresta, na folha, a árvore e na escala da bacia hidrografica. Alguns estudos vêm mostrando que a adubação, em particular com potássio, também pode aumentar a demanda de água por parte das árvores.O K propicia aumento de biomassa foliar e IAF das plantas, sugerindo aumento da transpiração das árvores. Estudos na escala da bacia hidrográfica, mostraram que bacias hidrográficas ocupadas por florestas de eucalipto podem reduzir o fluxo superficial dos cursos da água como também o estoque de água nas diferentes camadas do solo, o que pode comprometer a disponibilidade hídrica numa escala local. Por outro lado, alguns trabalhos mostraram que os impactos das florestas plantadas nos recursos hídricos dependem do regime pluviométrico local. Outros fatores podem influenciar o uso da água pelas florestas tais como a capacidade de retenção de água no solo, condições atmosféricas, espaçamento e genótipos. O principal objetivo dos gestores florestais, frequentemente, tem sido a busca por uma técnica de manejo florestal que permita o alcance de altas taxas de produtividade dos genótipos plantados. Atualmente, o manejo florestal não leva em conta a disponibilidade dos recursos ambientais, principalmente a água por meio do regime pluviométrico. Essas estratégias são importantes para aumentar a sustentabilidade das florestas plantadas, principalmente em áreas em que a disponibilidade de água é crítica. A compreensão da dinâmica dos parâmetros fisiológicos pode ajudar os gestores florestais a entenderem as respostas das árvores em função das condições climáticas e planejar estratégias que possam aumentar a sustentabilidade dos plantios florestais de *Eucalyptus* sp.

Palavras-chave: Uso da água; Eficiência do uso da água; Fotossíntese; Plantações de eucalipto; Condutância estomática; Condutância estomática.

ABSTRACT

Water use and wood productivity of *Eucalyptus grandis* plantations: effects of management, water supply and soil texture

Water availability is a strategy resource for forest plantation. Many authorities and researches are concerned about the water use and the impact of forest plantation. Water use and water use efficiency have been measuring in all over the world. High productivity of Eucalyptus sp plantation are depend of environmental resources such water, nutrients, light and also genotype resource efficiency. High water use is also associated with high productivity and water use efficiency. Many reports were published about water use in eucalyptus plantation. Water use might vary among 1100 mm to 1300 mm for eucalyptus plantation. Climate conditions, soil characteristics and forest management might influence water use. Currently, studies aim to understand hydrology cycle at the leaf, trees and on landscape scale. Previous studies showed that K fertilizer increase water demand. Leaf biomass and LAI also increase due to K fertilizer application, which suggest increase in water use. Eucalyptus forest plantation may also decrease water discharge in catchment, which might decrease water availability in local scale. However, transpiration varied within soil moisture, precipitation, temperature, atmosphere conditions, fertilizer application, genotype and latitude. The main objective of forest managers was often find a technique regime which forester may use to achieve the target growth rate. Nowadays, it is important to manage the forest taking to account local resource supply, local climate condition and resource availability. These strategies might increase the sustainability of forest plantation. Measurement of physiological functioning might help forests manager to understand the growth response of our forest as result of climate condition and also planing new strategies to increase forest sustainability.

Keywords: Water use; Water use efficiency; Photosynthesis; Eucalyptus forest management; Water deficit; Stomatal conductance

LIST OF ABREVIATION

MAI – Mean annual increment (m^3 ha^{-1} ano^{-1})

VPD – Vapour pressure déficit (kpa)

LAI – Leaf area index

LMA – Leaf mass area

WUE – Water use efficiency (kg m^{-3})

WUE_{ab} – Water use efficiency for aboveground biomass (kg m^{-3})

WUE_p- Water use efficiency for stemwood (g DM kg^{-1} H_2O)

GPP- Gross primary production

DBH – Diameter at breast height

Et- Evapotranspiration (mm)

ET_{EC} – Evapotranspiration measured by Eddy Covariance (mm)

T_{SF} – Transpiration measured by Sap Flow (mm)

ET_{TDR} – Evapotranspiration measured by Time Domain Reflectometer (mm)

PPFD- Constant photosynthetic photon flux density (µmol m^{-2} s^{-1})

1. INTRODUCTION

Eucalyptus productivity increased by a factor of 4 from 1970 to 2015, due to intensification of management, genetic improvements (Resende et al., 2012) and better silvicultural practices (Gonçalves et al., 2004). Eucalyptus forest plantation are among the most productive forest in the world. In Brazil, the majority plantation are locate on Tropical and Subtropical regions (Flores et al., 2016; Stape et al., 2010). Stem production across tropical region are among 9 to 45 Mg ha^{-1} $year^{-1}$ and 19 to 44 Mg ha^{-1} $year^{-1}$ on subtropical regions (Binkley et al., 2017).

Eucalyptus stands are planted on a short rotation (6-8 years) for pulp and paper and also for biomass (4 years). After the clear-cut, coppice management is common worlwide. Historically, Brazilian Eucalyptus coppice management consisted of three cycle, which one high forest clear cut followed by two coppice clear cuts (Souza, 2001). However, at high quality sites there are also possible to manage the forest for three to four cycles (Gonçalves et al., 2008). Two cycles of coppice management have been considering profitable and also lower establishment cost (Rezende et al., 2005). The silvicultural technology used minimum cultivation methods and high fertilizer rates. Both silvicultural technology guarantees similar wood production volume to those at first rotation (Gonçalves et al., 2008).

High growth rates and high forest productivity are result from genetic improvement and also silvicultural pratice such as site preparation, fertilization, spacing and weed control (Gonçalves et al., 2013). Climate and environmental characteristics influences the growth of planted forest of Eucalyptus. Water, nutrients and light are the main factors which affect forest productivity (Landsberg and Waring, 2014). In particular, water availability might limit tree growth in many tropical regions. A study conducted in the state of Bahia, Brazil, showed that double precipitation to 800 mm year-^{1} to 1600 mm $year^{-1}$ led to a 3-fold increase in wood growth, from 10 Mg ha^{-1} $year^{-1}$ to 30 Mg ha^{-1} $year^{-1}$ (Stape et al., 2004a). Suplemental water within sites increases Eucalyptus wood growth by 20-80% (Ryan et al., 2010; Stape, 2010). Hence, environmental changes in the future will determine the high rates of Eucalyptus growth. Water availability and annual precipitation regime may alter gross primary production and wood production by one-third to one-half (Stape et al., 2008). Regional changes in climate might change regional production (Binkley et al., 2017).

The main objective of forest management was often to maximizing forest productivity by manipulating resource supply, capture and resource use by the forest. The selection of the best genetic materials takes into account biotic and abiotic resistance and wood quality of materials (Mafia, 2016). Silvicultural researches improved our understanding about the responses of tree growth and wood quality to soil quality, fertilizers, spacing, weed control and soil preparation, which result high rate of growth for Eucalyptus plantation (Gonçalves et al., 2004).

Many sites currently used for Eucalyptus production were selected based on their ability to sustain forest productivity. The effect of silvicultural practices on physiological processes have been widely studied, especially the relationship between tree growth and environmental resource seasonality (Battie-Laclau et al., 2016; Binkley and Stape, 2004; Stape et al., 2010). Water, nutrients and light are the main factors that limit forest productivity. All these factors have a strong effect on tree physiological functions and forest productivity.

Water is important to physiology of plants and its also crucial role in all physiological process. It is responsible to transport carbohydrates, nutrients and phytohormones which are required for growth. Plants require a vast quantity of water. Aproximately 90% of N, P and K are absorbed and 10% to 70% of photosynthetically fixed carbon into new tissues. Water retained on biomass are less than 1%. The remainder is lost by transpiration (Lambers, 1998; Landsberg, 1999; Taiz and Zeiger, 2013).

Water is an important factor affecting forest productivity. Fast growth species, generally have high water use, which raises questions regarding both the ecological impacts of plantations and sustainability of wood production (Lima, 2011). These questions are important for forest sector. Quantifying both the water use and water use efficiency of fast-growing plantations in contrasting environmental contexts and management is thus a major research goal. Water use efficiency quantify the productivity of forest per amount of used water. It is therefore a good indicator of tree performance (King et al., 2013; Lévesque et al., 2014). Water use efficiency has been shown to vary across environmental conditions. It may be estimated at various spatial and temporal scales (Binkley and Stape, 2004).

Fast growth rates are commonly associated to high water use. Measurement of rainfall was correlated with mean annual increment (MAI), suggesting that water availability controls productivity in tropical regions. Many studies recognized the role of water in the control of forest productivity (Gonçalves et al., 2004; Santana et al., 2000; Stape et al., 2004b) but research quantifying water use and how it vary at different climate condition and contrasted soil texture are still unknown.

Water use by an Eucalyptus plantation in Southern Brazil has been shown to be 587 mm year^{-1}, 1397 mm year^{-1}, 1597 mm year^{-1}, 1491 mm year^{-1} and 1081 mm year^{-1} in the first 5 years after planting (Christina et al., 2016). Similar results were also reported in other experiments in Brazil such as 1371 mm year^{-1} (Battie-Laclau et al., 2016; Stape, 2010). Different results were also obtained in other studies in different environmental conditions (Forrester et al., 2010b; Hubbard et al., 2010, 2004). Vapour pressure deficit (VPD) and leaf area index (LAI) influence transpiration rate. VPD is the evaporative demand which determine evaporative rates from the forest canopies. It is also depend from energy balance, wind speed and radiant energy flow rates across the foiliage. Transpiration will be faster from forest canopies with large amounts of foliage (Landsberg and Waring, 2014) .

Large amounts of fertilizer are applied in Eucalyptus commercial plantations to reach high productivity (Battie-Laclau et al., 2016). In particular, potassium showed increase LAI (Stape, 2002), photosynthesis, stomatal regulation and transpiration (Battie-Laclau et al., 2014b, 2014a; Taiz and Zeiger, 2013; Whitehead and Beadle, 2004a) and also to change leaf water potential (Battie-Laclau et al., 2016). Potassium supply may also change nutrient use efficiency, light use efficiency (Christina et al., 2015; Stape et al., 2004b) and water use efficiency (WUE) (Battie-Laclau et al., 2016, 2014b, 2013; Laclau et al., 2009). Wood production, gross primary productivity (GPP) and photosynthetic capacity was also increase by potassium fertilization (Battie-Laclau et al., 2014a).

Potassium supply increased canopy transpiration and water use efficiency for stemwood production by 1.7 and 60%, respectively, 2 year after planting in a Eucalyptus plantation of Southern Brazil (Battie-Laclau et al., 2016). Water use efficiency results have shown ranged 1.1 to 1.9 stemwood m^{-3} of water transpired for potassium fertilizer. These results were corroborated by other experiments (Stape et al., 2004b). Resource use efficiency have been shown positively correlate with growth rate in tropical Eucalyptus forest plantations. Breeding programs have tried to select trees with high growth rates, which may have in turn led to select trees with high water use efficiency for stemwood production (Battie-Laclau et al., 2016).

A growing body of evidences suggest that intensive management practices aiming to increase the productivity of Eucalyptus plantations also increase resource use efficiency, including light use efficiency (Binkley and Stape, 2004; Campoe et al., 2012), water use efficiency and nitrogen use efficiency (Binkley and Stape, 2004). However, it remains clear that the total amount of ressource use by trees also increases with forest productivity (Battie-Laclau et al., 2014b).

Many authorities are concerned with the high rate of water use by eucalyptus plantation. Some strategies have been discussed to improve land-use management at the watershed scale with the objective to decrease total water use (Lima, 2011). Forest plantations and water resource are at the center of political concerns due to the potential strong impact of Eucalyptus plantations on water resource at regional scale (Lima, 2011). The impact of Eucalyptus plantations on water resource has however to be apreciated in the light of local environemental context.

Many strategies have been proposed to reduce the envionmental impact of fast-growing forest plantations, including the creation of reserves for native habitats, mosaics of natural and exotic vegetation, longer rotation length and management for multiple use on land use planning (Gonçalves et al., 2017). At the stand level it has been proposed alternative genetic materials and lower stocking density (Ferraz et al., 2013; Hakamada et al., 2017). Researchers have recommended to revisiting the management of Eucalyptus plantations, taking into account the characteristics of local area to reach the best trade-off among ecosystem services and wood production.

In the future, sustaining eucalyptus forest productivity under climate change will be the main challenge of practitioners. Research on climate change have predicted reduction in rainfall in most tropical regions, where forest Eucalyptus plantation are concentrated (IPCC, 2013). More intense and frequent drought periods will affect these plantations, which might reduce tree growth and pattern of water use (Wu et al., 2011). Drought events might reduce Eucalyptus plantation yield by as much as one third over the six to seven year of a commercial rotation in Brazil (Almeida et al., 2010). In addition to this, Eucalyptus plantations might be exposed to higher fire hazard and pest infestation. Elevated temperature and CO_2 concentration will also have independent impacts on Eucalyptus trees physiology (Booth, 2013). However, the interacting impacts of these various factors on forest productivity remain unknown. Adaptive strategies should be adopted by forest managers to overcome the challenges brought by climate change for sustainable wood production (Booth, 2013).

There are some gaps about the relationship of water use and water use efficiency in two contrasted soil texture and also physiological studies about Eucalyptus coppice management. It is defined two chapters on this thesis. In the first chapter, the objective is to evaluate the effect of soil texture (clayish and sand soil) on stand characteristics, water use and water use efficiency and comparison between Sap Flow measurements, Time Domain Reflectometer and Eddy Covariance system. The second chapter will address stand characteristics, water use and water use efficiency for stemwood production, photossynthetic rates, transpiration and intrisic water use efficiency on Eucalyptus plantation manage for coppice. The chapter are:

1) Effects of soil texture on transpiration and water use efficiency for stemwood production in a commercial Eucalyptus plantation.
2) Effect of potassium and water supply on productivity and water use in *Eucalyptus grandis* plantation managed as coppice.

With these two chapters, I expect to partially discuss the gaps of knowledge about the relationship of water, use and soil texture in commercial Eucalyptus stands and analyze the effect of potassium fertilizer applycation on psysiological parameter on Eucalyptus manage for coppice.

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2. EFFECTS OF SOIL TEXTURE ON TRANSPIRATION AND WATER USE EFFIENCY FOR STEMWOOD PRODUCTION IN A COMMERCIAL EUCALYPTUS PLANTATION

ABSTRACT

Water use by Eucalyptus forest plantation is associated to high growth rate and productivity. Forest productivity is strong related to environmental resource availability, climate conditions and soil characteristics. Information about water use and water use efficiency in different soil texture are scarce in the literature. This study set out to gain insights into the effect of soil texture on water use and water use efficiency in a commercial forest plantation. Transpiration and growth were measured at mid-rotation, between stand ages of 3.8 years and 4.8 years, with seedling of *E. grandis* in Ferrasol (FAO) at clay content of (22% to 25%) and sand (16%) soil texture, at the same total stocking density, in a randomized block experiment. Water use efficiency was also calculated. Granier sap flow probes were used to estimate the transpiration over one year. Measurement of sap flow, time domain reflectometer and eddy covariance were compared at daily, monthly and annualy scale. Regression was established among sap flow sensor and eddy covariance for evaluating the correlation an daily and monthly scale. The highest aboveground biomass at mid-rotation was obtained for both sites (121 Mg ha^{-1}). Annual transpiration was higher in clay content plot (1220 mm) than on sand soil texture (1170 mm). WUE was 1.7 kg dry matter (DM) m^{-3} in *E. grandis* at clay content plot and 1.5 kg dry matter (DM) m^{-3} in sand soil. Evapotranspiration method of eddy covariance was higher than evapotranspiration of TDR and transpiration measured by sap flow. Regression established between Eddy Covariance and sap flow have shown R^2 ranged among 0.64 and 0.63 at daily and monthly scale at clay content, while for sand soil texture R^2 ranged 0.52 and 0.6 for daily and monthly scale respectively. In our study, we did not find any differences of aboveground biomass, transpiration and water use efficiency across clayish and sandy sites. They were not statiscally significant, except volume increment. Future studies should be developed to compare tree functioning across larger texture gradient. The difference in texture did not affect transpiration, water use and water use efficiency, not the mean characteristics of the studied Eucalyptus forest plantation.

Keywords: Transpiration; Water use; Water use efficiency; Clay content; Sand soil texture

2.1. Introduction

Eucalyptus forest plantations are major tree crop covering more than 20 million ha globally (Booth, 2013). In Brazil, Eucalyptus forest plantation area cover approximately 5.8 million hectare. Others major areas for Eucalyptus plantations include India (3.9 million of ha), China (2.2 million of ha), Portugal (800 000 ha) and Spain (500 000 ha) (GIT Consulting, 2009).

Eucalyptus species are fast growing, with high rate of growth and productivity. The productivity per ha of Eucalyptus plantation has increased by a factor of 4 from 1970 to 2015, thanks to intensification of management, research in genetic improvements (Resende et al., 2012) and improvement of silvicultural practices (Binkley et al., 2017). Forest productivity is strongly related to environmental resource availability and physiological functioning. Water and nutrient are the main determinants of forest productivity (Mitchell, 1992). Availability of water and nutrients indeed increases leaf area index, stomatal conductance and photosynthesis rates, with positive impacts on tree growth. In addition, climate conditions and soil characteristics also influenced forest productivity (Caldato and Schumacher, 2013; Lambers, 1998; Lima, 2011).

Eucalyptus planted forests are currently found in very contrasting climates worldwide. Eucalyptus is common planted in Ferrasol that comprise a range of clayish to sandy textures (FAO). The water retention capacity in clay content soil is greater than in sand soils. Clay content soil has indeed a stronger matric forces and porosity than clay. Soils with high clay content has higher capacity to adsorbed and retain water. The water stock may reach 75 mm m^{-1} in soils with 10% of clay content to 200 mm m^{-1} in soil with 80% of clay (Binkley et al., 2017).

Many studies have demonstrated a strong relationship between soil water supply and wood production. Some studies estimated that water availability may increase wood productivity by 15-35% even in dry areas (Binkley et al., 2004). Doubling precipitation from 800 mm $year^{-1}$ to 1600 mm $years^{-1}$ led a 3-fold increase in growth rate (from 10 Mg ha $year^{-1}$ to 30 Mg ha $year^{-1}$) (Binkley et al., 2017; Campion et al., 2004; Stape et al., 2008).

High productivity is also associated to high water use (Binkley and Stape, 2004). Forest plantation of fast-growing species are characterized by deep root system, non-zero leaf area index over the whole rotation and high evapotranspiration (> 1100 mm), (Dye and Versfeld, 2007). Water use by Eucalyptus plantations is also a matter of debate by authorities, as it can lead to reduction in the discharge of catchments (Almeida et al., 2016) and decrease in soil water availability (Albaugh et al., 2013; Christina et al., 2016). Water use by fast growing species are an important subject to assess the sustainability of forest plantation and to define the best management strategy.

Many reports were published about water use of eucalyptus. Hubbard et al. (2004) reported the short and long-term effects of fertilization on transpiration in an experimental forest near Hilo. In this study, water use was estimated to reach *ca.* 487 mm $year^{-1}$ in fertilized plots during the 5 months following fertilization and remained 401 mm $year^{-1}$ greater than control treatment on the long run. In Brazil, measurements of water use in *Eucalyptus grandis* plantation reached 750 mm $year^{-1}$ in the first year, 1300 mm $year^{-1}$ in the third years (Battie-Laclau et al., 2016) and 1229 mm $year^{-1}$ in the fifth year after planting (Battie-Laclau et al., 2016). Measurements in plantations established in South Africa gave an estimated transpiration above 1100-1200 mm (Dye, 1996). Little is known about the variability of water use in Eucalyptus trees across soil conditions, weather and tree age. It is important to compare water use in a wide variety of genotype on uniform site to compare water use by trees and the interactions with local climate condition. It is also important to understand the relationship of environmental issues and resources, such as water use, with management and tree growth on long-term experiment (Albaugh et al., 2013).

Tree growth is affected by water and nutrient availability as well as resource use efficiency and biomass partitioning in planted forest (Binkley and Stape, 2004; Stape et al., 2004b). Water use efficiency is a simple parameter, which is define by the ability of ecosystem to capture carbon and produce biomass as a function of water use. WUE can be estimated in different ways, such as intrinsic water use efficiency (WUEi) (Osmond et al. 1980), leaf WUE with isotope signature (Farquhar and Richards, 1984) and water use efficiency for stem production (Battie-Laclau et al., 2016). Water use efficiency is also a useful metric to comparing species performances and it is crucial to understand the relationship between water use and wood production. Comparisons of the different methods (and associated scales) to estimate water use efficiency are scarce in the literature and commonly show that different methods lead to different results and conclusions.

Eucalyptus forest plantations generally have higher water use efficiency than other tree species (Binkley et al., 2004) and also use water more efficiently than species with lower growth rates (Otto et al., 2014). Stape et al. (2004) showed that water use efficiency increased with water supply. In this study, WUE was estimated for different tree productivity classes (low, medium and high), and was estimated to be *ca.* 1.59, 2.24, 3.21 kg m^{-3}, respectively.

Similar results was obtained in "throughfall exclusion experiment". WUE ranged of 0.8 – 1.2 kg of stemwood per m^{-3} water transpired in the second year after planting and 0.9-1.9 kg of stemwood m^{-3} water transpired at the third year (Battie-Laclau et al., 2016).

Eucalyptus genus are the most planted species in tropical and subtropical regions (Harwood and Nambiar, 2014). Information about water use and water use efficiency taking account different soil texture is scarce in the literature but highly needed. This study set out to gain insights into the effect of soil texture on water use and water use efficiency in a planted clonal forest of *Eucalyptus grandis*. Sap-flow measurments and dendrometric inventories were used to estimate WUE at two sites with contrasting soil textures, located within the footprint of an Eddy-covariance tower. Sap-flow derived WUE was subsequently compared to WUE derived from Eddy-covariance data at anuall, monthly and daily scales.

2.2. Material and Methods

2.2.1. Study area

The study was conducted in the commercial *Eucalyptus grandis* plantation, managed by the EucFlux project (www.ipef.br/eucflux/en), at Itatinga (22° 58' 04"S, 48° 43'40"W) in southeast Brazil, São Paulo State. Mean annual rainfall was 1340 mm. Rainfall are concentrated from October to May. Mean annual temperature is 19.5°C (16.3°C on average from June to August and 22.2°C on average from December to February). The mean annual rainfall from January 2010 to December 2014 was 1430 mm $year^{-1}$. The mean annual relative humidity is 76%. Soils were classificated as Ferrasols (FAO classification), developed on Cretaceous sandstone of the Marília formation, Bauru group. The relief is typical from São Paulo western plateaus, with a gently undulating topography. The slope was 5%. Maximum elevation was 760 m above sea level. Soil properties were representative of the most soil common type, where Eucalyptus plantation are established in Brazil. (Table 1). In the top soil, clay content ranged about 16% at the sand soil and 22% - 25% on clay soil from 2 – 10 m soil layer (Pinheiro et al., 2016b).

Table 1. Main physical and chemical soil properties across all the sampling positions.

Soil Layer	Sand	Silt	Clay	pH	O.M[a]	P_{resin}[b]	Base cations	CEC
(cm)	Particle size distribution (%)			$CaCl_2$	g kg^{-1}	mg kg^{-1}	$mmol_c$ kg^{-1}	
0-25	80.8 ± 2.0	3.5 ± 0.8	15.8 ± 0.1	3.89 ± 0.12	13.2 ± 3.2	4.2 ± 0.8	6.5 ± 0.5	51.0 ± 15.4
25-50	79.2 ± 1.6	3.2 ± 0.9	17.7 ± 1.0	4.12 ± 0.06	8.8 ± 0.6	4.7 ± 0.5	5.8 ± 0.5	34.6 ± 1.8
50-100	77.0 ± 1.6	4.2 ± 0.9	18.9 ± 1.0	4.10 ± 0.02	7.4 ± 0.4	4.7 ± 0.6	5.8 ± 0.8	29.9 ± 2.2
100-400	71.9 ± 1.6	4.8 ± 2.1	23.3 ± 1.1	4.46 ± 0.08	7.4 ± 1.6	4.8 ± 0.3	6.4 ± 0.5	22.0 ± 1.0
400-800	67.3 ± 1.0	8.0 ± 4.1	24.7 ± 3.2	4.61 ± 0.12	4.7 ± 0.6	5.1 ± 0.6	6.4 ± 1.3	19.2 ± 1.6
800-1200	69.2 ± 1.9	9.1 ± 4.4	21.7 ± 3.1	4.37 ± 0.12	4.5 ± 0.2	4.5 ± 0.3	6.2 ± 0.3	21.0 ± 5.4
1200-1400	82.0 ±8.6	4.1 ± 1.6	14.0 ± 7.1	4.39 ± 0.04	4.1 ± 0.3	4.8 ± 0.7	5.9 ± 0.8	15.4 ± 1.1

[a] Organic matter (OM) determined used sodium dichromate.

[b] Resin extraction of phosphorus and exchangeable element determination.

2.2.2. Measurement sites

This study was carried out within the footprint of an Eddy-covariance tower, at two sites presenting contrasting soil textures.Both plots comprised 12 x 14 trees. The clone of *E. grandis* planted in the experiment parcel was widely used in the São Paulo State, and management followed commercial standards. Trees were planted with a spacing of 3 x 2 m, followed minimum cultivation techniques of site preparation (Gonçalves et al., 2004). Glyphosate was used (4 l ha^{-1}) to eliminate competing vegetation until canopy closure (~18 months). Leaf cutting ants were controlled. It was used sulfuramide-based baits (1.3 kg ha^{-1}) whenever necessary. Fertilization regime used in a commercial plantation were applied: 18 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, 30 kg K_2O ha^{-1} and 4 Mg ha^{-1} of dolomitic lime at planting, 31 kg N ha^{-1}, 36 kg K_2O ha^{-1} at age 4 months, 31 kg N ha^{-1}, 67 kg K_2O ha^{-1} at age 12 months and 151 kg K_2O ha^{-1} at age 24 months (Figure 1).

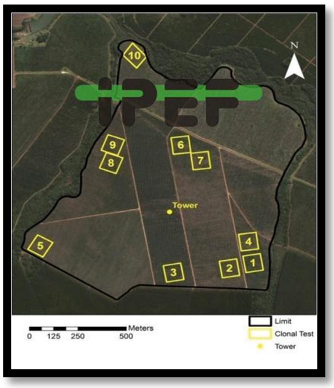

Figure 1. Experimental design of Eucflux project. The location of the tower plot corresponds to the sandy site, the plot number 8 corresponds to the claysish site.

2.2.3. Stand growth and aboveground biomass

Tree diameter at breast height (DBH) and total height were measured every three months from March 2013 to March 2014 at both clayish and sandy sites. Allometric equations were calibrated based on the destructive sampling of 10 trees at each plot, encompassing DBH distribution. Destructive sampling was realized on February of 2013. The selected trees were harvested and divided into components: leaves, branches, stemwood and bark. Tree stems were sawn and measure for volume estimation. Leaves was divided in three different sections of the crown (high, medium, low). All components were weighted in the field. Twenty leaves from each tree were scanned. Samples of all components were taken and dried at 65°C. Areas of the scanned leaves were estimated using the program Image J and leaf mass per area (LMA) was calculated. Average dry matter content (ratio of the sample dry mass over the fresh mass) were calculated combined to field-based fresh weight to calculate the dry biomass of all tree components. LMA was combined to fresh leaves weight to estimate LAI. Allometric equations between diameter at breast heigh (DBH) and aboveground biomass, LAI and volume were calibrated and combined to DBH inventories to estimate biomass components and LAI at plot level.

2.2.4. Soil Moisture Measurements

Soil water content was measured using TDR probes (CR100 datalogger, multiplexer AM16/32, CS616 Campbell Scientific) installed at the depths of 0.15, 0.50, 1.00, 2.00, 3.00, 4.00 and after in each 2.00 m until 10.00 m at both sites (Figure 2). The sensors were calibrated in the field by gravimetric soil water content and bulk density measurements. Soil water content was measured every 30 min. Soil water content was compared to sap flow measurements. Comparison of soil water content with sap flow was important to evaluate the reliability of transpiration estimates. Annual measurements of evapotranspiration (Et) were made by integrating soil water content down to the depth of 10 m, using the water balance equation (Equation 1).

$$\mathrm{Et} = (\mathrm{Rx}0.9) + \mathrm{S}_{\mathrm{wfinal}} - \mathrm{S}_{\mathrm{winicial}} - \mathrm{D} \qquad (1)$$

Where R (mm) is the rainfall, Sw_{final} (mm) and $Sw_{inicial}$ (mm) is soil water storage, D (mm) is drainage below 5 m depth. Interception losses were considered to be 10% of rainfall during the period (Almeida et al., 2007). Deep drainage was not considered.

Figure 2. Measurement of volumetric water content Time Domain Reflectometer (TDR) up to 10 meter at the study site. Source: Nouvellon (2010).

2.2.5. Sap flown, tree water use and stand transpiration

Sap flown measurement was conducted at both clayish and sandy sites. Sap flow density (v, g H_2O m^{-2} sapwood s^{-1}) was measured from March 2013 to February 2014 (365 days) in 15 trees at each site. The selected trees encompassed the DBH distribution of the stands. They were equipped with one sensor at ~1.30 m above the ground. The sap flow sensors were protected from external temperature variations and water intrusion by a reflective foil. Sensors output voltage was recorded every 30 min (CR1000 dataloggers; AM16/32 and AM416 multiplexer). Sap flow probes were checked every 15 days. All sensors out of reading and physically damaged were replaced. The probe direction was moved every 3 months for *Eucalyptus grandis* to newly drilled holes in another position round the trunk (selected at random) to prevent underestimation of sap flow due to fast tree growth. The sap flow density was calculated using a calibration equation determined in a preliminary study (Delgado-Rojas et al., 2010). The sapwood area at ~1.30 m above the ground was determined by dye injection in 10 trees felled at the age of 4.5 months at both sites (total of 50 trees). The allometric relationship between DBH and sapwood area was determined for each site and applied to the trees equipped with sap flow sensors. Transpiration for each tree was calculated as the product of the sap flow density and the sapwood area estimated from successive measurements of DBH over the study period. The sensors were assumed to measure the instantaneous sap velocity integrated over the average sapwood thickness

of each tree. For each tree equipped with a sap flow sensor at both sites, the relationship between the daily total transpiration (dependent
variable) and DBH^2 (independent variable) was determined by linear regression and were applied in all trees at both sites (Kunert et al., 2012).

There were gaps in the readings of the sap-flow sensors in one week for the clayish site. Missing values were estimated using empirical relationship between estimated stand evapotranspiration (ETo; (Allen, 1998)) and measurements of daily transpiration for the same trees before and after sensor failure. The R^2 between daily total transpiration and DBH^2 over the study period ranged from 0.60 to 0.80 at both sites. These regressions were then used to predict daily stand transpiration in all the trees of the plots (Figure 3).

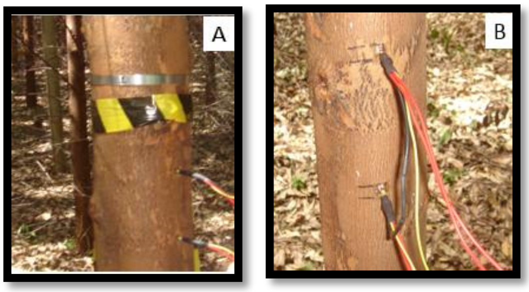

Figure 3 Measument of growth (A) in the trees and sap flow probes installed at 1.30 meter above the ground at both clayish and sandy sites (Eucflux project) (B).

Sap flow density calculated by Granier equation sub-estimated stand transpiration in *Eucalyptus* sp trees in preliminary tests. Delgado-Rojas (2008) thus established an equation for sap flow density estimation in *Eucalyptus grandis* (Equation 2) that was used in this work.

$$u = 478.017 \times 10^{-6} \times k^{1.231} \times SA \qquad (2)$$

Where "k" is a constant between sap flow density and temperature, "SA" is sapwood area.

2.2.6. Eddy Covariance measurement

Eddy Covariance (EC) system was installed above the canopy of the plantation in 2009. The EC system was equipped with 3D sonic anemometer (Young Ultrasonic 3D Anemometers), net radiation, wind speed, air temperature, relative humidity, diffuse PAR and rainfall sensors. The 3D sonic anemometer measured the three components of wind speed at very high temporal resolution (20 Hz). Licor (Li-7500) was used to measure the atmospheric fluctuations of latent heat (H), sensible heat (HE) and CO_2. Raw data were processed using the software Eddy Pro, and evapotranspiration was calculated from LE using constant latent heat of vaporization and density of water. The measurement of Eddy Covariance was compared with sap flow at daily and monthly scales. Annual measurements of evapotranspiration were compared with sap flow-derived estimates and TDR installed down to 10 m at the tower plot (Figure 4).

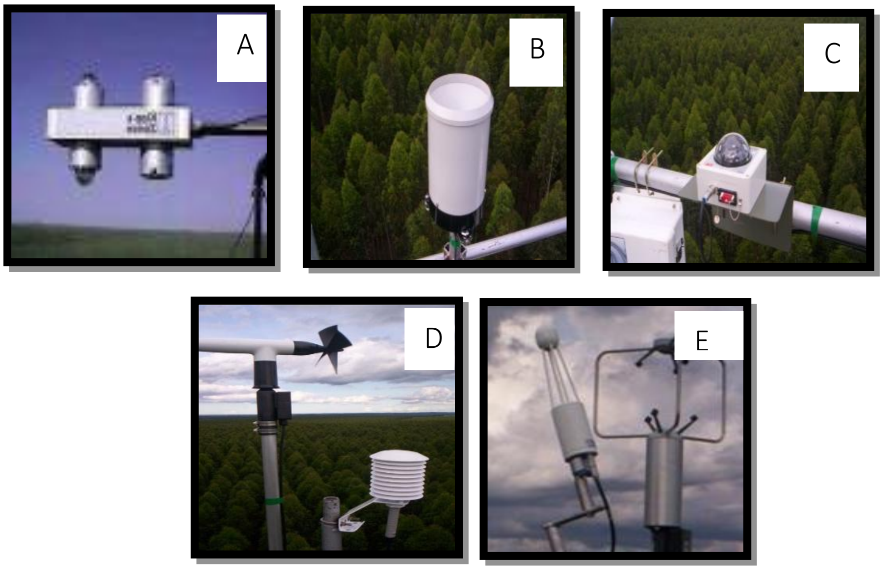

Figure 4. Eddy Covariance system installed at the experiment area. Incident shortwave and long-wave IR radiation (A) Rainfall measurement (B), Difuse PAR sensor (C) , wind speed, air temperature and relative humidity (D) and LI-7500 and 3D Somic anemometer (E). Source: Nouvellon (2010).

2.2.7. Water use efficiency

Stem growth variation was measured once a week in all the treatments by dendrometers (Botosso, P.C. ; Tomazello-Filho, 2001). DBH was measure at the beginning of the study. The variation of growth measured by dendrometer was added to initial DBH to obtain actual DBH. Allometric relationship between stemwood (kg) and DBH^2 (cm^2) of each treatment were applied in each trees at both sites. Stemwood and transpiration of each tree were calculated as the sum of each period. Water use efficiency was calculated in each period sharing stemwood biomass (kg) by the amount of water transpired (m^{-3}) over the same period (Equation 3).

$$WUE = \frac{\Delta \text{ Biomass (kg)}}{\text{Transpiration } (m^{-3})} \qquad (3)$$

2.3. Results

2.3.1. Biomass Production

Eucalyptus forest plantation on clayish soil showed higher volume, volume increment, and aboveground biomass increment than in sandy soil. Volume, volume increment and aboveground biomass increment were 9%, 29% of and 20% higher in clayish than in sandy soil, respectively. Sapwood area and sapwood area increment was comparable across sites (Table 2).

Table 2 Main characteristics of the stands: volume, volume increment, aboveground biomass, aboveground biomass increment, sapwood area and sapwood area increment monitored from 3.8 years to 4.8 years after planting.

Parameter	Site	
	Sand	Clay
Volume (m^3/ha)	188(a)	201(a)
Volume Increment (m^3/ha/year)	25(a)	35(b)
Aboveground biomass (Mg/ha)	121(a)	121(a)
Aboveground biomass increment (Mg/ha/year)	17(a)	21(a)
Sapwood area (m^2/ha)	12(a)	11(a)
Sapwood area increment (m^2/ha/year)	1.2(a)	1.1(a)

*Significant effect on parameter was evaluate at $p<0.05$ at both sites.

2.3.2. Transpiration and water use efficiency

Leaf area index (LAI) measured on destructive sample biomass was 10 % higher on clayish than on sandy soil. Both site showed the same transpiration (Table 3). There were not found statiscally diference on mean characteristics of leaf area index, transpiration and water use efficiency for aboveground biomass (WUE_{ab}) and stemwood (WUE_p). Both WUE_{ab} and WUE_p was not significant. The results showed that water use efficiency for aboveground was 1.5 in sandy soil plot and 1.71 kg of aboveground biomass per m^{-3} of water transpired on clayish soil. WUE_p for stemwood was 2.9 on clayish soil and 2.1 dm^3 stemwood m^{-3} of water transpired on sandy soil, respectively (Table 3).

Table 3. Mean characteristics of the stand. Leaf area index, transpiration, water use efficiency from 3.8 to 4.8 years after planting.

Parameter	Site	
	Sand	Clay
LAI (m^2/m^2)	5.5 (a)	6.0 (a)
Transpiration (mm)	1179 (a)	1221 (a)
WUE_{ab} (kg of aboveground biomass/m^3 of H_2O)	1.5 (a)	1.7 (a)
WUE_p (dm^3 stemwood/m^3 of H_2O)	2.1 (a)	2.9 (a)

*Significant effect on parameter was evaluate at $p<0.05$ at both sites.

Highest values of transpiration followed evapotranspiration. Maximum and minimum values of transpiration over the study period was comparable across sites, reaching 6.5 mm day^{-1} and 0.3 mm day^{-1}, respectively (Figure 5a, 5b and 5c). Time series of transpiration on clayish and sand soil showed positive correlation among global radiation and vapour pressure deficit (Figure 6a, 6b, 6c and 6d).

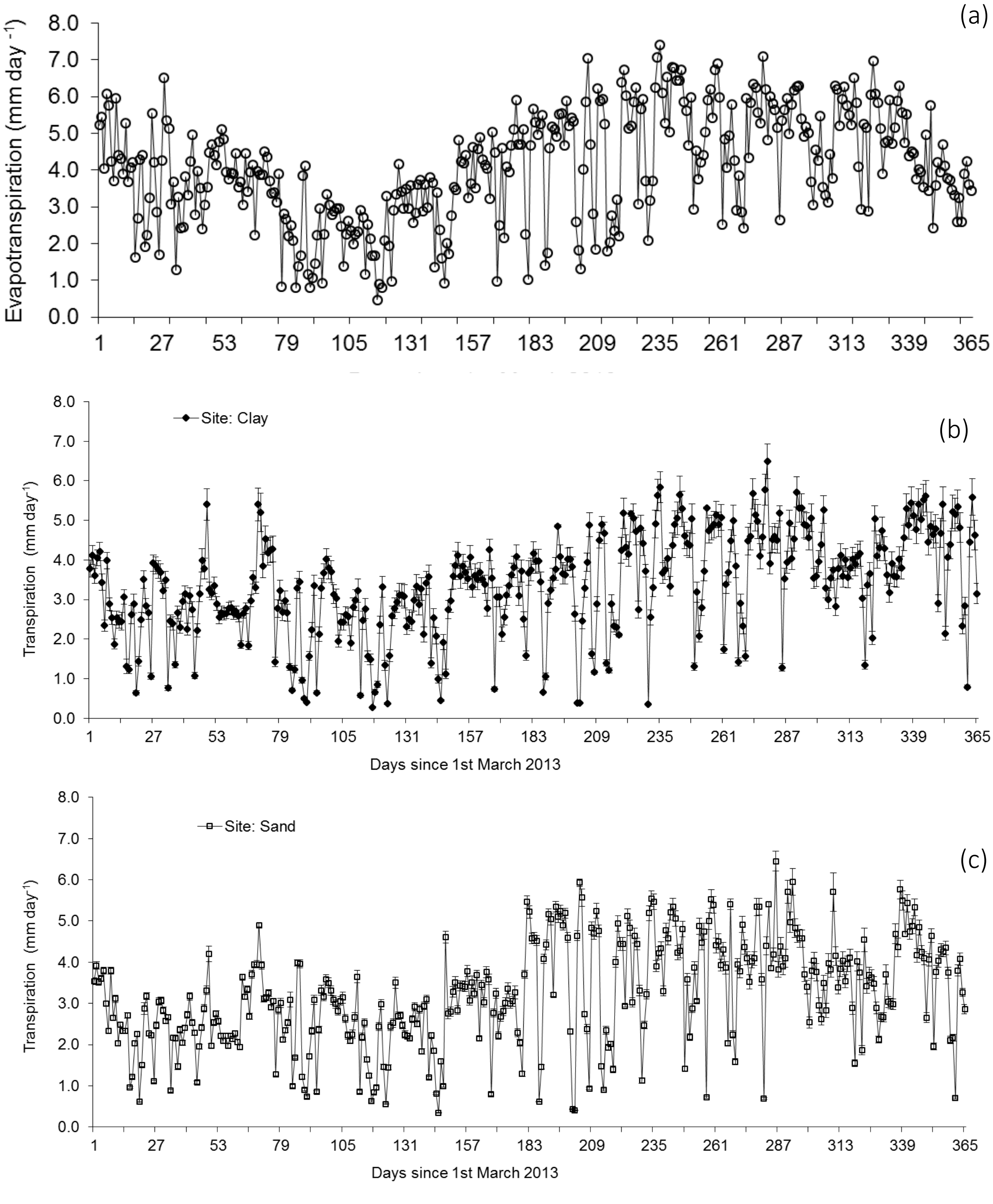

Figure 5. Daily potential evapotranspiration measured by Eddy covariance system at the study site (a), daily stand transpiration in the Eucalyptus sp at clayish and sand soil from March 2013 to February 2014.

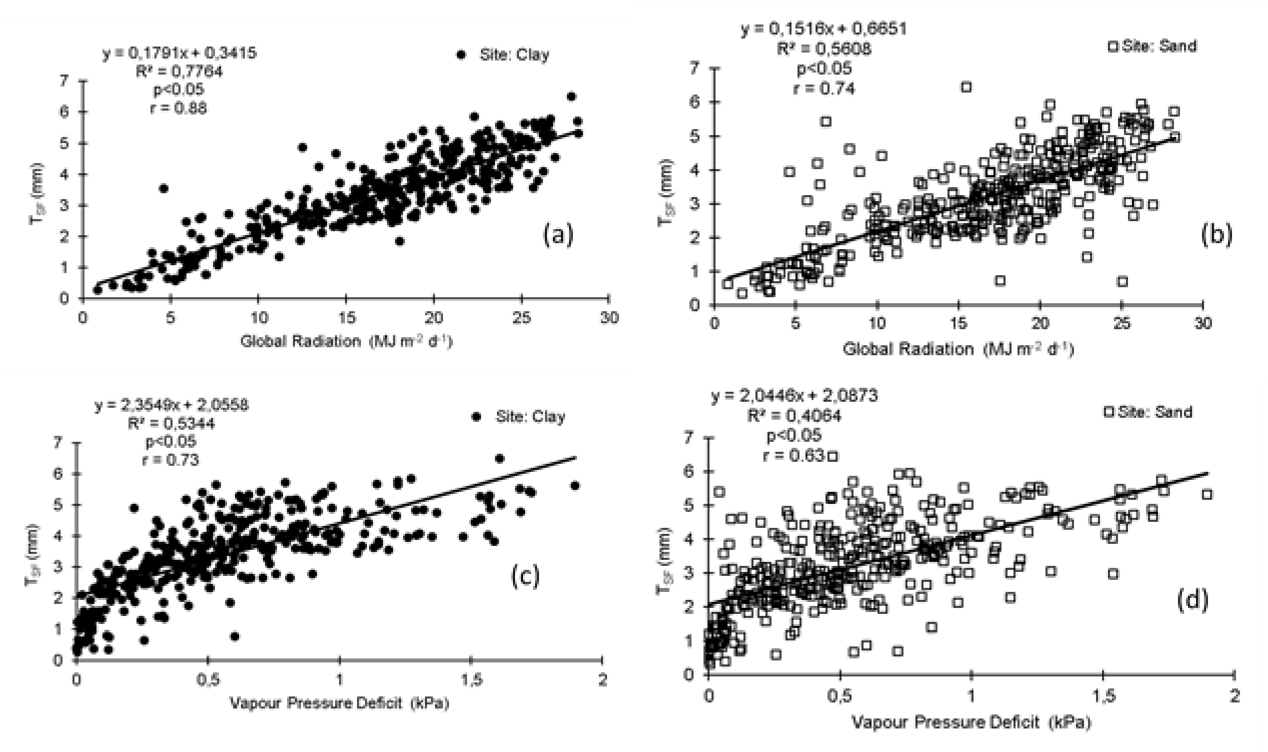

Figure 6. Relationship among transpiration measured by sap flow (T_{SF} mm) on clayish and sand soil among Global radiation (a and c) and vapour pressure deficit (c and d).

Accumulated transpiration over the study period reached 1220 mm and 1170 mm for clayish and sandy soil, respectively. Total precipitation reached 1372 mm over the same period. Transpiration at clayish and sandy sites was equivalent to 78% and 75% of total annual precipitation, respectively (Figure 7).

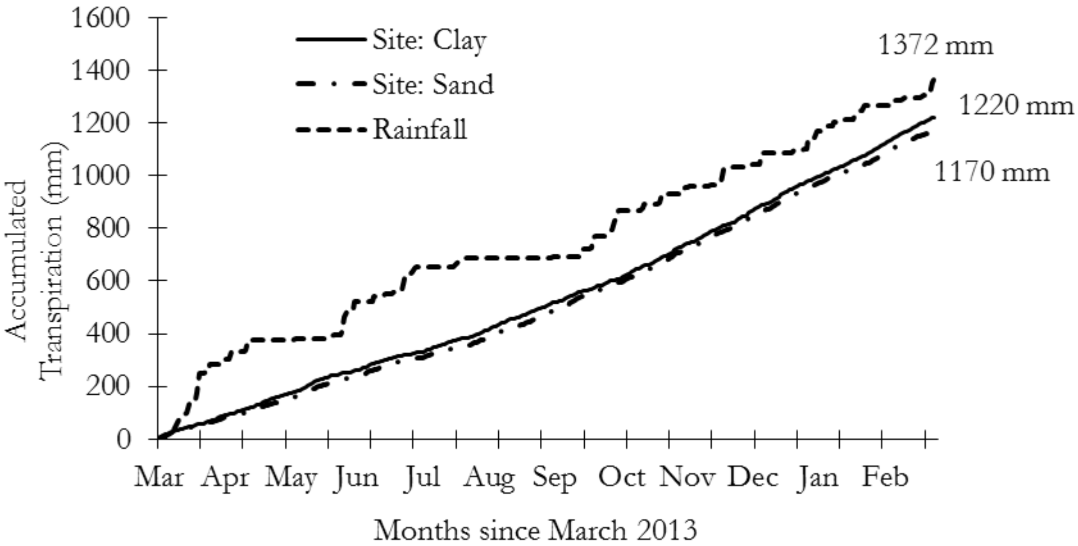

Figure 7. Accumulated transpiration of seedling of Eucalyptus *grandis* at clay texture site and sand texture site from March 2013 to February 2014.

2.3.3. Intra-annual variation of water use efficiency through the year

Time series of water use efficiency for stemwood on *Eucalyptus* sp plantations at sand and clay soil content was comparable. The highest WUE observed in sandy soil was 3.67 ± 0.9 g DM kg^{-1} H_2O and 3.5 ± 0.7 g DM kg^{-1} H_2O on clayish soil. Minimum WUE was also comparable in both treatment, Eucalyptus on clayish soil showed 0.31-± 0.9 g DM kg^{-1} H_2O and 0.37 ± 0.7 g DM kg^{-1} H_2O on sandy soil. The stemwood biomass production of *Eucalyptus* sp on clayish and sand soil followed patterns comparable to other measured variable. *Eucalyptus* sp on clayish soil showed average of 41-± 15.9 g $tree^{-1}$ day^{-1} and 40 ± 20 g $trees^{-1}$ day^{-1} in sandy soils, respectively (Figure 8a and 8b).

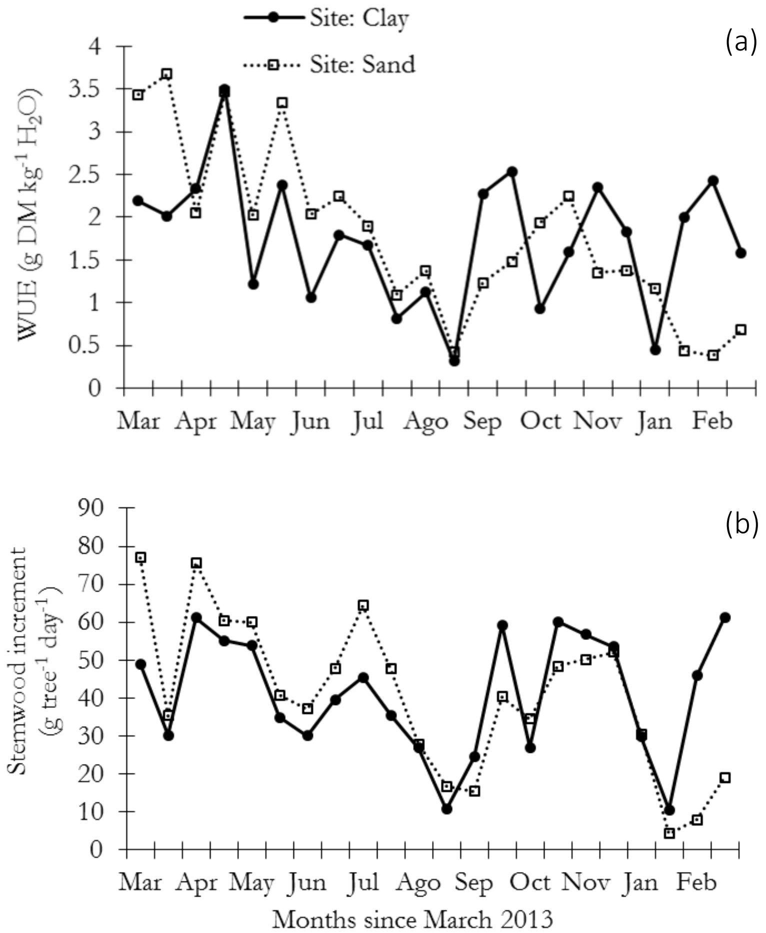

Figure 8. Water use efficiency (a) and stemwood increment (b) of seedling Eucalyptus *grandis* plantation in Ferrasol (FAO Classification) on clayish and sandy soils from 1° March of 2013 to March 2014.

2.3.4. Comparison of Eddy Covariance, Sap Flow and TDR probes

Eddy Covariance measurements showed greater maximum value (7.4 mm day^{-1}) followed by transpiration from sap flow (6.5 mm day^{-1}). The minimum evapotranspiration value measured by Eddy Covariance was 0.46 mm, followed by sap flow estimate at sandy soil (0.34 mm) and clayish (0.28mm) site. Annual total evapotranspiration measured by Eddy Covariance was 1487 mm, while sap flow probes measurement was 1220 mm at clay site and at Tower site was 1170 mm. Measurement by Time Domain Reflectometer (TDR) was 1147 mm at clay content site and 1109 mm at sand content site (Figure 9).

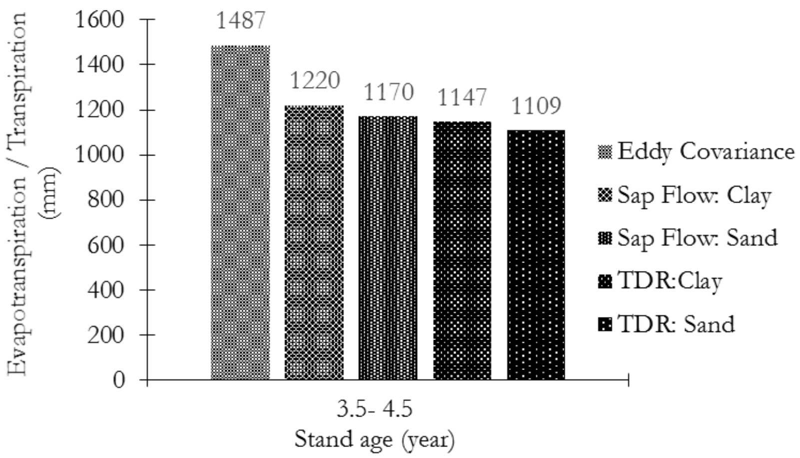

Figure 9. Annual evapotranspiration measured by Eddy Covariance method, transpiration measured by Sap Flow at both site (clay content and sand content) and evapotranspiration measured by TDR probes (clay content and sand content) from March of 2013 to February of 2014.

A regression was established between evapotranspiration (ET_{EC}) measured by Eddy Covariance and Sap Flow measurement (T_{SF}) at daily and montly scale. Sap Flow-derived estimate of transpiration were lower than EC-derived evapotranspiration (ET_{EC}). The determinants coeficients at daily and monthly scale for clay content was 0.6 for both site (Figure 10a and 10b). Similar results were found at the sand soil content between evapotranspiration measured by Eddy Covariance (ET_{EC}) and transpiration at sand soil by sap flow measurement (T_{SF}) (Figure 11a and 11b).

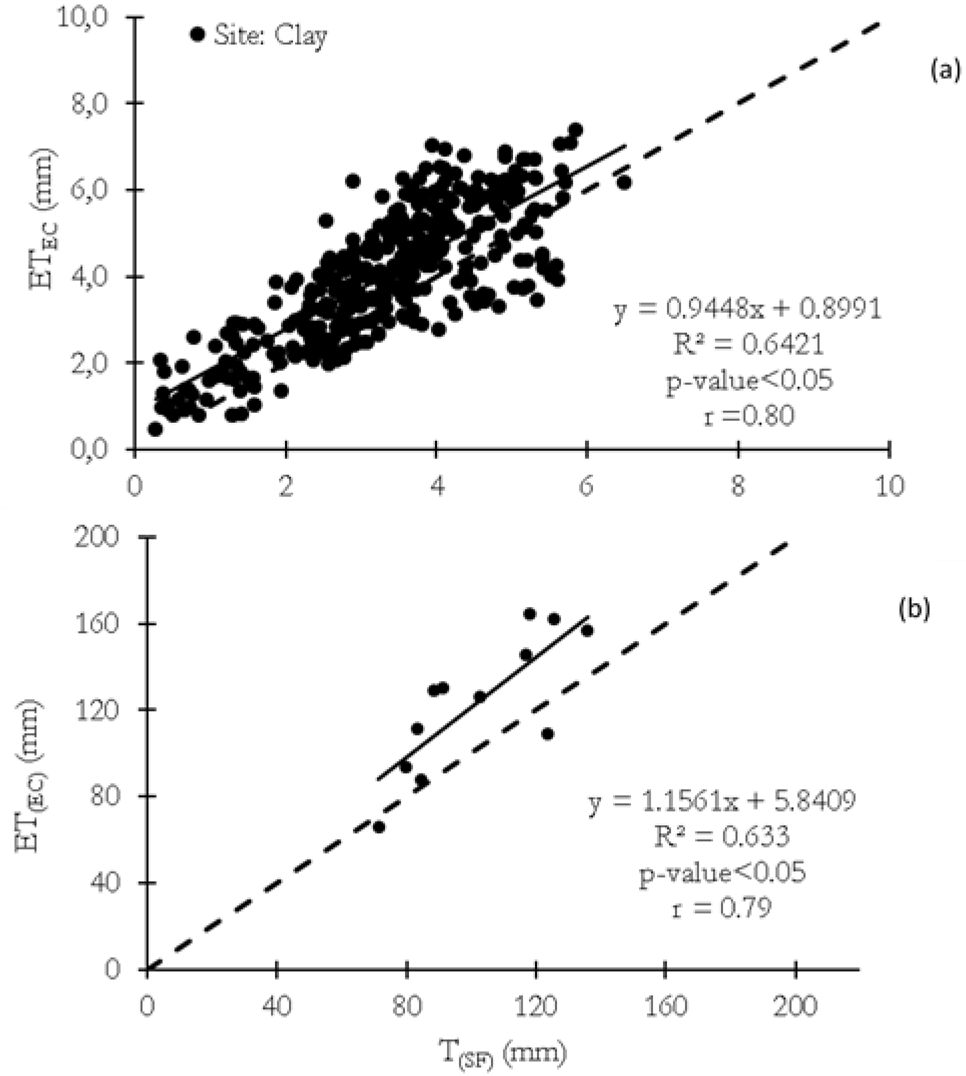

Figure 10. Measurement of Evapotranspiration (ET_{EC}) and Transpiration of the trees (T_{SF}) at the study site of clay content of Eucflux project on daily (a) and monthly scale (b).

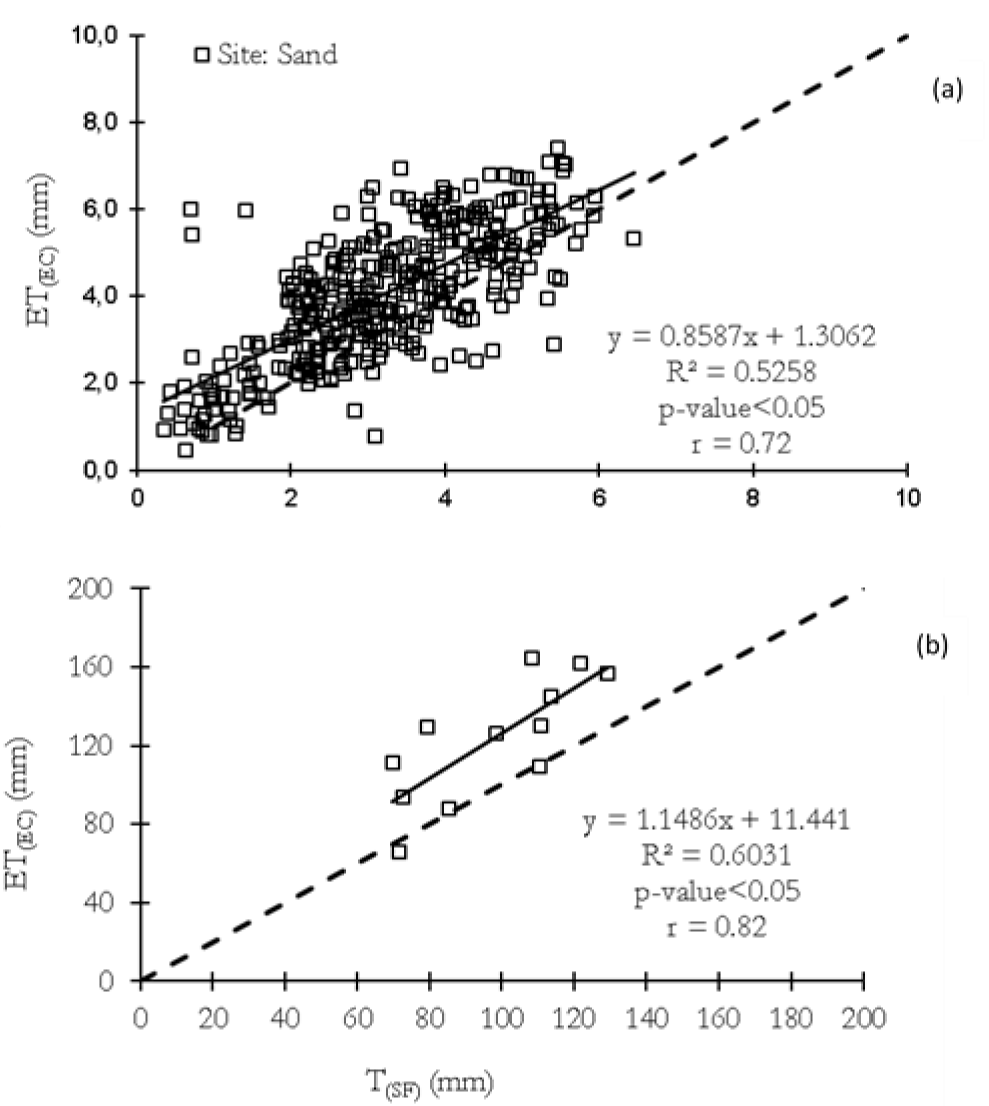

Figure 11. Measurement of Evapotranspiration by Eddy Covariance (ET_{EC}) and Transpiration by sap flow (T_{SF}) on daily and monthly scale at the study site on sand content at Eucflux project on daily (a) and monthly scale (b).

Comparison among Evapotranspiration measured by Eddy Covariance (ET_{EC}) and time series of Time Domain Reflectometer (TDR) was also studied. A regression established at daily scale showed a lower determinat coefficient for both site (R^2= 0.25 on clayish and R^2=0.05 for sand soil) (Figure 12a and 12b) and also for monthly scale (R^2=0.42 clayish and R^2= 0.07 for sand soil) (Figure 12c and 12d). On drought period, the highest determinat coefficient was found among transpiration (T_{SF}) and evapotranspiration (ET_{EC}) (Figure 13a). Evapotranspiration by Eddy Covariance (ET_{EC}) and TDR (ET_{TDR}) showed a lower determinant coefficient (R^2= 0.32) (Figure 13b). Lower determinant coefficient was also obtained with transpiration (T_{SF}) and evapotranspiration (ET_{TDR}) (Figure 13c).

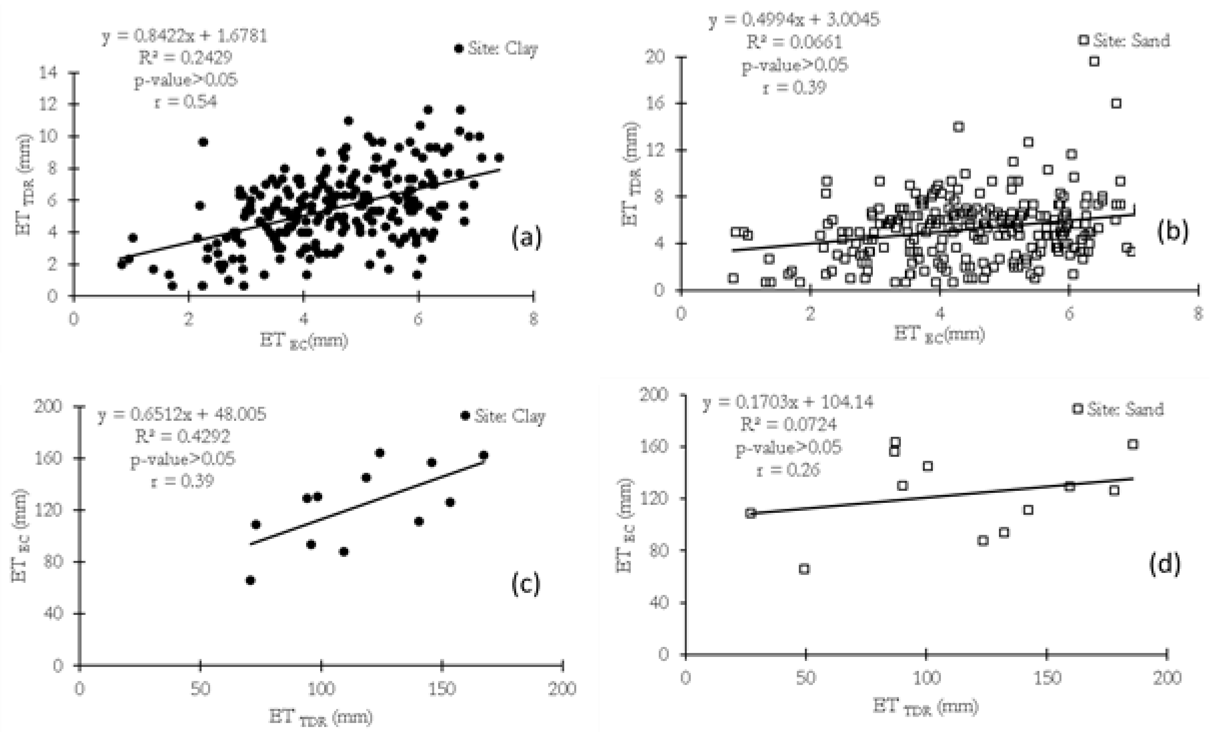

Figure 12. . Measurement of Evapotranspiration by Eddy Covariance (ET_{EC}) and Evapotranspiration by TDR (ET_{TDR}) on daily (a) and (b) and monthly (c) and (d) scale on clayish and sand soil at the study site.

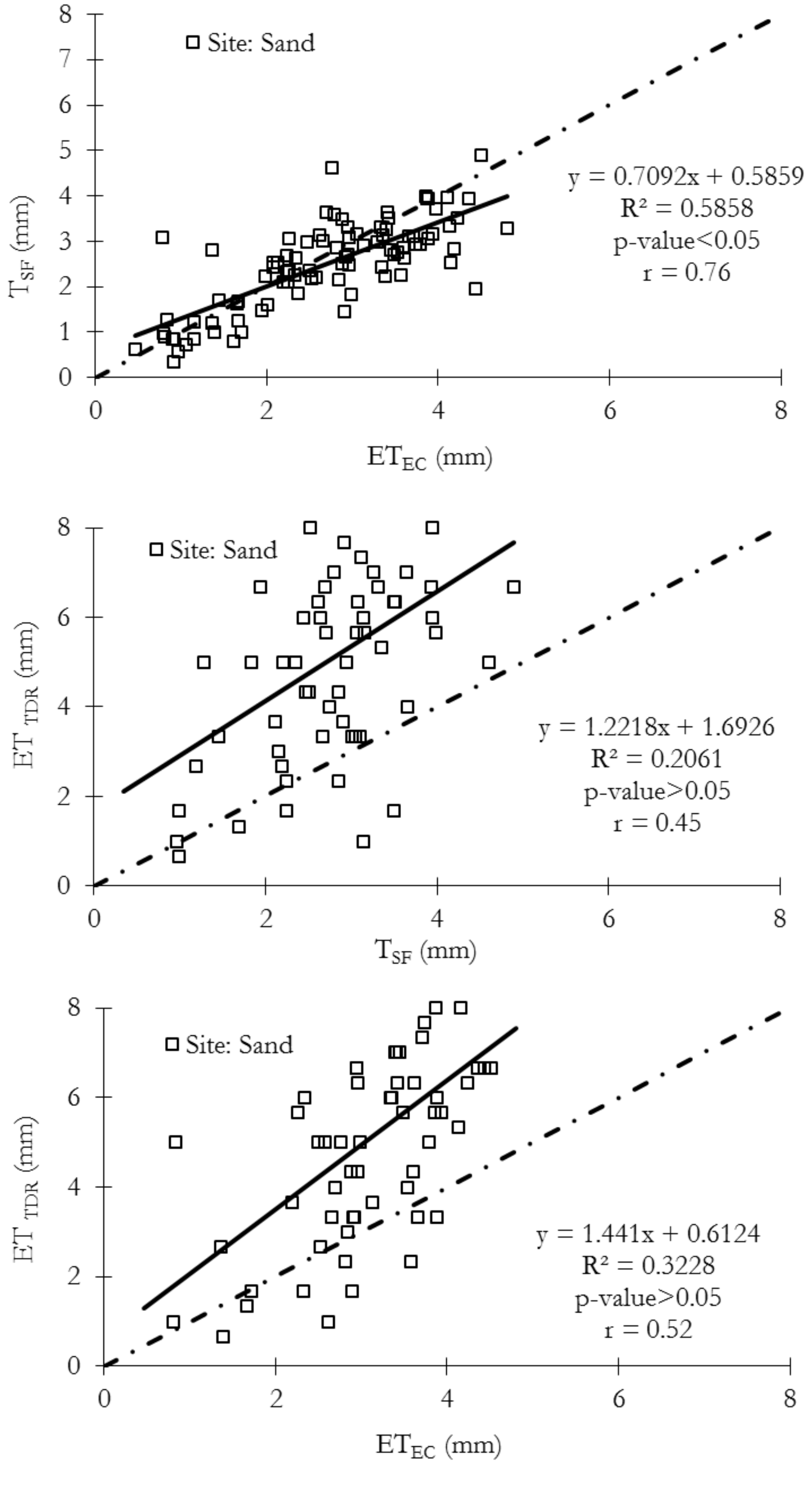

Figure 13. Regression established among Transpiration measured by sap flow (T_{SF}), Evapotranspiration (EC) (ET_{EC}) (a), Evapotranspiration (TDR) (ET_{TDR}) and Evapotranspiration (EC) (Eto_{EC}) and Evapotranspiration (ET_{TDR}) and Transpiration by sap flow (T_{SF}) at 3 month (May, June and July) without rainfall of 2013 at sand soil.

2.4. Discussion

2.4.1. Biomass Production

Biomass productivity was highest on clayish soil for volume, volume increment, and aboveground biomass increment. Aboveground biomass, sapwood increment and sapwood area was also equal in both sites. *E. grandis* trees are well adapted to the climate of mean temperature of 20°C and precipitation between 1000 and 1750 mm (Booth, 2013). Many clones of *Eucalyptus grandis* showed high productivity in many sites in Brazil. However, *Eucalyptus grandis* plantation also showed productivity reduction due to climate and soil characteristics. High productivity is also linked to LAI and resource use efficiency such as water, light and environmental resource availability. High LAI is associated to increased canopy light capture. Most productive clones have shown high LAI and high light interception. However, high LAI might not result in high productivity, due to changes in light resource efficiency across clones and environmental contexts. Water availability and water use efficiency might increase the productivity of forest plantation. Different gradients of water availability and soil water retention capacity might support highest taxes of tree growth. A study conducted in Brazil, Bahia state, showed that productivity was increased by a factor of 3 (10 Mg ha^{-1} and 30 Mg ha^{-1}) as a result of an increase in precipitation (800 mm year^{-1} and 1600 mm year^{-1})(Stape et al., 2004b). Stape et al., (2010), presented similar results. Supplemental water within sites also can increase Eucalyptus wood growth by 20-80% (Hubbard et al., 2010; Ryan et al., 2010).

Resource use efficiency is also important for tree development. Resource such as water, nutrients and light might contribute to a better use, capture of CO_2 and to produce biomass. The capacity to capture resource, use of resource more efficiently and allocation of biomass are different between each type of forests and genotypes in different sites (Binkley et al., 2004). Trees on clayed soil content plot could have more water availability on the soil superficial layers, which might support the trees with an adequate water supply. The trees also might fix more CO_2 per unit of light intercepted than a water stressed trees.

Soil characteristics also influence *Eucalyptus* sp productivity. Our results have shown no significant difference in productivity across clayish and sandy sites. However, clay content soil might retain water in their macrospore structure. This water retention could be important for tree survival along the rotation. On the other hand, sand soil texture has high capacity to drain water form precipitation to deep soil layers, which could obligate the tree develop their root system in deep soil layer. Water availability in sand soil texture are less available than soil with clay content. High correlation of productivity was found with clay content such as >0.70 in *Eucalyptus* sp plantation at Itatinga station (Gonçalves et al., 2012). Clay content has strong correlation with soil properties such as water retention and aeration capacity (Gonçalves, 2002). Sand soil content has less capacity to water retention, which could not support high rates of growth and productivity at this site.

2.4.2. Transpiration and water use efficiency

Many studies have shown different transpiration results for Eucalyptus plantation (Battie-Laclau et al., 2016; Christina et al., 2016). Annual transpiration measurement were often between 1100-1350 mm (Almeida et al., 2007; Battie-Laclau et al., 2016; Christina et al., 2016), other reports measured different results in another part of the world such as China and Australia (Forrester et al., 2010a; Ouyang et al., 2018). However, the magnitude of transpiration is also impacted by the local precipitation, the climate conditions, soil moisture and the planted species on the landscape.

Transpiration varied as result of changes evapotranspiration, precipitation, vapor pressure deficit and solar radiation (Gharun et al., 2014). Lower values of transpiration was measured during dry seasons, approximately 0.3-0.5 mm day^{-1}, while higher transpiration was measured on wet seasons 5.5-6.5 mm day^{-1} for both sites. In the currently study, it is also observed that transpiration followed evapotranspiration and vapor pressure deficit variation. Highest values of evapotranspiration occurred on sunny days and are correlate with high temperature in the wet seasons, which might contribute to increase transpiration in the scale of the trees. Similar results was reported in the literature, it was found a linear correlation of transpiration and vapor pressure deficit above 80% (Ouyang et al., 2018).

Transpiration also varied as a result of soil moisture. In our study, transpiration was higher in clay soil content than in sand soil. Clay content could retain water near the surface, which might facilitate the water absorption by the trees. Sand soil has different characteristics, and has higher capacity to drain water to deep soil layer, which might obligate the trees to allocate carbon to the roots growth in deep soil layers (Pinheiro et al., 2016b). Leafs with an adequate water supply might fix more CO_2 per unit of light intercepted than a water stressed trees. A plant with an adequate water supply may develop a high leaf area index, which has a strong correlation with transpiration. Transpiration of *Eucalyptus* sp used to follow the variation of evapotranspiration. This behavior suggest a weak stomatal control by the *Eucalyptus* sp trees and a possible anisohydric regulation and non-conservative water use (Ouyang et al., 2018).

In our study soil moisture availability did not influence the transpiration, water use efficiency and stemgrowth of the trees at the study site. All the results was very similar for both sites. The difference between clay and sand content was not sufficient to cause differences in transpiration, stemwood growth and leaf area index. Soil moisture effect on tree growth developed should be investigated in a higher clay content than 16%.

2.4.3. Comparison between Eddy Covariance, Sap Flow and Time Domain Reflectometry

Comparison between $ET_{(EC)}$ and Transpiration by TDR showed higher values of transpiration by Eddy Covariance technique than Sap Flow and TDR. The ratio of $ET_{(TDR)})/ET_{(EC)}$ and $T_{(SF)}/ET_{(EC)}$ at annual scale was 89% and 84%, respectively. These results suggest that $ET_{(EC)}$, Sap Flow and TDR are relatively accurate to estimate evapotranspiration and transpiration an annual scale. The correlation analyses between $ET_{(EC)}$ and $T_{(SF)}$ was not close to 1 in both daily and monthly scale. The determinant coefficient was 0.6 for clay soil for daily and monthly scale, while at sand soil was 0.5 at daily and 0.6 for monthly scale. Both measurement of Sap flow at the study site might underestimate the transpiration compare to evapotranspiration measured by Eddy Covariance.

The accuracy of methods vary significantly in many studies. The ratio between $ET_{(com, TDR, SF)}/ET_{(EC)}$ also reach 0.9 in the forest evapotranspiration experiments an annual scale (Ra et al., 2019; Shimizu et al., 2015). Eddy Covariance method is a powerful technique to measure evapotranspiration in different type of vegetation and crop at different spacial and temporal scale. Sap Flow and TDR are very useful, however measurement of them are possible in a local scale. Time domain reflectometer (TDR) and Sap Flow techniques underestimated evapotranspiration compared to evapotranspiration (ET_{EC}) measured by Eddy Covariance. Both techniques, TDR and Sap Flow has a limited operation and are subjected to some errors. Sap flow probes are subject to some electrical problems due to energy supply and lower probe durability. Sap flow measurement is also subject to influence by thermal gradients from the environment (Do and Rocheteau, 2002). In addition to this, sapwood area might vary among the trees. Some sensors might be installed on non-conductive area, which underestimate sap flow density (Ecologie and Foresti, 2011). Time Domain Reflectometer (TDR) might be subject to some errors either. Based on infiltration and deep drainage. The soil depth limits TDR measurement. Shallow soil layer are easier to install than deep soil layer. Probes were installed near the surface on shallow soils, which make easy to measure evapotranspiration in many annual crops. Some crops develop root system in deep soil layer such as Eucalyptus plantation. Root front deph of eucalyptus might reach 85% of stand height (Christina et al., 2011). Some genotype of eucalyptus have showed root front depth ranged between 8 to 12 meters at 2 years after planting (Pinheiro et al., 2016b). From 3 years after planting up to clear cut, root front depth of eucalyptus plantation might reach 16 to 18 meters under the surface (Christina et al., 2016). Deep rooting strategy can increase the survival rate of seedling in drought period (Padilla et al., 2007) and the access of water availability for tree growth (Oliveira et al., 2005). Measurement of volumetric water content under eucalyptus plantation in deep soil layer might be difficult due to root front depth.

Sap Flow sensors has an advantage to estimate water use by the forest and different species, tree specie composition and for physiological response and stands specific responses environmental control. Relationship between transpiration and evapotranspiration measured by eddy covariance showed higher determinat coefficient than TDR and EC measurement. Both sap flow and TDR has methodological limitation. Eddy Covariance system should be apply to measured evapotranspiration in eucalyptus forest landscape. This technique adequately quantify water use by the forest and might reduce uncertainties.

2.5. Conclusions

Water use and water use efficiency at the clayish and sandy soil was similar. Soil texture between the sites was not sufficient to modify the transpiration at the tree scale. Volume, Abouveground biomasss, Sapwood area, sapwood increment, leaf area index was also similar between clayish and sandy soil;

Sap flow and Time Domain Reflectometer underestimate transpiration and evapotranspiration compare to Eddy Covariance System. Sap Flow is subject to some errors such as electrical supply. Electrical supply has to be well dimensioned. This technique is useful to estimate water use and to understand physiological response at the stands from the environment. Time Domain Reflectometer is also limited and might be subject to some errors based on infiltration and deep drainage and dependent of the type of soil. Eddy covariance has the capacity to measure evapotranspiration of eucalyptus forest plantation. This technique might adequately quantify water use and reduce uncertainties.

Water use efficiency and water use are important for forest planning at the land scale. There are few information about the water use and water use efficiency in different climate and species of *Eucalyptus* sp. Information about water use and water use efficiency are important to understand the relation of forest growth, resource use and resource use efficiency.

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3. EFFECT OF POTASSIUM AND WATER SUPPLY ON PRODUCTIVITY AND WATER USE IN AN EUCALYPTUS GRANDIS PLANTATION MANAGED AS COPPICE

ABSTRACT

Changes in pattern of precipitation and temperature are frequently in the world, which is causing water deficit in many regions. Water deficit might cause decrease in wood productivity. Forest managers often apply potassium fertilizer to mitigate the impact of water deficit to improve the plant resistance to water stress. Eucalyptus forest plantation are clear-cut after seven years planting. During economic crisis forest managers manage forestry for coppice stand. Many results were published about physiological adjustment of Eucalyptus on seedling forest stand. However, there are not many information about the effect of water deficit and K fertilizer application in a forest manage for coppice of Eucalyptus. The main objective of this work is to study the interaction of water deficit and potassium fertilizer on forest manage for coppice. To compare physiological adjustment of forest manage for coppice seedling forest stand of *Eucalyptus grandis* clone. The main hypothesis of this project is that forest manage for coppice are less susceptible to water deficit than seedling forest stands of *Eucalyptus plantation*. The study was conducted at Experimental Station of Itatinga (ESALQ/USP) on an experiment of "Water exclusion". This is a cooperative project with Esalq/USP and CIRAD/FR. Forest manage for coppice showed higher annual net primary production (ANPP) on K+W fertilizer plot with 2.9 kg m^{-2} $year^{-1}$, followed by K-W with 2.3 kg m^{-2} $year^{-1}$, 1.15 kg m^{-2} $year^{-1}$ for control plot C+W and 0.85 kg m^{-2} $year^{-1}$. Stemwood production, leaf net primary production, water use efficiency for stemwood and intrinsic water use efficiency also showed higher values for K application plot. Stand water use was 1112 mm on K+W, followed by 873 mm K-W, 673 mm for C+W and 614 mm for C-W. Forest manage for coppice has an advantage to produce aboveground biomass. Root system developed in deep soil layer provided access to water storage in deep soil layer, suggesting that trees has high potential to produce biomass on first years. In our study K fertilizer application increased tree water demand, however, water use was less than seedling forest stand.

Keywords: Potassium; Water use efficiency; Water use; Photosynthesis; Stomatal conductance; Water stock

3.1. Introduction

Eucalyptus forest plantations are spread all over the world. Eucalyptus is a fast growing species with high adaptability to contrasting environmental conditions (Booth, 2013; Turnbull, 1999). Currently, Eucalyptus plantations cover more than 20 million hectares in the world (Booth, 2013). Brazil has 5.8 million hectares covered by Eucalyptus forest plantations, followed by India (3.9 million hectares), China (2.2 million hectares) (GIT Consulting, 2009), Portugal (800 000 ha) (INCF 2013) and Spain (500 000 ha) (Pita and Pardos, 2001). Eucalyptus productivity is strong linked to environmental resources. Nutrients, light and water are the main limiting factors of eucalyptus productivity. Many studies have shown that water is the most important limiting factor for eucalyptus productivity (Stape, 2010). Water stress impacts large areas of eucalyptus plantations.

Changes in precipitation and temperature patterns are a significant threat to forest plantation health, productivity and sustainability (Booth, 2013; Brando et al., 2008). Climate change predictions have shown that most of eucalyptus plantations around the world will be more exposed to more fire hazard, risk of pests' infestation and drought events in the future. These factors might cause a yield decrease of 30% the first six to seven years after planting (Almeida et al., 2010). In particular, water stress might induce leaf growth inhibition, stomatal closure, decrease photosynthetic activity, changes in photosynthetic pigments content and oxidative burst (Berenguer et al., 2018; Lambers, 1998). Water stress also might cause a decrease in biomass allocation to wood (Metcalfe et al., 1990), loss of turgor and osmotic adjustment (White et al., 1996), decrease of water potential (Battie-Laclau et al., 2014a), stomatal closure (Silva et al., 2004), cell wall reinforcement and changes in antioxidants and antioxidant enzymes, chlorophylls and carotenoids (Silva et al., 2004).

Adaptive strategy might reach sustainable high growth and mitigation of the impact of water deficit, which is urgently needed. Throughfall exclusion experiments using gutter to prevent a total percentage of the total canopy from reaching the soil have been used to examine the trees response to drought in temperate (Hanson et al., 2001), tropical forest (Costa et al., 2010) and eucalyptus plantation on forest stand (Battie-Laclau et al., 2016, 2013; Christina et al., 2015). Throughfall exclusion was used to study physiological adjustment mechanism of the trees in combination with nutrients application and water stress in a tropical Eucalyptus plantation in Brazil at Itatinga Experimental Station (USP/ESALQ) (Battie-Laclau et al., 2016). Physiological parameters were measured such as photosynthesis, transpiration, water use efficiency and water stock. Although these studies provided clear advances in our knowledge of Eucalyptus functioning, they were only conducted on seed-origin plantations, while little is known about the physiological adjustment in coppice of forest plantation of *Eucalyptus* sp.

Eucalyptus forest plantation have been increasingly managed as coppice in Brazil because of growing economical constraints. Coppice is however less productive than seed-origin plantations in the majority of sites. Coppice productivity is often 30% lower than seed-origin plantations (Guedes et al., 2011). Nowadays, Brazilian forests company managed annually 49.8 thousands hectare of Eucalyptus plantations as coppice system, which corresponds to 16% of Brazilian total area of Eucalyptus forest plantation (Ferraz Filho et al., 2014). *E. camaldulensis, E. grandis and E globulus* are the species most used for coppice system. Environmental resource such as water, nutrients and light are the most important factors that influence the coppice capacity and growth rates. In addition to this, it is also important to taking account health factors and the damages on the forest stump for coppice management.

Physiological adjustments to throufall exclusion and potassium fertilization of seed-origin plantations have been evidenced (Battie-Laclau et al., 2016, 2014a, 2013; Christina et al., 2015). The results have shown that highest aboveground biomass accumulated at 3 years after planting in a plot fertilized with potassium and without througfall exclusion. Biomass reduction, gross primary productivity (GPP), light use efficiency, carbon absorption was also decreased in plot unfertilized with K. Eucalyptus productivity unfertilized by K and throughfall exclusion produce 25% less than plots with potassium deficiency without through fall exclusion (Christina et al., 2015).

Studies on physiological adjustments in coppice of Eucalyptus are extremely scarce in the literature. Forests managed as coppice have different morphological characteristics such as root system development in a high deep soil layer. It might be a potential advantage for the coppice during water stress periods. Root development in deep soil layer might provide the access to water storage in deep soil layer, which could allow high initial development and growth taxes at some sites (Christina et al., 2015, 2011). Roots system by *Eucalyptus* sp can reach 16 meters belowground, depending on the genotype characteristics (Christina et al., 2011; Pinheiro et al., 2016a). Water absorption by deep soil layer in eucalyptus plantations has been shown to be important for sustaining aboveground biomass increment, water use and productivity (Drake et al., 2009; Fleck et al., 1996; Poorter and Nagel, 2000).

Comparison and knowledge about physiological adjustment and the dynamic in ecophysiology of coppice and seed-origin plantations are therefore highly needed.

Fertilization is a silviculture management technique used to reach high productivity in plantations, which strongly affects tree functioning (White et al., 2009). In particular, potassium can increase the water stress tolerance (Battie-Laclau et al., 2014a; Ramachandra and Viswanatha, 2004). Potassium fertilizer application play an important role in plant physiology as it plays a key role in metabolic processes, enzyme activation, protein synthesis and photosynthesis of plants (Taiz and Zeiger, 2004). Potassium is also found in the vacuole. Approximately 90% of potassium is found in the vacuole where it has a role in the maintenance of osmotic potential, cell turgor and leaf expansion (Wakeel et al., 2011). Potassium is also involved in osmotic driven process and contribute to long distance transport as a charge carrier (Marschner, 2012). On the other hand, potassium increase water demand by the trees, which is compromising the sustainability of eucalyptus plantation in water stress sites (Battie-Laclau et al., 2016, 2013; Christina et al., 2015). High growth rates, and high leaf area index lead to high rates of transpiration in potassium-fertilized plantations (Landsberg, 1999).

Physiological variation and different water regimes might provide physiological variation in many plants (Egilla et al., 2005). Many studies relating physiology to water stress and soil properties have been conducted in seed-origin eucalyptus plantations (Ryan et al., 2010). However, the outcome of the interaction of potassium and throughfall exclusion in a coppice plantation is unknown.

Our study aims to quantify the effects of potassium and water supply on wood productivity and water use effieincy in eucalyptus plantation managed as coppice. We study the dynamic of physiological responses as results of forest management and the adaptability strategies of the eucalyptus plantation at different level of potassium fertilization and water regime. We hypothesized that (1) Eucalyptus productivity and water use is higher in the coppice than fist rotation between 1 to 2 years after planting; (2) Potassium fertilizer application might increase water stress in a coppice plantation as showed at the seed-origin plantations; (3) The effect of potassium are less pronunciated in water exclusion treatment.

3.2. Material and Methods

3.2.1. Study area

The study was conducted at the Itatinga experimental station belonging to University of Sao Paulo. The area is located at latitude 23° 02′ S and longitude 48° 38′ W, with the maximum altitude of 863 m above sea level. The study site presented an annual rainfall of 1360 mm. The wet season is usually from December to March and the dry season is from June to September. The mean annual temperature in the study period was 25°C with variation of ±5°C. The study site has mildly undulating topography that is typical of the São Paulo Western Plateau. The experiment was conducted on a plateau (slope<3%). The soils were Ferrasols (FAO Classification) developed on cretaceous sandstone. The textural uniformity of the soil was high (clay content around 13% in the A1 horizon and 20%-25% in 1-6 m depth). Marília formation, Bauru group, with a clay content ranging from 14% in the A1 horizon to 23% in deep soil layers. The mineral content was dominated by quartz, kaolonite and oxyhydroxides and the soil was acid (pH between 4.5 and 5). Exchangeable K and Na concentrations were on average 0.02 cmolc kg^{-1} in the upper soil layer and <0.01 cmolc kg^{-1} from 5 cm to 1500 cm (Laclau et al., 2010).

3.3. Experimental design

A split-plot was designed in June 2010 with a highly productive *Eucalyptus grandis* clone used in a commercial plantation by Suzano Company (Brazil). It was evaluate four treatments (2 types of nutrient supply x 2 water regimes) replicated in three blocks. The experiment was managed for coppice in June 2016 (Figure 14).

The dominate spouts was selected taking account the DBH in each treatment. All sub-dominate sprouts was cut. The experiment studies the water supply regime (exclusion plots, -W, vs "exclusion free plots", +W) and the fertilization regimes (control, C and potassium fertilization, +K). The treatment were:

+K-W: 335 kg/ha applied KCl, approximately 37% of throughfall exclusion;

C-W: control, without K Fertilization and 37% of trhougfall exclusion;

+K+W: K fertilization and no throughfall exclusion;

C+W: control, no throughfall exclusion;

The plot were 864 m^2 in area, with 144 trees per plot in space of 3 x 2 m. The inner plot were 216 m^2. One plot of each treatment was reserve for biomass destructive sampling without disturbing tree growth in the inner plot where the measurement were taken. The dose of KCl was applied in single dose at 3 months after forest manage for coppice and start to growth. Other nutrients were also applied in the plot such as 3.3 g m^{-2} of Phosphorous, 200 g m^{-2} of dolomitic lime and micronutrients) and after three month 12 g m^{-2} of N was applied. Micronutrients were applied in not limiting for three growth (Gonçalves et al., 2008; Laclau et al., 2009).

Throughfall exclusion was also maintain on plantation managed for coppice. Water supply by the rainfall was partly exclude from the plot (-W) from September 2010 onwards. Plastic panel made of greenhouse plastic sheet realized throughfall exclusion. These panels was mounted on wood frames at heights varying between 1.6 to 0.5 m. Plastic panel covered approximately 37% of the plot area, water exclusion was approximately 450 mm per year in throughfall exclusion plot (–W) (Figure 14).

Figure 14. Experiment area of Throughfall Exclusion project installed in a *Eucalyptus grandis* plantation at Itatinga station University of São Paulo.

3.3.1. Stand growth and abouveground biomass

Tree growth was monitored between 1.2 to 2.2-stand ages. Tree diameter and height were measured each 6 months from January to December of 2018 in the inner plot of each treatment. The circumference at breast high of each stem was measured in each inventory.

Aboveground biomass was estimated by sampling 10 trees of each treatment on January of 2018 (K-W, C-W, K+W, C+W). The trees were selected based on an inventory of the experiment realized at 22 months. The diameter and height of each tree were measured in the field. The trees were sampled into components: leaves, living branches, dead branches, stemwood and stembark. The stem of *Eucalyptus grandis* was sawn into 0, 0.3, 1.0, 1.3, 3.0 and after three meters intervals until the canopies. The diameters, lengths and masses were measured in the field, as well as the circumference over and under bark at each section for both treatments. The foliage was collected at three different section of the crown. Sub-samples of the foliage were dried at 65°C (Laclau et al., 2009). Twenty leaves from each treatment were scanned. Leaf area was estimated by the program Image J and leaf area index (LAI) was calculated for each treatment. Allometric equation of aboveground biomass, LAI and volume were established between DBH^2 (m^2). These regressions were applied in all trees of the subplot.

3.3.2. Leaf water potential

The predaw leaf water potential (ψpdw) was measured of four trees accessed by scaffold tower in each treatment in block 2. The measurement was realized between 5:00 to 7:00 using a nitrogen pressure chamber (PMS Instrument Company, Albany, OR, USA). It was selected one leaf fully expanded leaf per tree in the upper third of the canopy and on the northern side of each tree (approximately two months). Diurnal measurement were also made in the upper third of the tree, on the northern side, approximately from 7:00 to 18:00 (four to five measurement througout the day) on sunny days (Figure 15).

Figure 15. Leaf water potential (predaw (ψ_{pdw}), midday (ψ_{mid}) and diurnal measurement on fully expanded leaves of the trees using PMS Intruments Schollander at the study site.

3.3.3. Diurnal dynamic of leaf gas exchange and hydric potential leaf

Diurnal time course of gas exchange was measured monthly on fully expanded leaves. It was collected one leaf of four tree in the upper third of the crown. Four scaffold was mounted in a subplot of each treatment. Measurements was made four to five times along the day. It was also measured leaf water potential of each measurement of gas exchange. Diurnal time course of gas exchange was made taking account photon flux density of the environment (PAR) along the day of each month during one year. It was assumed a constant CO_2 concentration (Ca=400 µmol mol^{-1}) with a portable gas exchange system (li-Cor 6400; Li-Cor Inc., Lincoln, NE, USA) and a chamber equipped with Li-Cor quantum sensor. Both, leaf water potential and gas exchanges was made to evaluate the dynamic on time course series of the physiological parameters in potassium fertilizer and water exclusion on the tree development (Figure 16).

Figure 16. Diurnal time course gas exchange measurement on fully expand leaves of the tree with Infrared Gas Analyser.

3.3.4. Seasonal survey of leaf gas exchange and hidric potential

Measurements of net CO_2 assimilation (A_{max}) and stomatal conductance (g_s) was measured monthly on fully expanded leaves. Leaves were collected in the upper third of the crown of four trees per subplots. The crown was accessed by scaffold tower mounted in subplot with 15 to 18 meters of height. Gas exchange was measured for six leaves per treatment between 8:00 to 10:00 at PAR flux density of 1600 µmol m^{-2} s^{-1}. Measurement of leaf water potential was made until the sunset at 5 to 7:00 and between 11:00 to 13:00. All measurement of gas exchange was made with a constant CO_2 concentration (Ca=400 µmol mol^{-1}) with a portable gas exchange system (li-Cor 6400; Li-Cor Inc., Lincoln, NE, USA) and a chamber equipped with Li-Cor quantum sensor. Measurement of net CO_2 assimilation and stomatal conductance were used to calculated intrinsic water use efficiency (WUE_i) in each treatment. The intrinsic WUE_i in each treatment was estimated according the equation below (Equation 4).

$$WUEi = \frac{Amáx\ (\mu mol\ m^{-2}\ s^{-1})}{gs\ (\mathrm{mol}\ m^{-2} s^{-1})} \qquad (4)$$

Where "Amax" is the maximum photosynthetic rate (µmol m^{-2} s^{-1}) at saturating Constant photosynthetic photo flux density (PPFD) and g_s (mol m^{-2} s^{-1}) is stomatal conductance.

3.3.5. Micrometeorological data

Micrometeorological data such as air temperature (°C), relative humidity (%) and precipitation (mm) were measured by a tower 22 meters above the ground in a clear area and 300 m from the experimental area (Figure 17). These parameters were measured using a datalogger (Campbell Scientific Inc., Logan, UT, USA) in each 30 minutes.

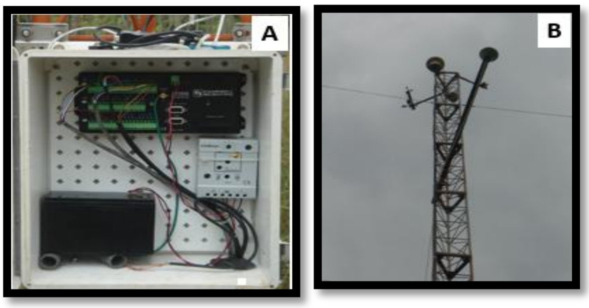


Figure 17. Meteorological station in Itatinga station at ESALQ/USP. (A) Datalogger equipment for storage the data and (B) Tower equipped of solar radiation, humidity, wild direction and velocity far 300 m from Water Exclusion experiment.

3.3.6. Sap flux measurement and stand transpiration

Sap flux measurement was conducted in all treatments K-W, C-W, K+W, C+W. Sap flow density (v, g H_2O m^{-2} sapwood s^{-1}) was measured from January to December of 2018 (365 days). Six trees were equipped by two probes in a random direction. The trees were selected taking account the range of cross section areas. The probes were installed in 1.30 m above the ground. The sap flow sensors were protected from external temperature variations and water intrusion by a reflective foil. Sensors output voltage was recorded every 30 min (CR1000 dataloggers; AM16/32 and AM416 multiplexer). Sap flow probes were checked in each 1 to 2 weeks. All the sensors out of the reading and physically damaged were replaced. The probe direction was moved every 3 months for *Eucalyptus grandis* in each treatment newly drilled holes in another position round the trunk (selected at random) to prevent underestimation of sap flow due to fast tree growth. The sap flow density was calculated with calibration equation determined in a preliminary study (Delgado-Rojas et al., 2010). The sapwood area at ~1.30 m above the ground was determined by regression established in each treatment in a previous study and was apply to the data collected on the inventory. The allometric relationship between circumference at breast high (CBH) and sapwood area was determined for each treatment and applied to the trees equipped with sap flow sensors. The estimated sap flow for each tree was calculated as the product of the sap flow density and the sapwood area estimated from successive measurements of CBH over the study period. The sensors were assumed to measure the instantaneous sap velocity integrated over the average sapwood thickness of each tree. A regression was established between the daily total transpiration (dependent variable) and CBH^2 (independent variable) and was determined by linear regression. The regression were applied in all trees in each inner subplot obtnaied by experiment inventory (adapted from (Kunert et al., 2012).

There were gaps in the readings of the sap-flow sensors. The sensor errors was produced by electrical problems in the experiment. The treatment C-W showed many electrical problems during the experiments. The gaps was corrected adopting one-sap flow probes per trees and regression established between transpiration and evapotranspiration (ETo; (Allen, 1998)). The R^2 between daily total transpiration and CBH^2 over the study period was 0.60 for all treatments. These regressions were then used to predict daily stand transpiration in all the trees of inner subplot. Sap flow density calculated by Granier equation sub-estimated stand transpiration in *Eucalyptus* sp trees in the previous tests. Delgado-Rojas (2010) established an equation for sap flow density estimation for *Eucalyptus grandis (*Equation 5).

$$u = 478.017 \times 10^{-6} \times k^{1.231} \times SA \qquad (5)$$

Where "k" is a constant between sap flow density and temperature, "SA" is sapwood area.

3.3.7. Water use efficiency for biomass production

Stem growth variation was measured two times in each month in all the treatments. Circunference at breast high (CBH) was measured at the beginning of the study. The variation of growth measured was sum with CBH during the study period. Allometric relationship between stemwood (kg) and diameter at breast high (DBH^2) (cm^2) of each treatment were applied in each trees in all subplot. Stemwood and transpiration of each tree were calculated as the sum of each period. Stemwood production and transpiration per day were calculated as the sum of each factor in a period of 15 days. Water use efficiency was calculated in each period by sharing stemwood biomass by the amount of water transpired over the same period (Equation 6).

$$WUE = \frac{\Delta\, Biomass\ (kg\ day^{-1})}{Transpiration\ (m^{-3}\ day^{-1})} \qquad (6)$$

3.3.8. Statistical Analysis

Homogeneity of variances was checked by the test of Bartlett whereas the normal distribution of residues was assessed via the Shapiro-Wilk test. Effect of fertilization and water regime was analysed using analysis of variance (ANOVA) on statistical software SPSS. The parameter analysed was annual net primary production, stemwood, leaf net primary production, water use efficiency for stemwood and water use efficiency for abouveground biomass. Microsoft Excel 2013 performed linear regression.

3.4. Results

3.4.1. Aboveground net primary production (ANPP)

Aboveground net primary production (ANPP) was highest in K fertilized plot (+K+W) with 2.9 kg m^{-2} $year^{-1}$, followed by +K-W with 2.3 kg m^{-2} $year^{-1}$, (C+W) with 1.1 kg m^{-2} $year^{-1}$ and 0.8 kg m^{-2} $year^{-1}$ (C-W). Aboveground net primary production (ANPP) in +K+W was 1.2 fold higher than +K-W, 2.6 than (C+W) and 3.6 (C-W) (Figure 18a). Similar result have showed for leaf NPP leaf. Potassium fertilized plot produced higher leaf NPP than unfertilized plot. The highest leaf NPP was for +K+W with 0.48 kg m^{-2} $year^{-1}$, followed by +K-W with 0..46 kg m^{-2} $year^{-1}$, 0.28 kg m^{-2} $year^{-1}$ for C-W and 0.23 kg m^{-2} $year^{-1}$ for C+W (Figure 18b). The stemwood was highest for K fertilized plot with 1.75 kg m^{-2} $year^{-1}$, followed +K-W with 1.45 kg m^{-2} $year^{-1}$, C+W with 0.5 kg m^{-2} $year^{-1}$ and 0.35 kg m^{-2} $year^{-1}$ for C-W. ANPP, Leaf NPP and stemwood was more affected by fertilization regime than throughtfall exclusion regime (Figure 18c).Statically analysis did not showed interaction between fertilization regime and throughtfall exclusion. The statistical analysis showed an effect of fertilization on ANPP, stemwood, stemwood water use efficiency and leaf NPP (Table 1). The proportion of ANPP between stemwood was higher in K fertilized plot with 60% for K+W, 62% for K-W, 46% in C+W and 42% C-W (Figure 18d).

Tabela 1. Effect of fertilization regime (Fertilizer: control, supply of K), water supply regime (+W: undisturbed rainfall vs 37% throughtfall exclusion, -W) on annual net primary production (ANPP), annual stemwood net primary production ($NPP_{stemwood}$), water use efficiency for aboveground biomass (WUE) and water use efficiency for stemwood (WUE_p), annual leaf net primary production (NPP leaf). * and ** indicated significant effect at P<0.05 and 0.01.

Parameter	Fertilizer	Water	Fertilizer x Water
ANPP	$F_{1,6}$=45.276***	$F_{1,6}$=0.297	$F_{1,6}$=2.971
NPP stemwood	$F_{1,6}$=69.158***	$F_{1,6}$=0.215	$F_{1,6}$=2.537
NPP leaf	$F_{1,6}$=24.497***	$F_{1,6}$=0.174	$F_{1,6}$=0.519
WUE	$F_{1,5}$=2.095	$F_{1,5}$=0.144	$F_{1,5}$=0.117
WUE_p	$F_{1,5}$=8.876***	$F_{1,5}$=0.464	$F_{1,5}$=0.01

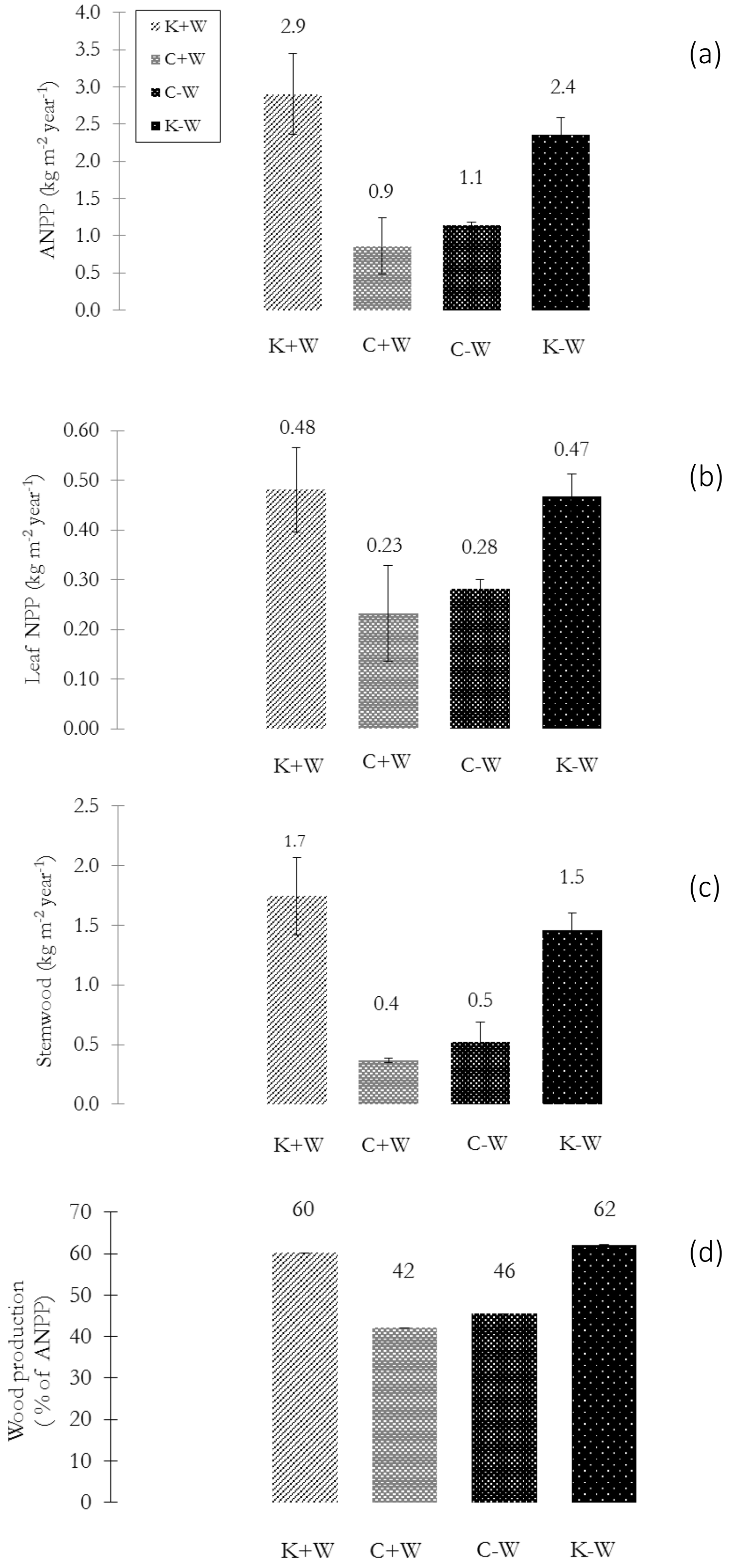
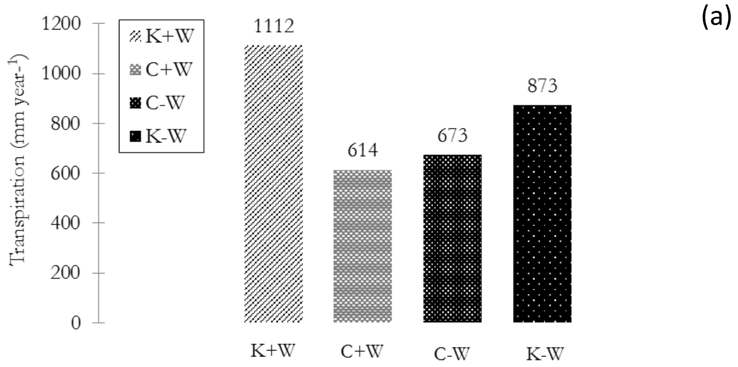


Figure 18. Aboveground net primary production (ANPP) (a), leaf net primary production (b), Stemwood production (c) and proportion of wood production on annual ANPP (d) in a coppice of *Eucalyptus grandis* stand. C refer to the control and K-fertilized plots. +W refer to undisturbed rainfall and – W to water exclusion of 37%.

3.4.2. Stand water use

Canopy transpiration was highest for potassium fertilized with 1112 mm for K+W and 873 mm K-W. Canopy transpiration on unfertilized plot was highest for control plot, C+W, with 673 mm, followed 614 mm in C-W (Figure 19a). Canopy transpiration fertilized by K used 1.2, 1.6 and 1.8 more water than others treatments at this study site. On the other hand, K fertilized results was significantly, suggesting increase in tree water demand. Potassium fertilization significanted affected water use efficiency for stemwood production, according the statiscally analyses (Table 1). However, throughfall exclusion had no effect on water use efficiency for stemwood production (Table 1). The highest water use efficiency for stemood (WUE_p) was for K fertilized plot (K+W) with 1.2 kg stemwood m^{-3} H_2O, followed by K-W with 1.0 kg stemwood m^{-3} per H_2O, 0.7 kg stemwood m^{-3} H_2O by C+W and 0.6 kg stemwood per m^{-3} H_2O by C-W (Figure 19b).



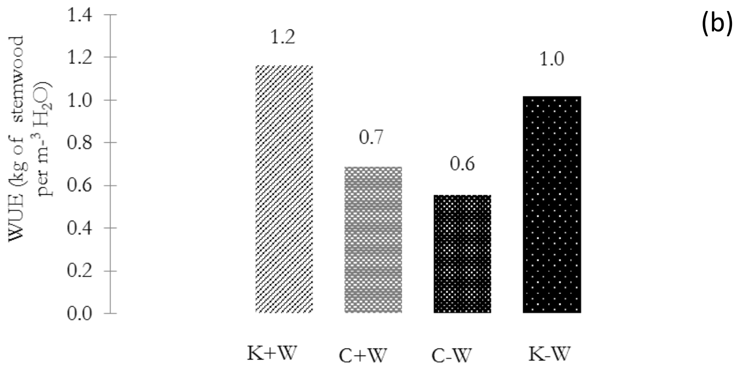


Figure 19. Annual canopy transpiration (a) and water use efficiency for stemwood (b) (WUE_p (ratio of stemwood biomass accumulation to stand water use) in coppice plantation of *Eucalyptus grandis* trees with 1.2 to 2.2 years. C and +K refer to the Control and K-fertilized plots, respectively, +W and –W refer to undisturbed rainfall and 37% exclusion throuhgfall exclusion, respectively.

3.4.3. Water use efficiency for stemwood production (WUE_p) and intrinsic water use efficiency (WUE_i)

Time series of intrinsic water use efficiency (WUE_i) and water use efficiency for stemwood production (WUE_p) have shown different results. The mean WUE_p was among 0.96 to 4.94 kg of stemwood per m^{-3} H_2O for K+W, 0.65 to 4.04 kg of stemwood per m^{-3} H_2O for K-W, 1.21 to 4.12 kg of stemwood per m^{-3} H_2O for (C+W) and 0.57 to 3.02 kg of stemwood per m^{-3} H_2O (C-W). WUE_i showed mean values of 45 μmol CO_2 m^{-2} s^{-1} K+W, 56 μmol CO_2 m^{-2} s^{-1} for K-W, 43 μmol CO_2 m^{-2} s^{-1} C-W and 35 μmol CO_2 m^{-2} s^{-1} C+W. WUE_p and WUE_i have not showed positive correlation. Both time series course showed different variation during dry and rainy season along the study period. The highest values of intrinsic water use efficiency (WUE_i) was presented in the dry season, while WUE_p have shown lowest values at the same periods. Over the rainy season, from September to December, both water use efficiency increase. (Figure 20a, 20b).

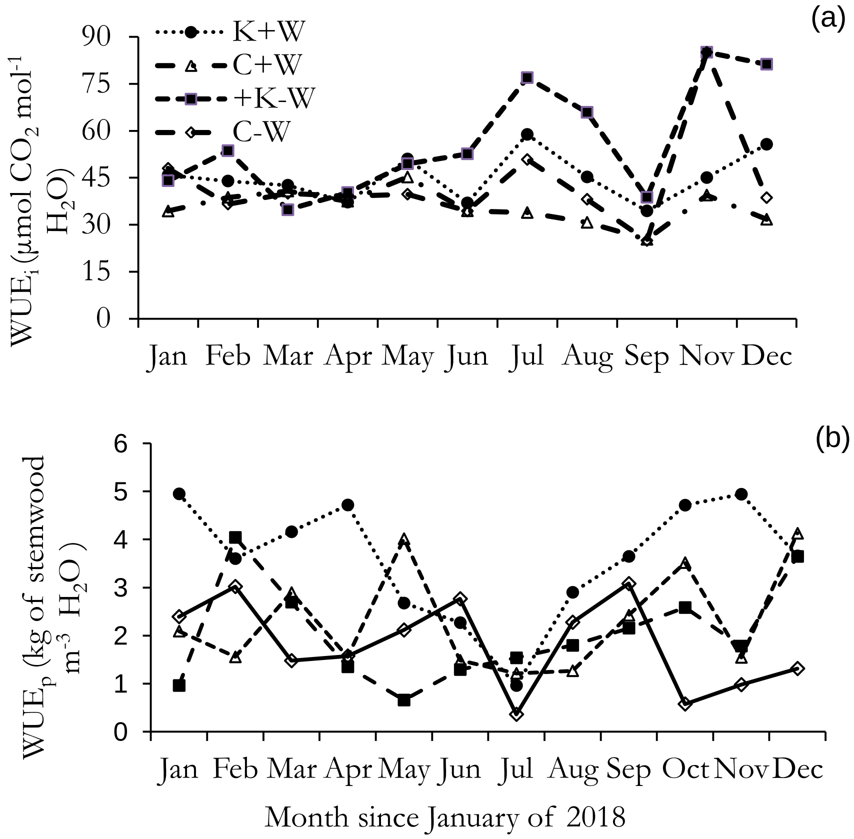


Figure 20. Intrinsic Water use efficiency (WUE_i) (a) and water use efficiency for stemwood (WUE_p) (b) in a coppice of the 1.2 to 2.2 year of *Eucalyptus grandis*.

A regression established between WUE_i and stomatal conductance showed a negative correlation. WUE_i sharp decrease with increase stomatal conductance (Figure 21a). Relationship between WUE_i, leaf water potential and vapour pressure deficit was lower (Figure 21b and 21c).

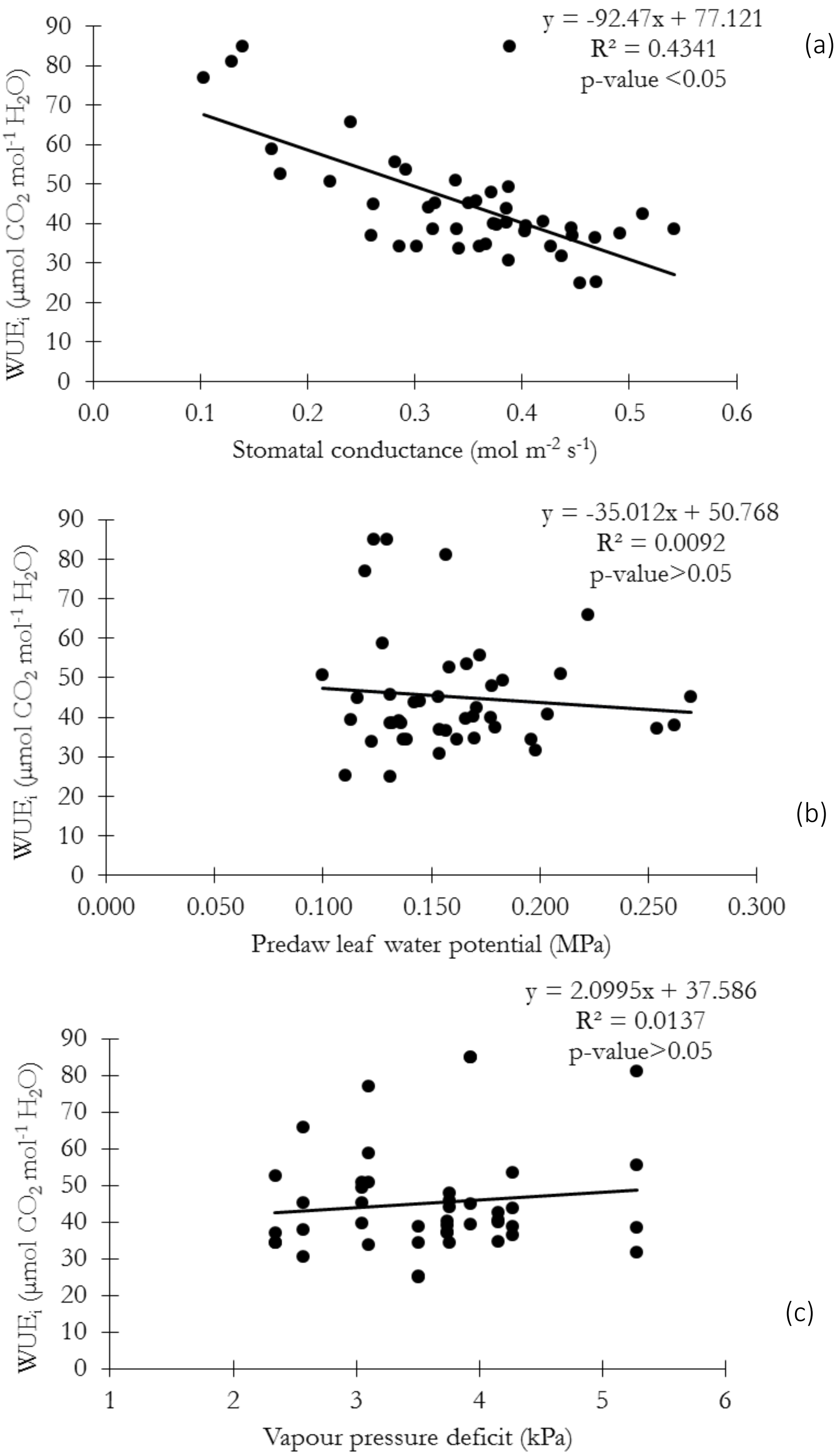
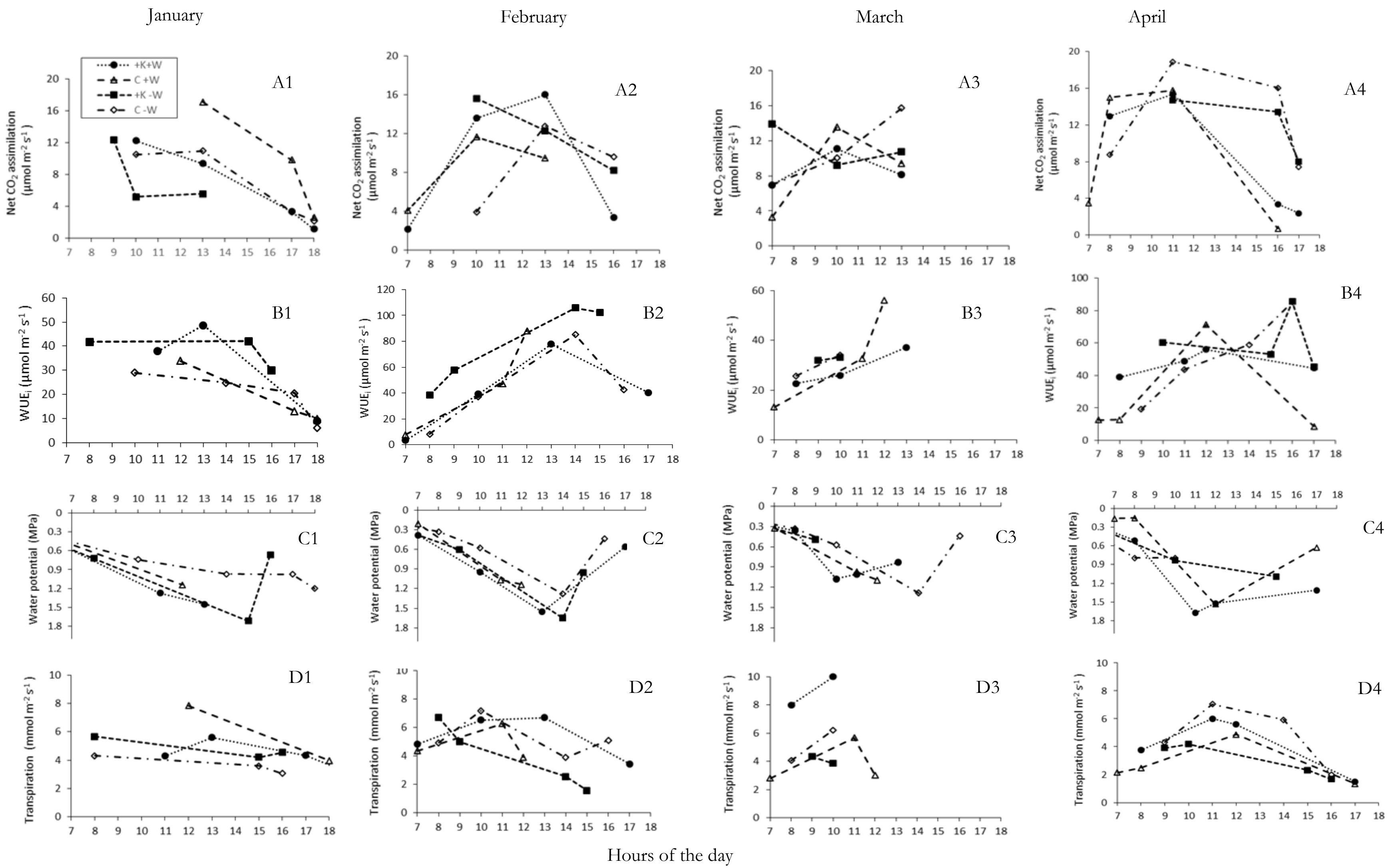
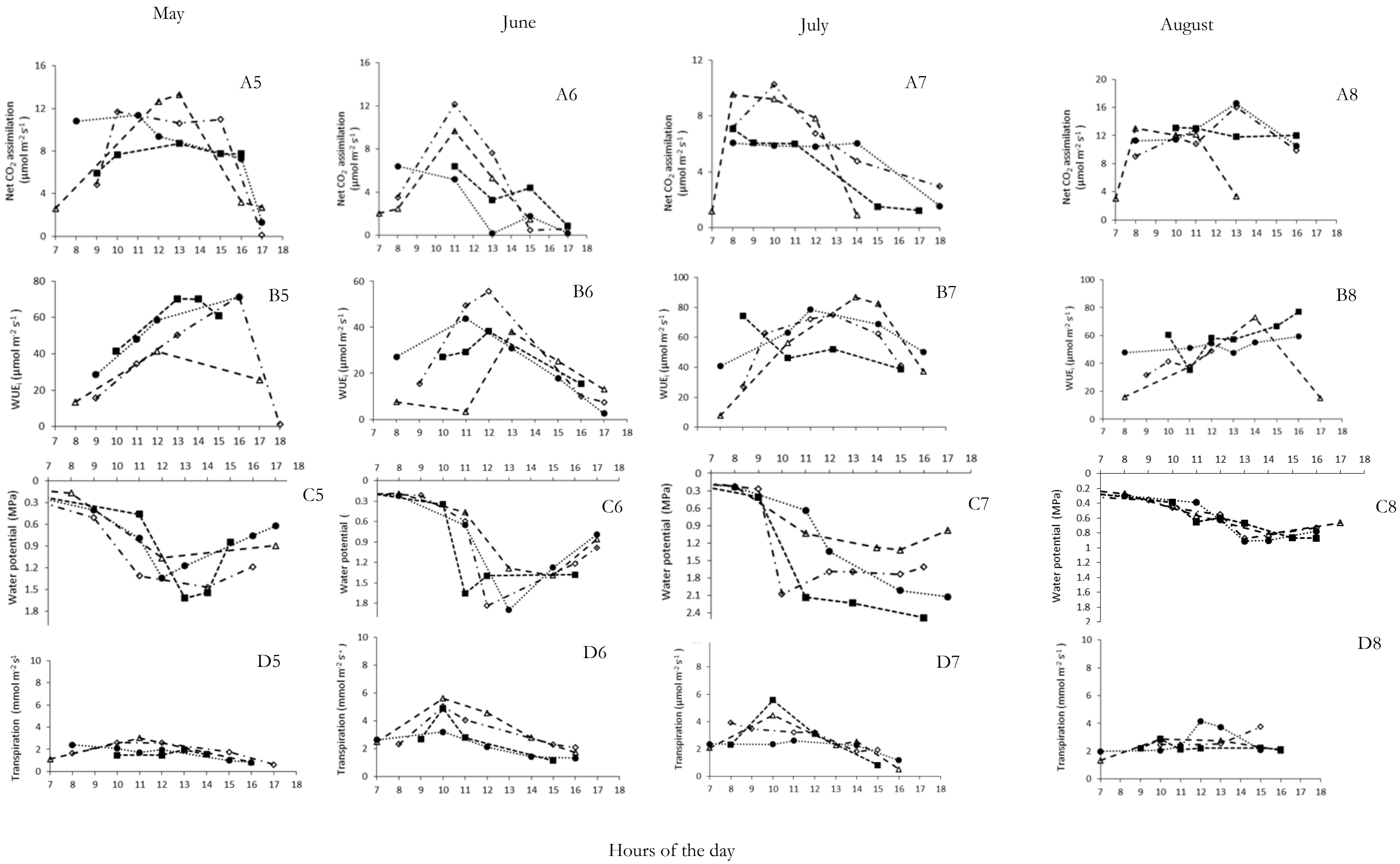


Figure 21. Relationship between intrinsic water use efficiency (WUE$_i$ – ratio of instantaneous of net CO_2 rate assimilation rate to stomatal conductance) (a), predaw leaf water potential and vapour pressure deficit (c) for all treatment at the study site.

3.4.4. Diurnal photosynthesis, stomatal conductance, leaf water potential and transpiration

All treatment showed a decrease in leaf water potential at the early in the morning. Leaf water potential increase along the day and reach the minimum values approximately at midday. Slow recovery in the afternoon has happened for all treatment in each month (Figure 22). Minimum leaf water potential values was reached on May, June and July by potassium fertilized plot with -1.5 MPa for K-W, -1.8 MPa for K+W on June and -2.1 to -2.4 MPa on July (Figure 22). Photosynthesis, stomatal conductance and transpiration have shown similar results in each month. The diurnal measurement was measured taking account the environment radiation, which might variety in each measurement at the same day. This dynamic might explain the difference between the measurements in each treatment. Despite of this, in the early of the morning photosynthesis increase slowly and followed the increase of temperature and relative humidity dynamic during the day. Taxes of photosynthesis decreased at the dry season in all treatment. At the dry season the temperature, also decrease. Highest taxes of photosynthesis were possible in the early in the morning. The taxes of photosynthesis follow the radiation and the temperature. At the midday, high temperature provide a decrease of photosynthesis taxes and transpiration as consequence of the stomatal conductance. This behaviour suggests a decrease in taxes of photosynthesis, transpiration and an increase in intrinsic water use efficiency (Figure 22). Potassium fertilized plot have shown highest values in the early of the measurement on each month for both treatment (Figure 22). Throughfall exclusion on potassium-fertilized plot has not caused effect on photosynthesis. On the other hand, unfertilized plot have shown slower values of photosynthesis in each month of the measurement, somewhat measurement reached almost higher taxes of photosynthesis than potassium fertilized plot. (Figure 22).


January
February
March
April
+K+W
C +W
+K -W
C -W
A1
A2
A3
A4
B1
B2
B3
B4
C1
C2
C3
C4
D1
D2
D3
D4
Net CO2 assimilation (µmol m-2 s-1)
WUEi (µmol m-2 s-1)
Water potential (MPa)
Transpiration (mmol m-2 s-1)
Hours of the day


May
June
July
August
A5
A6
A7
A8
B5
B6
B7
B8
C5
C6
C7
C8
D5
D6
D7
D8
Net CO_2 assimilation (µmol m^{-2} s^{-1})
WUE$_i$ (µmol m^{-2} s^{-1})
Water potential (MPa)
Transpiration (mmol m^{-2} s^{-1})
Transpiration (µmol m^{-2} s^{-1})
Hours of the day

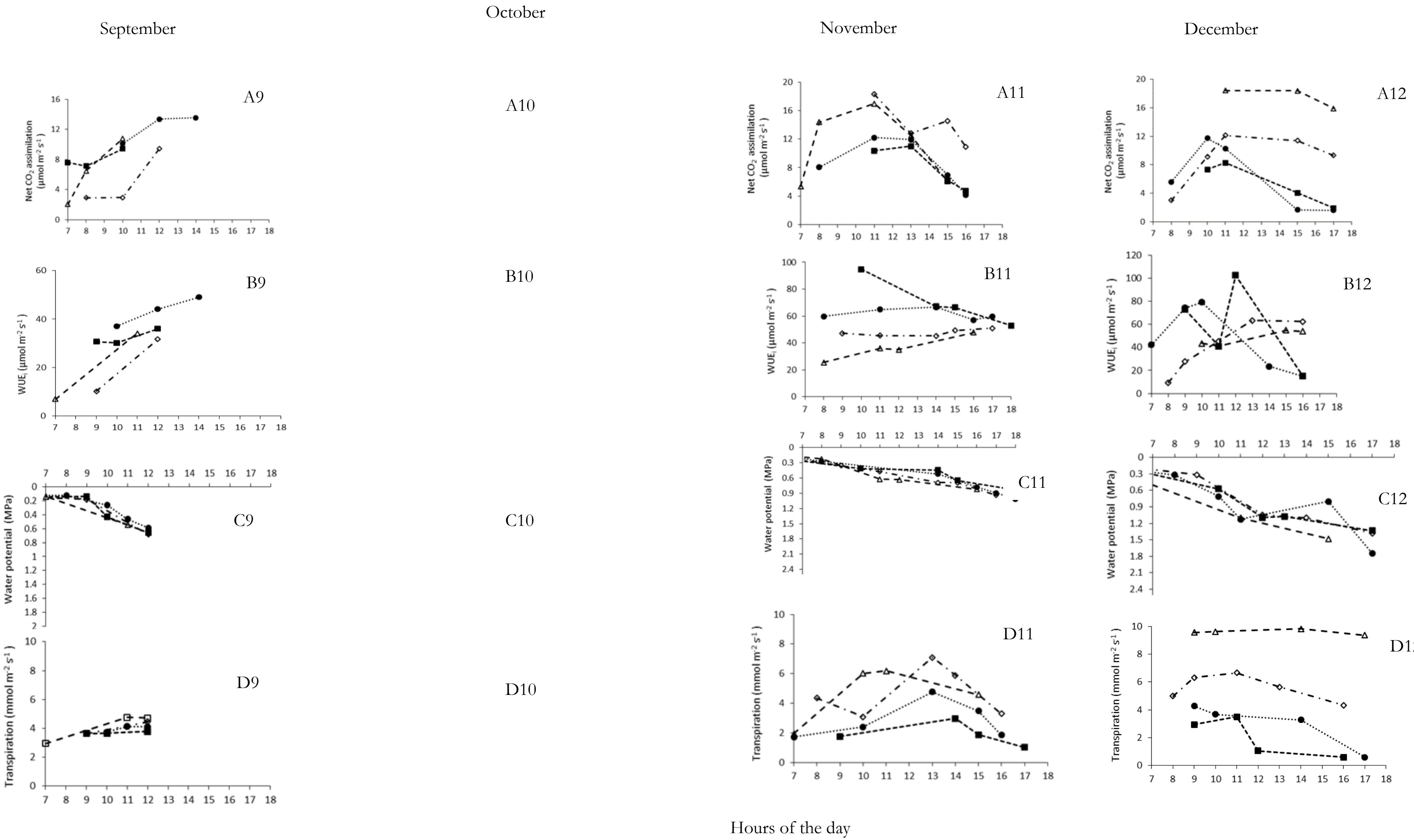


Figure 22. Diurnal dynamic of photosynthesis (A1-A12), intrinsic water use efficiency (B1-B12), leaf water potential (C1-C12), transpiration (D1-D12), of coppice of Eucalyptus grandis since January from December of 2018. Each values is the mean of four to five measurement made of fully expand leaves at track ambient radiation. Measurements of March and September were not completed due to an event of precipitation during the measurement. In the month of October was not possible to realize the measurment. C refer to the control plot, K-fertilized plot and +W undisturbed rainfall and –W is rainfall exclusion of 37%of rainfall.

3.5. Discussion

3.5.1. Effect of fertilization and throughfall exclusion on tree growth

Potassium fertilizer increase ANPP, leaf NPP and stemwood production compare to unfertilized treatments. Similar results were reported in a previous study at the same site on seed-origin-plantation of *Eucalyptus grandis*. The results showed that gross primary production (GPP) increase with application of potassium fertilizer as result of higher photosynthetic capacity, a lower stomata and mesophyll resistance diffusion of CO_2 (Battie-Laclau et al., 2014b) and also some increase in leaf area index and light use efficiency (Christina et al., 2015). In this study, K fertilizer plot of *Eucalyptus grandis* manage for coppice showed the same aboveground net primary production (ANPP), stemwood production and leaf net primary production (leaf NPP) than clone of *E. grandis* in forest stand. Throughtfall exclusion not affected ANPP, leaf NPP and stemwood production in the coppice management. Previous study with seedling forest stand showed that throughfall exclusion reduced the beneficial effect of K in aboveground net primary production (Battie-Laclau et al., 2016) and total leaf area (le Maire et al., 2013). In addition to this, wood production also might be reduce in the first two years of the experiment due to the fine roots achieve the water storage in deep soil layers (Christina et al., 2011; Laclau et al., 2013). Coppice management of Eucalyptus has different morphological characteristics. Root system development might reach deep soil layer. Root system in deep soil layer might be an advantage to access water storage in deep soil layers, which provide for trees drought tolerance, high aboveground biomass production and tree growth (Christina et al., 2015, 2011). Eucalyptus manage for coppice usually produce 70% of seedling (Carlos et al., 2014). However, it is also possible that coppice produce higher productivity than seedling of Eucalyptus. Productivity might also vary by environmental factor such as thermal regime, water resource availability, soil and physiographic condition, fertilization, weed control and genetics characteristics (Carlos et al., 2014). In this study, statistically analysis was significantly for fertilized application, suggesting that fertilizer application are an important silviculture practice to apply at coppice management for Eucalyptus. Throughfall exclusion was not sufficient to decrease trees growth on K fertilized plot in this initial growth phase. Root developed of all treatment could access water storage in deep soil layer even unfertilized plot.

3.5.2. Stand water use and water use efficiency for stemwood production

Water use by coppice of *Eucalyptus grandis* at the study was higher for K fertilizer plots. Similar results was found on seedling forest stands at the same study site (Battie-Laclau et al., 2016). However, eucalyptus manage for coppice fertilize by K use 18% to 32% less water than eucalyptus seedling forest stand on previous study. Control plot of coppice use less water than seedling forest stand. Water use by eucalyptus manage for coppice on control plot use 9% to 20% less water than seedling forest stand. Both results are relate to the sprouting capacity of *Eucalyptus grandis*, low vitality of the species and the stock density at the study site. Not all species could be manage for coppice. The high capacity to regenerate is relate to genetic characteristics of the species (Ferraz Filho et al., 2014). *E. grandis* in this study showed a relative lower capacity to regenerate. Control plot, one of that treatment in this study, could not regenerated at the same time. Stumps of *Eucalyptus grandis* regenerated after 5 months (November) and not all stumps could sprouting.

Water use efficiency on forest stand seedling and coppice at two years was very similar. WUE_p vary from 0.9 to 1.2 kg of stemwood per m^{-3} in the forest stand seedling and 0.6 to 1.2 kg of stemwood per m^{-3} of H_2O for coppice. Similar results were found in previous study (Battie-Laclau et al., 2016; Stape et al., 2004b). Forest manage for coppice water use efficiency was not changed in the first years of coppice at this study site compared to seedling forest stands. These results is due to low increment of the trees in this study on stemwood increment and carbon allocation from other compartments. Differences in carbon allocation between the compartments such as leafs and roots, stemwood and respiration induced by treatments might affected WUE for stemwood. Carbon water use efficiency might be reduce due to water stress and nutrients (Zhang et al., 2014), partioning of assimilates carbon to wood production (Ryan et al., 2010) and thus reducing WUE for stemwood production.

3.5.3. Water use efficiency for stemwood and intrinsic water use efficiency for forest management

Water use efficiency is define by the amount of water lost during the production of biomass or the fixation of CO_2 in photosynthesis. There are three types to measured WUE. WUE of productivity, photosynthetic water use efficiency (A/E) and intrinsic water use efficiency (A/g_w). WUE_p is similar to water use efficiency for productivity. Recent reports have shown many estimation of water use efficiency with different species, ages and management. The majority of the studies has the objective to maximize the water use by plants on the landscape, trying to find superior genetic material, understand the effect of management and plant responses. In this study, a time series water use efficiency was higher for K fertilizer than unfertilized plot. Time series of WUE was higher for K+W fertilized plot from January to March (rainy season). WUE_i at same time showed lower values (January to Mach) and also decrease during the dry season. From August to December the results for both WUE increase. Water and nutrient supply might increase stemwood production and water use efficiency. Previous study on seed-origin plantations showed that K fertilized increase photosynthetic capacity (Battie-Laclau et al., 2014b), leaf area index, light use efficiency (Christina et al., 2015) and ANPP. Throughfall exclusion was not statiscally significant at this study site stand characteristics (Table1). The drought season reduced water use efficiency in K-W compare with K+W. These effects were reported in other studies. Drought season might affect total leaf area (Whitehead and Beadle, 2004b) as consequently light absorption and whole tree carbon assimilation (Christina et al., 2015).

Increase water availability increased water use efficiency for stemwood (Stape et al., 2004b) and abouveground biomass such as leafs, branches and stemwood. Forest manager for coppice has developed roots in deep soil layers, which is an advantage for the sprouting regenerate for absortion water, nutrients and high production of aboveground biomass. In addition to this, carbon allocation from below to aboveground is likely to contribute to some increases in resource use efficiency. Carbon allocation in forest stands seedling occur for both compartment above and belowground at the plant inicial growth, while carbon allocation in forest manage for coppice might be allocate for aboveground on initial growth phase.

Intrinsic water use efficiency showed lowest values on rainy season (January, February and March), whereas on dry season (April to July) intrinsic water efficiency was highest for all treatments. This result was found in the previous study at the same study site (Battie-Laclau et al., 2016). Intrisic water use efficiency is direct relate to regulation of stomata closing. Lower stomatal conductance decrease both water loss and photosynthesis, which suggest an increase in water use efficiency through the stomata conductance. Stomata closing decrease carbon concentration in the chloroplast (C_c), however the difference between carbon concentration in the chloroplast (C_c) and the carbon concentration from the atmosphere (C_a) increase. This increased CO_2 concentration gradients across the stomata counteracts the decrease in stomata conductance, which is decline more photosynthesis than transpiration. Photosynthesis decline less than transpiration due to decrease in CO_2 concentration in the chloroplast (C_c) and intercellular CO_2 concentration (C_i) (Cernusak et al., 2003; Lambers, 1998).

Forest manage for coppice showed little increase in WUE_i than forest stand seedling. K application showed higher values than the control plot. Statistically analysis indicated significance effect for K fertilizer application (Table 1). Forest manage for coppice are dependent for fertilizer application for this initial growth on *Eucalyptus grandis*. Nutrients application is one of the key factor that determine the succeful of coppiced management (Carlos et al., 2014). Stumps mortality also occur in this study on unfertilized plot. Both treatment C+W and C-W could not regenerated after the clear cutting in all block.

3.6. Conclusion

Our study did not find different result between forest manage for coppice and seed-origin-plantation. Both type of management showed the same magnitude of stemwood, annual net primary productivity and leaf net primary productivity at 1 to 2 years after clear-cut. These results suggest that root system development in deep soil layer might be an advantage for water and nutrient absorption in the initial regeneration. Tree carbon allocation in forest manage for coppice might be partioning in leaf, branches and on stemwood production. However, carbon allocation in seedling forest stand might be partionining among below and aboveground biomass. Potassium fertilized application improve production of aboveground biomass. Throughtfall exclusion did not affected significantly aboveground production. Potassium fertilized plot with throughfall exclusion (K-W) produce less aboveground biomass than K fertilized plot without throughtfall exclusion (K+W).

Stand water use was highest for all K fertilized application in forest manage for coppice. However, water use was less than forest stand seedling measured in the previous study. Water use efficiency for stemwood was similar on the initial growth phase of forest manage for coppice. The difference above water use among forest management is due to tree diameter and stock density on forest manage for coppice. Not all stumps regenerated even in K fertilized plot. The dominate sprout on the stump was left to regrowth. In addition to this, the sprouts was not uniform for all plot. Forest manage for coppice use less water than seedling forest stands, approximately 18% less water use than seedling forest stand. Our estimation of transpiration might be understimate due to electrical problems. Hence, the first hypothese of this study was not confirmed.

Leaf water potential in dry season range -2.1 and -2.4 MPa in K fertilized plot and was more pronunced at the throughfall exclusion on dry season. This result was similar to the previous study. Both treatment on K fertilized plot showed highest leaf water potential than control plot, suggesting that potassium fertilizer application might increase water stress and water demand by the tree. The second hypotheses was confirmed.

Stand water use was higher on both plot fertilized by K application, suggesting that K fertilized increase water demand by the trees. This result was similar to the previous studies. The results have shown that water use were less K fertilizer plot with water exclusion than on K fertilized without water exclusion. Troughfall exclusion might decrease water availability for water absorption. Leaf water potential reached -2.4 MPa on dry season, suggesting that both treatments with potassium fertilizer have shown increase in water demand by fertilization. Hence, the third hypothese was not confirmed in this study.

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