THE STUDY ON TRANSPIRATION AND WATER USE EFFICIENCY OF *SHOREA PARVIFOLIA* UNDER ELEVATED CARBON DIOXIDE CONCENTRATION IN THE TROPICAL FOREST

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ABSTRACT

Tropical forest plays an important role in gas exchange regulation. It is interesting to know how plants respond to a changing environment especially fluctuation in carbon dioxide. How would this fluctuation affect their transpiration and water use efficiency (WUE) at the leaf level? Plants leaf is subjected to water deficit and therefore show different response in physiological parameters. Much as we know that the fluctuation of WUE at leaf levels is directly related to the physiological processes which control the gradients of water and carbon dioxide between the leaf and air surrounding the leaf. This paper will report the preliminary results of the physiological measurement of Shorea parvifolia sapling under the elevated carbon dioxide concentration in Tekam Forest Reserve, Pahang.

Keywords: Gas exchange, transpiration, vapour pressure deficit, tropical forest, *Shorea parvifolia*

INTRODUCTION

People are interested to know how plants respond to a changing environment especially fluctuation in carbon dioxide. How would this fluctuation affect their water use efficiency (WUE) at the leaf level? Plants leaf is subjected to water deficit and therefore show different response in physiological parameters. Much as we know that the fluctuation of WUE at leaf levels is directly related to the physiological processes which control the gradients of water and carbon dioxide between the leaf and air surrounding the leaf (Hatfield & Dold, 2019).

The tropical rain forest response to any disruption and environmental change is a critical issue in view of the importance and potential of tropical rainforests to mitigate the effects of climate change. Increased carbon dioxide (CO_2) gas levels in the air may have an impact on future water demand by plants. Most previous studies focused on the agricultural sector and found that the size and productivity of the plants were proportional to the increase in CO_2 concentrations in the air but the reduction in CO_2 concentrations resulted in plant productivity reducing and causing disruption to plant distribution (Siebke et al., 2002; Kimball, 1983; Street- Perrott et al., 1997). This shows that the plants did not react in the same way to the concentration of CO_2 gas due to differences in photosynthesis pathways in plants (Monteith, 1978; Kimball; 1983; Ghannoum et al., 2000). The two types of photosynthetic pathways are light reaction and dark reaction that form 3 types of photosynthesis known as C3, C4 and CAM photosynthesis. Theoretically, given that CO_2 gas is a greenhouse gas, increase CO_2 in air will cause air temperatures to rise. This occurred because of the nature of CO_2 gas that is capable of trapping heat and this will cause an increase in surrounding temperature. The increase in ambient temperature causes the difference in water vapour pressure (VPD) to increase. Increased VPDs will cause plant stomata to be open to release water into atmosphere during the photosynthesis processes. However, at the same time with increased VPD, it will affect stomatal behaviour to control the loss of water. The stomatal closure that controls the loss of water or the water use efficiency (WUE) by plants. When VPD is high, WUE will decrease and vice versa. Stomatal closure affects the process of photosynthesis. At physiological stage, WUE is defined as the rate of photosynthesis to transpiration (Liu-Kang & Theodore, 2004). The change in the plant WUE or transpiration should be measured as a response to environmental changes. This is important because plant transpiration is closely linked to photosynthesis activity. Transpiration rate in tropical forests is influenced by the amount of energy and VPD as well as the amount of groundwater content (Marryanna et al., 2017; Kosugi et al., 2012). The effect of changing CO_2 gas concentration on water efficiency by plants should be investigated to better understand the effects of climate change on tropical forests.

WUE is the molar ratio of CO_2 uptake (A) to transpiration (E) and can be written as $A/E= (c_a\text{-}c_i)/1{:}6\Delta w$ where c_a is external and c_i is the internal partial pressure of CO_2, respectively Δw is the leaf-air VPD (Griffith, 1993). Factors affecting WUE are increasing carbon dioxide (CO_2) concentrations, increasing temperatures, more variable precipitation, and variations in humidity. To analyse WUE, one must collect several important data that includes photosynthesis, transpiration, stomatal conductance (gs) and temperature. Stomatal conductance is dependent on environmental variables and essential for analysing water losses by transpiration and CO_2 uptake for photosynthesis (Granier et al., 2000). Hence, *gs* have been widely considered as a good plant-based indicator for irrigation purposes (Hernandez-Santana et al., 2016). WUE (or transpiration efficiency) describes the intrinsic trade-off between carbon fixation and water loss that occurs in dry land plants because water evaporates from the interstitial tissues of leaves whenever stomata open for CO_2 acquisition. The transpiration efficiency of crop plants is generally low as they typically lose several 100-fold more water than the equivalent units of carbon fixed by photosynthesis (Helen et al., 2013). VPD is an important regulator for plant growth. It influences several physiological parameters such as stomatal opening, CO_2 uptake, and transpiration, nutrient intake at root and plant stress. Theoretically, stomata will get closing as VPD increase and CO_2 uptakes get reduced. As VPD increases, the plant transpires (evaporates from leaves) faster due to the larger difference in vapour pressures between the leaf and the air.

Forest is a primarily climate regulator because it regulates the gas exchange between land and atmosphere under changing environment, however susceptible to climate change. We must investigate the resilience of tropical forest under changing environment. Hence, we should understand its transpiration and WUE respond to elevated CO_2 because increase in CO_2 concentration will potentially increases air temperature. This paper is aimed to share the preliminary result of transpiration monitoring at Tekam Forest Reserve (FR) where the free air CO_2 enrichment (FACE) was initiated to monitor the effect of elevated CO_2 on forest ecosystem.

MATERIAL AND METHOD

Site description

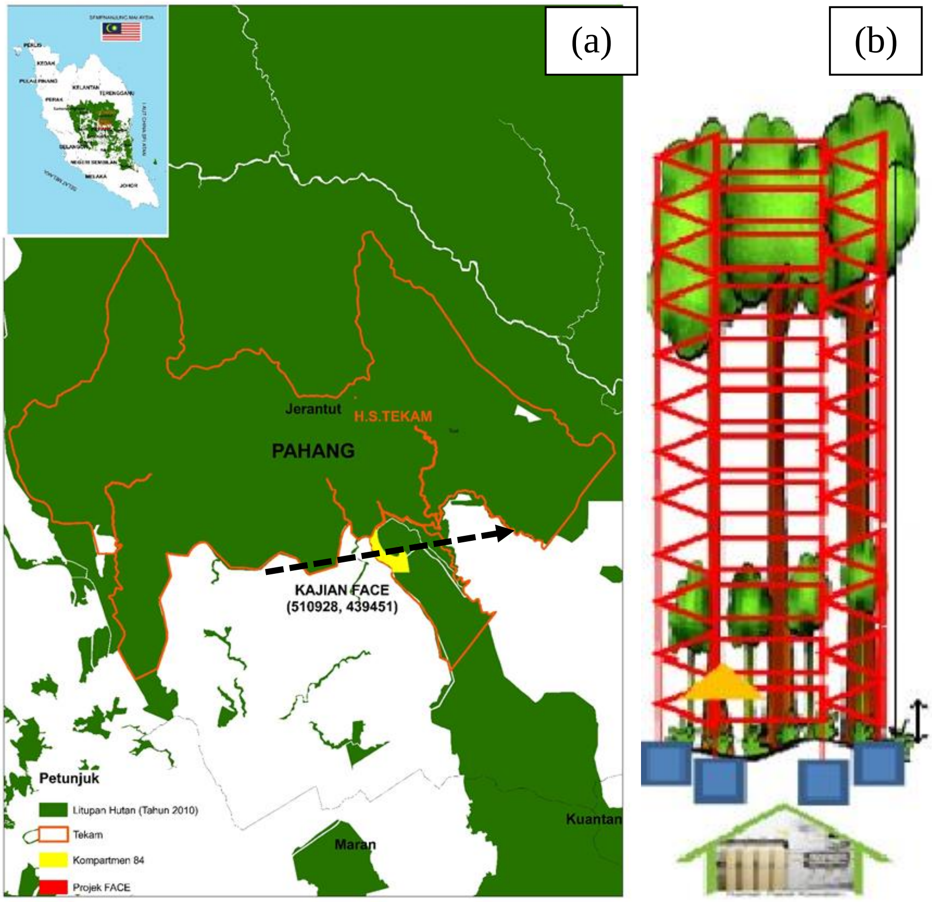
The study was conducted at the compartment 84 Tekam FR in Jengka, Pahang Peninsular Malaysia (Figure 1). It is situated at about 4" 15' N latitude and 102" 37' E longitude with elevations ranging from 80 to 280 m above sea level. The topography of the area can be described as undulating to hilly with an average slope of 28% (Baharuddin & Rahim, 1994). The soil in this area was derived from pyroclastic and volcanic rocks which are interbeded mainly with shales and sandstones of argillaceous strata and arenaceous materials which rise to clayey and sandy texture respectively (Amir Husni 1989). Middle soil layer (40-60cm) characterised with 42% clay, 25% silt and 37% sand. Monthly rainfall distribution of the study area generally showed a two-peak pattern which normally coincided with the northeast monsoon and the transitional period (Abdul Rahim, 1983). This pattern occurred in the months of November and May. The annual rainfall totals ranged from 1789 to 3190 mm with an average of 2508 mm (Baharuddin & Rahim, 1994) with the average temperature of 26^0C based on the nearest Meteorological station at Batu Embun.

The vegetation is typical of hill dipterocarp forest comprising a three-layered canopy, namely upper, middle and lower layers. The dominant species are *Shorea leprosula*, *Shorea bracteolate*, *Dipterocarpus cornutus*, *Euglina* species and *Cryptocarya* species. The lower layer is dominated by saplings of the upper canopy species and includes palms and shrubs. A hexagon of 12- meter height and 6-meter edge Free Air Carbon dioxide (CO_2) Enrichment (FACE) chamber was built in this area for monitoring the effect of elevated CO_2 on the forest ecosystem. It was estimated about 922 individuals inside the chamber which comprises of all vegetation including Dipterocarpaceae and non-dipterocarpaceae species (Table 1). Non-dipterocarpaceae dominated the hexagon of 419 individual followed by liana (n=272), dipterocarpaceae (n=204) while the rest (rattan, herb, fern, pandan, and palm) comprises of less than 15 individuals. The ratio of dipterocarpaceae and non-dipterocarpaceae within the FACE hexagon chamber is 1:2 (D=204, ND=419). The dipterocarpaceae family is dominated by *Shorea parvifolia* (9%) or is equavalent to 83 individuals of the total 922 individuals.

Table 1 The individual species distribution across the FACE chamber

Family	N of individual	%
Non Dipterocarpaceae	419	45.44
Liana	272	29.50
Dipterocarpaceae	204	22.13
Rattan	13	1.41
Herb	9	0.98
Fern	2	0.22
Pandan	2	0.22
Palm	1	0.11
Total	922	100.00

Figure 1 (a) Location of the study site at Jengka Research Station, Compartment 84 Tekai Forest Reserve, Pahang and (b) the design of Free Air CO_2 Enrichment (FACE)



Species selection
The FACE hexagon composed of eight families (Table 1), however, this paper report only on *Shorea parvifolia* (Figure 2). The *S. parvifolia* dominated 9% (83 individuals) of the total individuals (922 individuals) within the FACE hexagon (Table 2). The selected *S.parvifolia* was at diameter ranges between 13 to 18 mm with height range between 1 and 2.2 meter. It is locally known as *meranti sarang punai* and is widely distributed dipterocarp occurring in extreme south-east peninsular Thailand, throughout Peninsular Malaysia (except Perlis, extreme north-western Kedah and the Langkawi Islands), Sumatra, and Borneo and intervening islands (Lee et al., 2016) and mainly significant for timber supply.

Table 2 The distribution of Dipterocarpaceae species within FACE hexagon

Dipterocarpaceae Species	N of Individual	%
Shorea parvifolia	83	9.00
Shorea lepidota	54	5.86
Dipterocarpus cornutus	29	3.15
Shorea leprosula	21	2.28
Hopea dryobalanoides	12	1.30
Dipterocarpus baudii	3	0.33
Shorea ovalis	1	0.11
Shorea multiflora	1	0.11

Figure 2 The leaf of *S.parvifolia*

Leaf Gas-exchange measurement

Measurements were carried out in the field using LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA) connected to a standard 6 cm^2 cuvette. Fully expanded, sunlight leaves were clamped in the sensor cuvette, maintaining their natural position. The leaves were flushed with ambient air (flow rate 500 µmol s^{-1}), of which temperature and relative humidity were simultaneously recorded. Gas-exchange measurements, including CO_2 fixation rate (A), stomatal conductance to water vapor (gs), and transpiration rate (E), were logged after readings reached stable state (1–3 min). Infra-red gas analyser was matched to reach equilibrium before every measurement. Measurements were conducted with ambient temperature, while CO_2 reference concentration was maintained at 400 µmol CO_2 mol^{-1}. The photosynthetic photon flux density was set at 1600 µmol $m^{-2}s^{-1}$ to ensure that light-saturated photosynthesis rates were reached. Measurement was conducted between 10:00 a.m and 12:00 p.m, and the sample was logged three times for a measurement. These measurements were taken on 6 March 2019 at the FACE hexagon. Water use efficiency (WUE) at the leaf level was calculated as the ratio of carbon uptake (A) and water loss through transpiration (E) (WUE=A/E).

RESULTS AND DISCUSSION

Photosynthesis and transpiration in *Shorea parvifolia* sapling

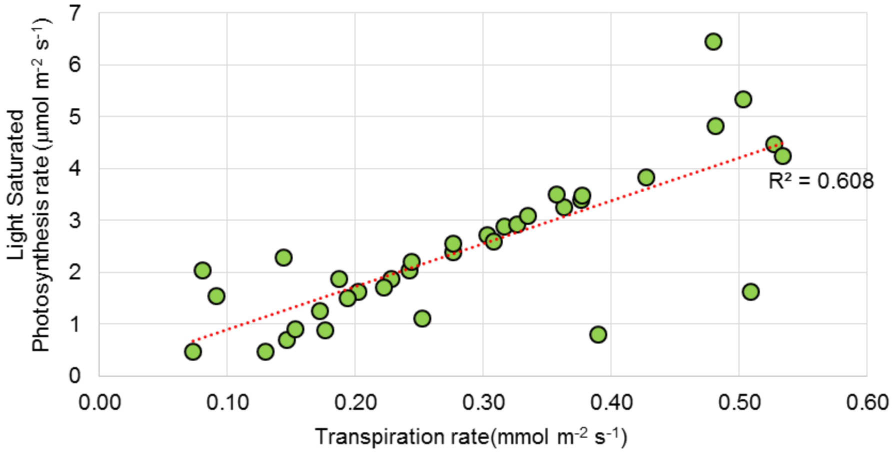
Physiological measurement found that the photosynthesis rates of *S.parvifolia* sapling was at the average of 2.46 µmol m^{-2} s^{-1} (±1.42) (Table 3). Lowest photosynthesis rates were 0.47 µmol m^{-2} s^{-1} and the maximum 6.45 µmol m^{-2} s^{-1}. The average stomatal conductance was 0.02 mol H_2O m^{-2} s^{-1} (±0.01), the VPD was at the average of 8.84 µmol CO_2 $mmol^{-1}$ H_2O (±4.09). The carbon intake (Ci) of measured *S.parvifolia* was at the range between 23.50 to 508.00 µmol CO_2 mol^{-1} averaging at 232.18 µmol CO_2 mol^{-1} (±81.53).

Table 3 The physiological measurement for *S.parvifolia*

	Average	Min	Max	SD
Photosynthesis (µmol m^{-2} s^{-1})	2.46	0.47	6.45	1.42
Stomata Conductance, *gs* (mol H_2O m^{-2} s^{-1})	0.02	0.01	0.04	0.01
Transpiration (mmol m^{-2} s^{-1})	0.29	0.07	0.53	0.13
Leaf Vapor Pressure Deficit (kPa)	1.49	1.41	1.57	0.05
Water use efficiency (µmol CO_2 $mmol^{-1}$ H_2O)	8.84	2.04	25.12	4.09
Carbon intake (ci) (µmol CO_2 mol^{-1})	232.18	23.50	508.00	81.53

The comparison of photosynthesis rate and transpiration in *S.parvifolia* (Figure 3) showed that transpiration increased with photosynthesis. The average photosynthesis rate was 2.46 µmol m^{-2} s^{-1} (n=36, ±1.42) while the average transpiration rate recorded was 0.29 mmol m^{-2} s^{-1} (n=36, ±0.13).

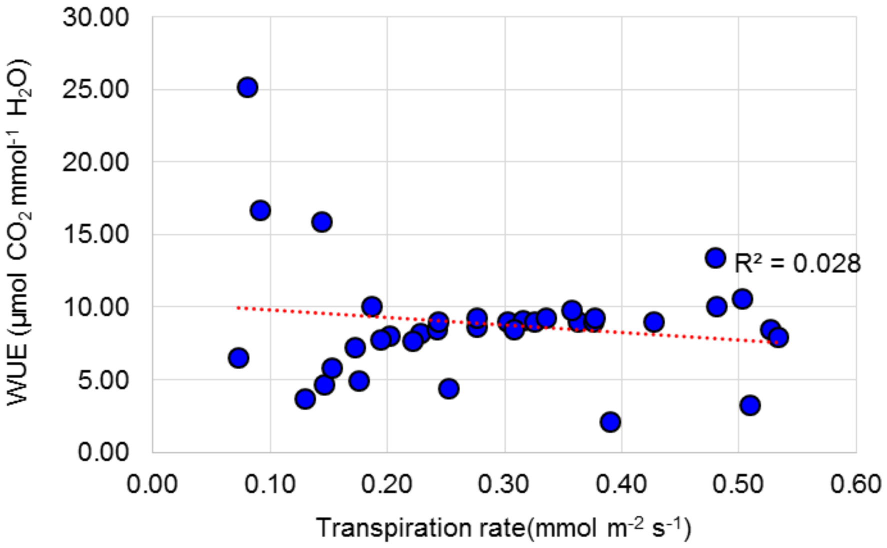
Figure 3 Comparison of photosynthesis and transpiration rates in *S.parvifolia* under the elevated CO_2 concentration



The relationship between transpiration rate and water use efficiency

The WUE of the *S.parvifolia* decreased as the transpiration rate increases (r^2=0.08). Maximum WUE was recorded at about 45.42µmol CO_2 mmol^{-1} H_2O occurred at the 0.12mmol m^{-2} s^{-1} transpiration rates. Most of the time WUE in *S.parvifolia* was in the range between 0 to 10 µmol CO_2 mmol^{-1} H_2O with transpiration varies from 0.12 to 0.50 mmol m^{-2} s^{-1} (Figure 4).

Figure 4 The comparison between transpiration and WUE of the *S. parvifolia*



The effect of stomata conductance and VPD on transpiration rate and WUE of *S.parvifolia*

There are two parameters underlying the transpiration and WUE in the plant; stomata opening and VPD. Stomata are important portals for gas and water exchange in plants and have a strong influence on characteristics associated with photosynthesis and transpiration (Yuping et al., 2017). As stomata control temperature and WUE, they are vital to the existence of the plant. Both stomata conductance (*gs*) and VPD seem to play important role in the transpiration regulation of *S.parvifolia,* however have weak effect on the WUE (Figure 5a,b and Figure 6a,b). Plants prefer different VPD at different stages in their growth. Usually young plant need lower VPD and moving to higher VPD as they mature. The plant monitored in this study is of sapling and shaded area, therefore VPD at leaf level is important. The VPD that plants feel is different than the air VPD because the temperature of leaves differs from the temperature of the air. The transpiration is strongly correlated (r^2=0.99) with *gs*, and maximum transpiration of 0.53 mmol m^{-2} s^{-1} was recorded at maximum *gs* of 0.04 mmol H_2O m^{-2} s^{-2}. The WUE decreases when *gs* increases indicating water stress. Under conditions of short-term water stress, plants increase their WUE by reducing stomata aperture and thereby transpiration rate; however, under conditions of prolonged water deficit, plants frequently also produce leaves with reduced maximum stomata conductance (Yuping et al., 2017). Plant stomata aperture play a predominant role in modulating the diffusion of CO_2 and H_2O vapour between leaf and atmosphere (Buckley and Mott, 2013), and optimizing photosynthetic and transpiration rates, hereby the water use efficiency (WUE) at leaf scale (Liu et al., 2009).

Figure 5 The relationship between leaf stomata conductance (*gs*) and (a) transpiration rate (b) WUE in *S. parvifolia*

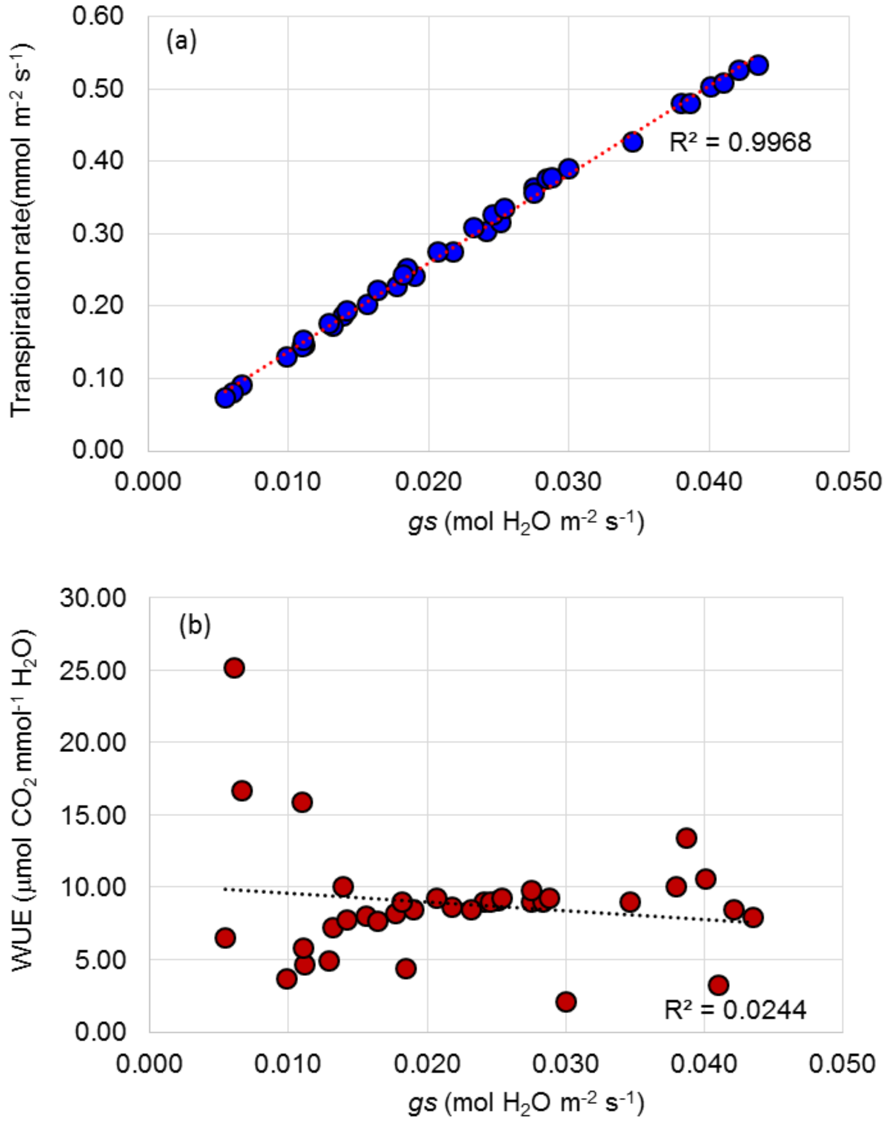
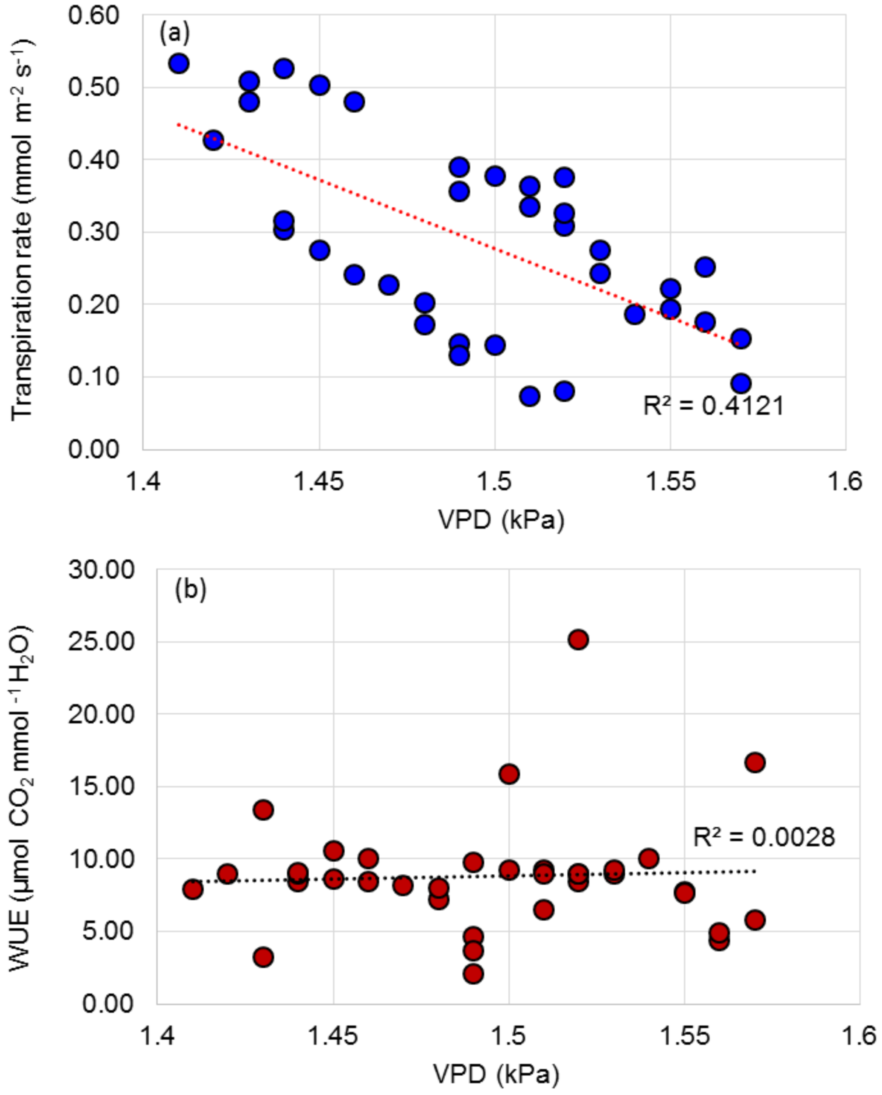


Figure 6 The effect of VPD on (a) Transpiration rate and (b) WUE of the *S. parvifolia*



CONCLUSION

Generally, transpiration rate is correlated proportionally with increase in stomatal conductance however; WUE is negatively correlated with stomatal conductance. At the certain level, plant will regulate stomatal opening to prevent excessive water loss and therefore, the ratio of photosynthesis to transpiration became smaller and thus WUE. Transpiration of *S.parvifolia* in this site is dependent with VPD and thus stomatal conductance. Elevated CO_2 in the FACE hexagon might also cause plant to reduce stomata opening as plant does not need to put much effort to capture CO_2 from the atmosphere because of abundance concentration is available. Regular monitoring should be conducted to obtain more data and strengthen the current findings. This observation is also important to address the effects of global warming on tropical forest growth. Since the effects of carbon storage on forest growth are still uncertain, we need longer term data to justify it. Some technical limitation faced in this study is due to rapid power drained of the Li6400 XT. The battery is able to sustain at least less than 4 hours on the light saturated measurement.

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