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Effects of Soil Compaction on Growth and Physiological Characteristics of *Azadirachta excelsa* Seedlings

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ABSTRACT

Urban landscape trees have always been very much exposed to the stresses of soil compaction. Trees in such condition need high flexibility in both morphological and physiological attributes to survive well. This study was conducted to determine the effect of soil compaction on *Azadirachta excelsa* seedlings by imposing different levels of compacted medium with bulk density at 1.2, 1.4 and 1.6 g cm^{-3}. Growth and physiological characteristics were assessed and repeated measures analysis was used to analyze the differences between treatments. The results showed that the seedlings were seen struggling to survive but still sprouting new shoots even after showing dying symptom. Significant decrease in leaf elongation also occurred in this experiment resulting from root damage thus initiated reducing physiology performance in leaf traits. However, biomass data showed persistent relation towards root sensitivity of the system architecture. Gas exchange attributes were also found to decrease significantly between treatments but not for other water related parameters such as predawn and midday water potential, hydraulic conductance and water use efficiency. It was decisively found that this species is partially tolerable towards soil compacted condition due to its ability to resist permanent damage and stress avoidance.

Key words: Repeated measures ANOVA, root shoot ratio, chlorophyll fluorescence, gas exchange, water use efficiency

INTRODUCTION

Plant growth and physiological processes are affected by soil compaction which is already discussed in millennia. In urban landscape, soil compaction is one of major physical factor that inconvenience to the tree and unfortunately trees are really highly exposure to the causes of soil compaction presence. One of the causes can be vehicles pass by along the road. Management of urban landscape tree requires heavy equipment during stockpiling which also can be the factor for soil to be compacted. Other than that, minimal management maintenance, raindrop effect, fire and trampling by animal and even footstep of human can give vibration into the ground and it expands until reach to root of trees.

Soil is composed by solid material and pores where solid fractions consist of mineral and organic mineral, meanwhile pores contain amounts of water and air. Soil compaction can be measured by using bulk density where the dry weight of soil divided by its volume and is expressed by g cm^{-3}. Whereas, the soil volume is including volume of soil particles and pores among soil particles. There are two important characteristics of porosity which are total pore spaces and the size distribution of pores. The pore spaces is the soil volume which occupied by air and water. Soil pores are generally grouped to two categories which are micropores and macropores. The micropores are pores less than 0.06 mm in diameter that important for water retention meanwhile macropores are greater than 0.06 mm diameter and it is important for aeration and saturated water flow. In compacted soil, the pores have been lacking due to tight soil.

Soil compaction reduces pore spaces between soil particles as they are pressed together (Horn *et al.*, 1995). As consequence, soil contains only few large pores which reduce the rate of both water infiltration and drainage and also the exchange of gases become slowly done. Other than that, soil strength increase where ability of soil to resist being moved is high which cause tree root difficult to penetrate to compacted layer. Both study by Alberty *et al.* (1984) and Day and Bassuk (1994) found that woody plant roots experienced restricted growth at bulk densities greater than 1.5 g cm^{-3}.

As compaction increases, physically inhibit roots struggle for elongation and favour to generate thick and short lateral roots limited by oxygen presented, decreasing pore sizes and increased soil strength. Short roots is resulted from less occupation of soil volume and this scenario lead to lacking of resources collection such nutrient and water for internal physiology processes and growth (Andrade *et al.*, 1993). The situation makes trees in stress condition and leading to decreasing growth rate where more energy uses for associated roots facing compacted soil. The soil compact also contributes to have anaerobic layer due to less pore which survival roots is impossible. Moreover, leaf area and shoot biomass also significantly affected by compacted soil by reducing of plant development (Coder, 2000).

Soil compaction effects are covering beyond than plant morphology but also including their physiology process. Various studies showed that stress root produced chemical signal such as abscisic acid and ethylene to the shoots via xylem sap. As consequence, tree physiological activity decreases in photosynthetic activity due to drop in stomatal conductance on leaves (Tubeileh *et al.*, 2003). Thus, plants involved developed to absorb less water and tuned water deficit leaf. Due to unable to uptake enough nutrient, the plants tend to have for nitrogen and potassium deficiency (Kozlowski, 1999). In this experiment, thirty three seedlings of *A. excelsa* were tested to figure out their morphological and physiological response towards soil compaction. It was hypothesized that growth rate and physiological performance of *A. excelsa* decrease in higher bulk density.

MATERIALS AND METHODS

Study site: The experiment was conducted in a nursery (open area) of the Faculty of Forestry, University Putra Malaysia, Serdang, Selangor, from the end of October 2011 until March 2012. The average daily temperature varied from about 27-30°C, the relatively humidity varied from 60-71% and the irradiation range was around 609.48 µmol m^{-2} sec^{-1}, PAR as measured by a Watch Dog Model 2475 Plant Growth Station (Spectrum Technologies Inc., Aurora, IL).

Treatment preparation: Pot with size of 32 cm in height, 34 cm in inner diameter and volume of 16.2 L was filled with mixture of top soil, sand and peat (3:2:1). Hereafter, soil bulk density determined by soil weight inside pot and pot volume as demonstrates at Eq. 1. After that, the soil in the pot was compacted until it is in the line of volume of the pot taken. From here, it can be seen that the higher the level of bulk density, the heavier the soil weight and higher soil volume and as a result, it is harder to compact the soil.

$$\text{Bulk density}\,(\text{g cm}^{-3}) = \frac{\text{Soil wet weight}\,(\text{g})}{\text{Soil volume}\,(\text{cm}^3)} \tag{1}$$

There are three levels of bulk density as treatment in this experiment, 1.2, 1.4 and 1.6 g cm^{-3}. In the end of the experiment, soil bulk density was measured again by using cylindrical core method (Page-Dumroese *et al.*, 1999) to detect changes that may occur after transplanting seedling. After the pots were prepared with compacted mixture soil with bulk density, they were then left for two weeks before transferring to three years old of healthy *A. excelsa* seedlings. During that time, pot was still observed in order to have accurate bulk density for experiment. After two weeks, soil was taken away in order to transfer the seedlings inside the pots. Hereby, it was noticed that soil was prepared in the compact properties but not compacted during planting tree. This method selected to simulate the common situation in field whereby seedlings were planted in the compacted soil. In addition, it explains the situation where part of tree ultimately roots already face stress with soil compaction even in early stages.

Experimental design: Completely randomized experimental design was use during arranging pots in this experiment. Meanwhile, randomization was carried out by using random number generator used in order to prepare every treatment to have equal chance of being assigned to any experimental unit.

Growth measurement: Growth data were taken for every first week of month. Seedling diameters were recorded with a digital vernier caliper (Mitutoyo UK Ltd., Hampshire, UK) 10 cm above the collar and heights were measured with a meter ruler. The measurements support the data on growth increment throughout the experiment in terms of Absolute Growth Rate (AGR) includes the diameter and height growth using an average of three readings for each seedling. The average of the diameter or height was used to calculate the absolute growth rate, as follows:

$$\text{AGR}_{\text{diameter}}\,(\text{mm week}^{-1}) = \frac{D_2 - D_1\,(\text{mm})}{T_2 - T_1\,(\text{week})} \tag{2}$$

$$\text{AGR}_{\text{height}}\,(\text{cm week}^{-1}) = \frac{H_2 - H_1\,(\text{cm})}{T_2 - T_1\,(\text{week})} \tag{3}$$

Where:

$\text{AGR}_{\text{diameter}}$ = Absolute diameter growth rate
$\text{AGR}_{\text{height}}$ = Absolute height growth rate
D_1/ H_1 = Diameter or height at T_1
D_2/ H_2 = Diameter or height at T_2
T_1/ T_2 = Time

Biomass from each part of the seedlings was measured as an absolute growth estimator. The samples of the tree components were oven-dried at 75°C until a constant weight was reached. The components consisted of aboveground biomass, determined by calculating the sum of the biomasses of shoots, leaves and stems. Other components were root mass as the below ground biomass and total seedling biomass calculated as the sum of the aboveground biomass and root biomass.

Measurement of leaf ecophysiological traits and leaf characteristics: During measurement, three fully expanded leaves from each individual seedling were selected as sample for measurement. The measurements were collected in the similar time of data collection, to minimize changes through months which may occur in leaf water content and irradiance level. The chlorophyll contents of the composite leaf samples from each treatment were determined using a SPAD 502 Plus chlorophyll meter (Spectrum Technologies Inc., Aurora, IL). The index of chlorophyll content was known as SPAD value.

Using the same leaves from the *A. excelsa* seedling, photochemical efficiency was measured using a Handy Pea chlorophyll fluorometer (Hansatech Instruments, Norfolk, UK), wherein dark leaf clips were applied to stop any photosynthetic process. After 15 min, PAR was supplied and data on initial fluorescence (F_o), maximum fluorescence (F_m) and the maximum photochemical efficiency of photosystem II (PSII) indicated by the ratio of variable to maximum fluorescence (F_v/F_m where $F_v= F_m-F_o$) were recorded.

Leaf gas exchange rate was measured with a LI-6200 portable photosynthesis meter (LI-COR Inc, Lincoln, NE). Data was taken in the morning, from 800-1100 h, to avoid the state where they achieve optimum photosynthetic rate at midday (Marra and Heinemann, 1982). All measurements were taken at a flow rate of 250 μm sec^{-1}, reference CO_2 concentration of 360 μm CO_2 mol^{-1} (ppm) and 650 μm photons m^{-2} sec^{-1} of quantum flux. The responses were quantified by measuring the photosynthesis rate (A_{net}), stomatal conductance (G_s), intercellular CO_2 (C_i), transpiration rate (E) and leaf to air vapor pressure deficit (VpdL) calculated based on leaf temperature (D). The leaf samples was then picked to measure leaf area using a LI-3100 leaf area meter (LI-COR Inc., Lincoln, NE).

Whole seedling hydraulic conductance (K) and water use efficiency: Briefly, hydraulic conductance (K) determines water movement across the membrane (Hsiao, 1973; Wullschleger *et al.*, 1998). The changes occur during hydraulic conductivity and exposure of the woody stem to xylem cavitation and the significance of water supplies is evaluated. Typically, the larger the K value, the greater is the flow rate. K calculations were performed based on measured water potential and whole seedling transpiration as:

$$K(\text{kg kpa}^{-1}\,\text{sec}^{-1}) = \frac{E}{-\Psi_p\ (-\Psi_m)} \tag{4}$$

Where:

K_p = Whole-seedling hydraulic conductance

E = Transpiration rate

Ψ_p = Predawn leaf water potential

Ψ_m = Midday leaf water potential

Whole seedling transpiration rate was measured by using an equation that uses data on weight loss over time:

$$E = \frac{W_2 - W_1\,(kg)}{T_2 - T_1\,(h)} \tag{5}$$

Water potential (Ψ) was measured by taking one healthy leaf from each seedling, plucked at predawn and midday, to determine its water potential using a leaf pressure chamber (PMS Inc., Corvallis, OR).

Water Use Efficiency (WUE) can be classified into two types, intrinsic WUE (WUE_i) and instantaneous WUE (WUE_{inst}), according to the following equations derived from parameters gained from gas exchange measurements (Fischer and Turner, 1978):

$$WUE_i = \frac{A_{net}\,(\mu mol\,CO_2)}{g_s\,(mol\,H_2O)} \tag{6}$$

$$WUE_{inst} = \frac{A_{net}\,(\mu mol\,CO_2)}{E\,(mol\,H_2O)} \tag{7}$$

Generally, WUE_i only focuses on the situations inside the stomata opening, while WUE_{inst} is influenced by the changing evaporative demands on water flux outside the leaf.

Statistical analysis: All analyses were performed using repeated measure analysis of variance (ANOVA) carried out with SPSS version 21.0 statistical software (SPSS, Chicago, IL) to identify differences between time, treatment and their interaction except for biomass. Curve fitting and graph handling were then performed using Sigma Plot version 10.0 (SPSS Inc., San Rafael, CA). All tests were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Survival rate within treatment: Survival rate through experiment period was not significantly affected by bulk density treatment (F (3, 29) = 3.638, $p<0.05$, meanwhile significant differences found by repeated time (F (6,174) = 10.196, $p<0.001$). Figure 1 shows that magnitude of growth declined for seedling survival in the higher soil bulk density during the study period. However, entering to fourth month, it was found that the survival rates in all treatments including control have decreased constantly. To be highlighted in this experiment is new sprouting shoots were found in lower part stem of identified dead stem which was near to planting collar. In the end, treatment of 1.6 g cm^{-3} soil bulk density had no more seedlings survive due to higher stress. Meanwhile 1.2 and 1.4 g cm^{-3} soil bulk density still have seedlings survive at 10 and 40%, respectively.

Morphology performance within treatment: Repeated measures ANOVA correction determined that mean $AGR_{diameter}$ (F (5,145) =16.192, $p<0.001$) and differed statistically significant between months (Fig. 1). Other than that, for $AGR_{diameter}$ the growth of *A. excelsa* differed in time and treatment interaction at F (15,145)=2.114, $p<0.05$. There was highly significant in response between treatments where F (3, 29)=7.894, $p<0.001$ for $AGR_{diameter}$. Meanwhile, *post hoc* test carried

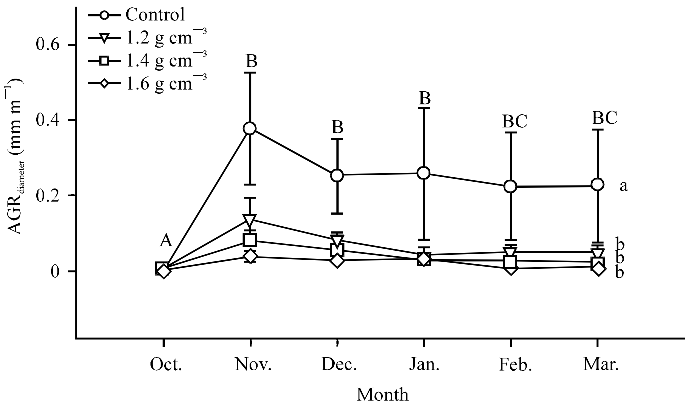

Fig. 1: Absolute growth rate diameter per month over time of *Azadirachta excelsa* seedlings planted at different levels of soil bulk densities. Data is the mean of each treatment replicates±SEM, shown by vertical error bars. Uppercase letters indicate significant differences for all means comparisons with pairwise comparisons within each month. Lowercase letters indicate significant differences for all means comparisons with Tukey's HSD within different treatments

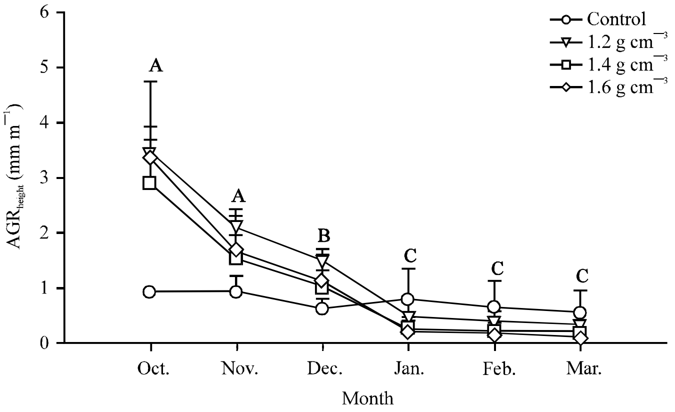

Fig. 2: Absolute growth rate height per month over time of *Azadirachta excelsa* seedlings planted at different levels of soil bulk densities. Data is the mean of each treatment replicates±SEM, shown by vertical error bars. Uppercase letters indicate significant differences for all means comparisons with pairwise comparisons within each month

out using the Tukey's HSD revealed that *A. excelsa* seedlings in control were significantly different from other treatments for $AGR_{diameter}$. It was found that higher bulk density affect the relative growth rate in diameter where no increment were found in seedlings.

Repeated measures ANOVA shows that mean AGR_{height} (F (5, 145) = 23.397, $p<0.0001$) differed statistically significantly between time of months. In spite of, there are no significant difference for interaction and between treatments for AGR_{height}. Expressively, the trend analysis throughout of experiment for both parameters AGR_{height} (Fig. 2). In early months of seedling planting in soil compaction condition, there was apparent increment by the seedlings. As opposed to entering the fourth month of experiment, seedlings significantly decrease in diameter growth. Nevertheless, further analysis of *post hoc* test using the Tukey's HSD revealed that in AGR_{height} and RGR_{height}, there were no differences among treatments.

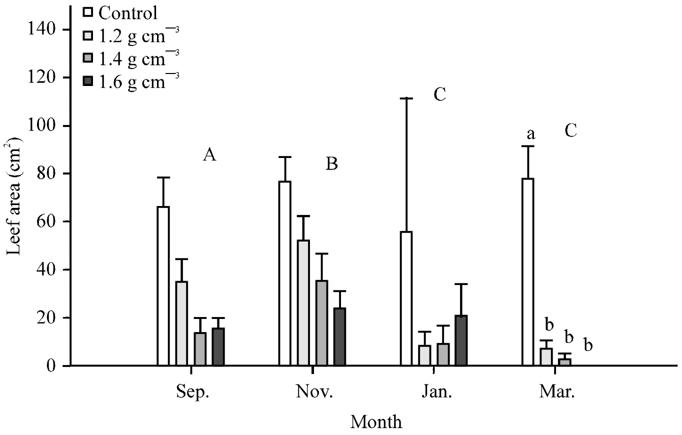

Fig. 3: Mean values of leaf area per month over time of *Azadirachta excelsa* seedlings planted at different levels of soil bulk densities. Data is the mean of each treatment replicates±SEM, shown by vertical error bars. Uppercase letters indicate significant differences for all means comparisons with pairwise comparisons within each month. Lowercase letters indicate significant differences for all means comparisons with Tukey's HSD within each treatments

The development of leaf area was measured four times throughout the experiment due to leaves number limitation (Fig. 3). The limitation was based on assuming soil compaction as stress factor to *A. excelsa* and to be watchful on photosynthesis factory of leaves. This assumption is really needed as considering the leaves limitation and hard time of the seedling were faced. The test between time effect yielded an F (4,16)=14.964, $p<0.001$, indicated the mean of month for each treatment was highly significant difference. Indeed, between soil bulk density treatments also same result after analysed with F (3, 29)=15.802, $p<0.001$. Whereby, leaf area in control seedlings were significantly different with other treated seedlings, proved by Tukey's HSD *post hoc* test at the end of experiment. Through observation, it was noticed that there were not many leaves in 1.6 g cm^{-3} as well as 1.4 g cm^{-3} which only had shoots remaining on a few parts of stem. After four months planted in higher bulk density, leaf area of *A. excelsa* seedlings was decreased until the end of experiment.

Biomass allocation: After seven months of experiment under soil compaction condition, *A. excelsa* showed slight variability in biomass yield. The shoots and leaves part from treated seedling do not have biomass weight except for treatment 1.2 g cm^{-3}. Averagely, treatment 1.2 g cm^{-3} have 3×10^{-5} g ($\pm7\times10^{-5}$) and 2×10^{-5} g ($\pm2\times10^{-5}$) of shoot and leaves mass, respectively. Despite, stem part do have significant difference between treatments, F (3, 9) =18.155, $p<0.0001$ which indicate that stem part really were affected by bulk density treatment. By the same token, root and total biomass do not allocate biomass in virtuous amount.

Leaf physiological traits: Treatment was significant to chlorophyll content between month F (6, 174)=14.93, $p<0.001$ and same goes between treatment, F (3, 29)=6.643, $p<0.001$ (Fig. 4). Repeated measures ANOVA that the all parameters was affected in increasing soil compaction level over the course of the study. Then, further analysis of *post hoc* test using the Tukey's HSD confirmed that all photochemical efficiency except F_o for treated seedling was pointedly affected by bulk density (Fig. 5).

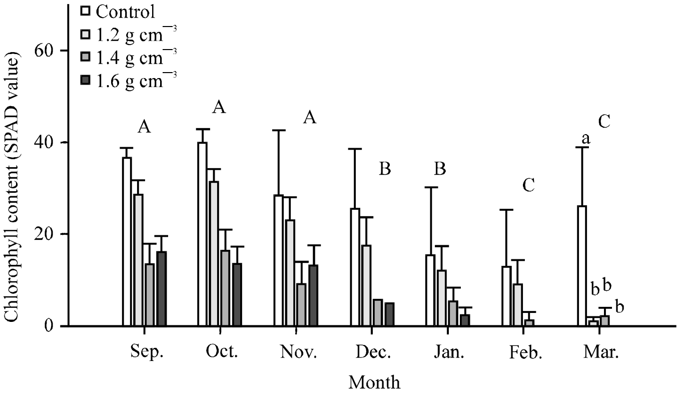

Fig. 4: Chlorophyll content using SPAD value per month over time of *Azadirachta excelsa* seedlings planted at different levels of soil bulk densities. Data is the mean of each treatment replicates±SEM, shown by vertical error bars. Uppercase letters indicate significant differences for all means comparisons with pairwise comparisons within each month. Lowercase letters indicate significant differences for all means comparisons with Tukey's HSD within each treatments. Lowercase letters indicate significant differences for all means comparisons with Tukey's HSD within different treatments

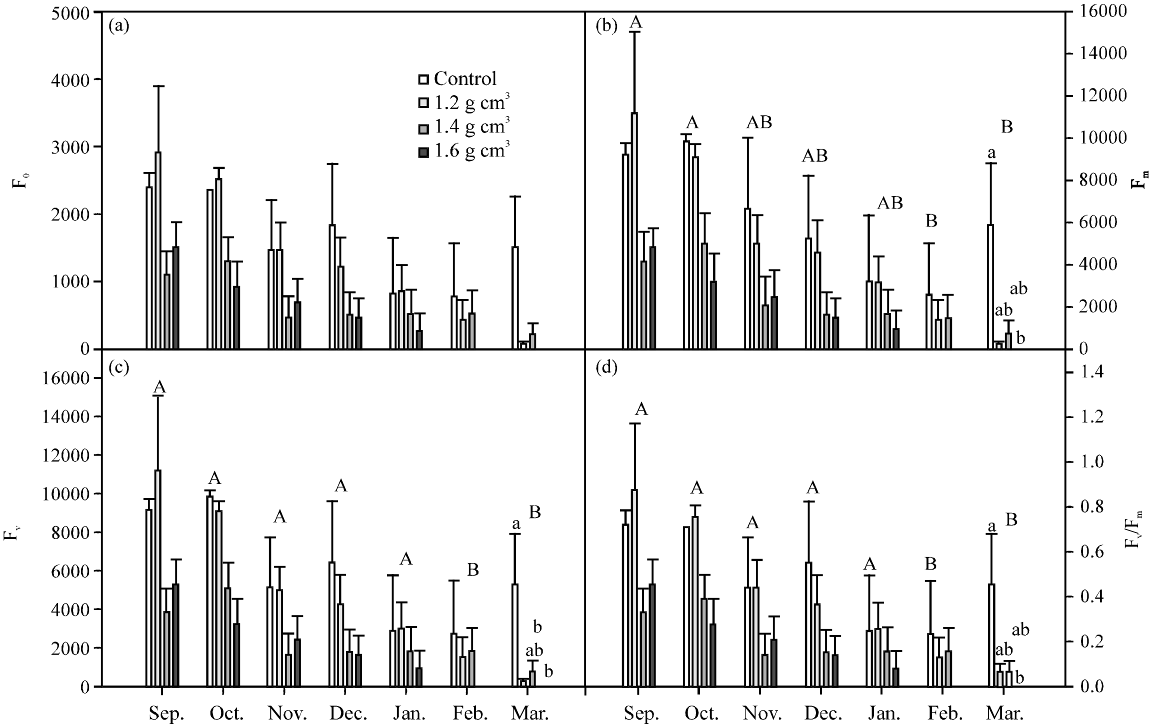

Fig. 5(a-d): Mean values of chlorophyll fluorescence parameters, (a) F_o, (b) F_m, (c) F_v and (d) F_v/F_m of *Azadirachta excelsa* seedlings planted at different soil bulk densities levels. Data is the mean of each treatment replicates±SEM, shown by vertical error bars. Uppercase letters indicate significant differences for all means comparisons with pairwise comparisons within each month. Lowercase letters indicate significant differences for all means comparisons with Tukey's HSD within each treatments

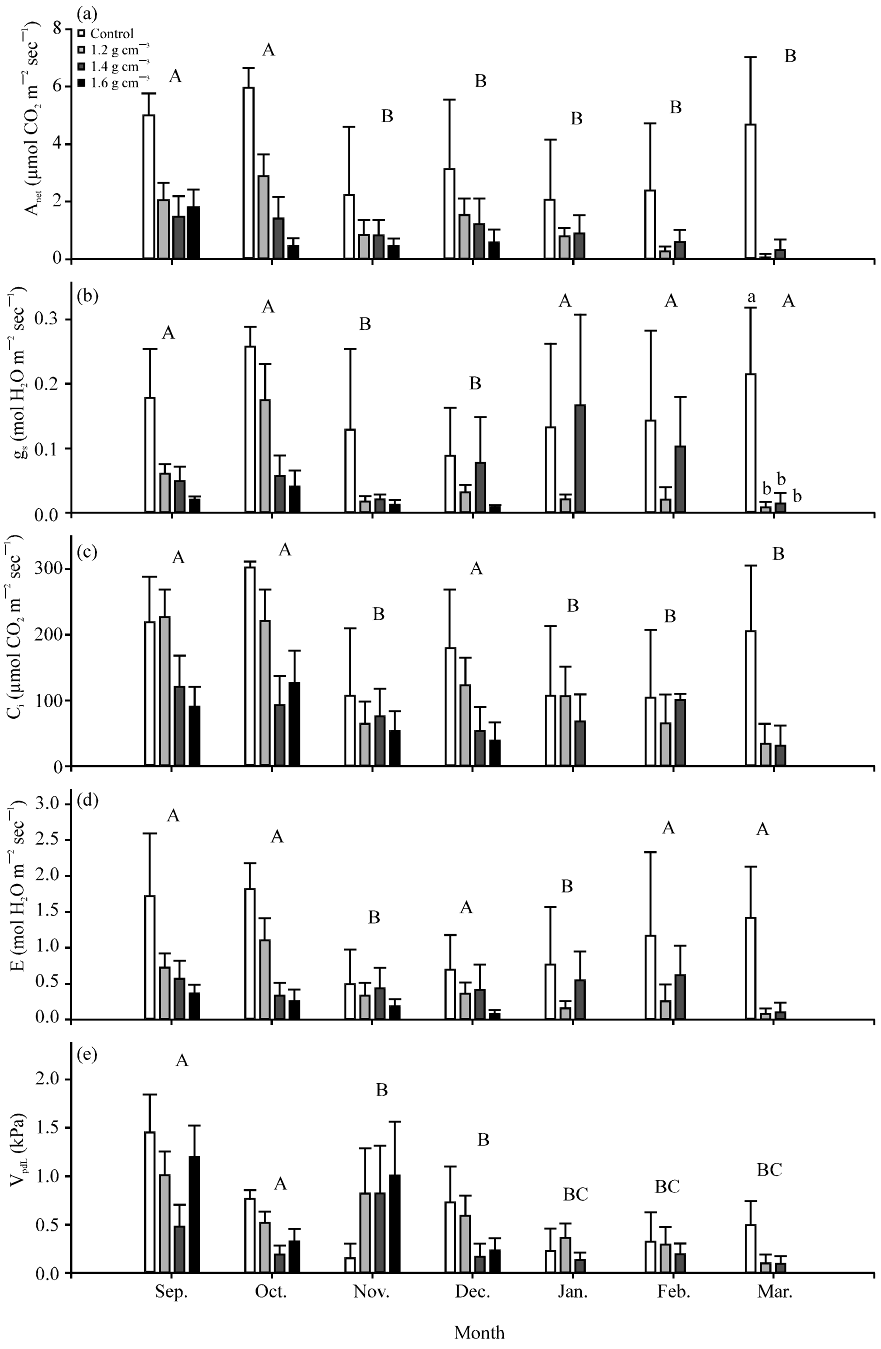

Fig. 6(a-e): Mean values over time of gas exchange parameters treatment (a) Net photosynthesis (A_{net}), (b) Stomatal conductance (g_s), (c) Intercellular CO$_2$ (Ci), (d) Transpiration rate (E) and (e) Leaf to vapour pressure deficit in leaves (VpdL) for each treatment of *Azadirachta excelsa* seedlings planted at different soil bulk densities. Data is the mean of each treatment replicates±SEM, shown by vertical error bars. Uppercase letters indicate significant differences for all means comparisons with pairwise comparisons within each month. Lowercase letters indicate significant differences for all means comparisons with Tukey's HSD within each treatments

Table 1: Repeated measures ANOVA within and between subjects of the effects of soil bulk densities and time on the gas exchange parameters for each treatment whereas net photosynthesis (A_{net}), stomatal conductance (g_s), intercellular CO2 (Ci), transpiration rate (E) and leaf to vapour pressure deficit in leaves (VpdL)

SOV	MS	df	F
A_{net}			
Time	1.995	6,174	10.879***
Time*Treatment	0.273	18,174	1.491^{ns}
Treatment	2.462	3,29	2.713^{ns}
g_s			
Time	0.060	6,174	4.348**
Time*Treatment	0.022	18,174	1.577^{ns}
Treatment	0.153	3,29	3.388*
C_i			
Time	11.684	6,174	10.32***
Time*Treatment	1.448	18,174	1.279^{ns}
Treatment	12.591	3,29	1.893^{ns}
E			
Time	0.749	6,174	7.788***
Time*Treatment	0.139	18,174	1.44^{ns}
Treatment	1.109	3,29	2.7^{ns}
V_{pdL}			
Time	1.002	6,174	9.268***
Time*Treatment	0.139	18,174	1.282^{ns}
Treatment	0.500	3,29	1.339^{ns}

ns = non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Gas exchange parameter attribution: ANOVA repeated measures across time for all the parameters showed significant differences (Table 1, Fig. 6). Meanwhile only g_s have significant different between treatment where control had substantial highest as compared to other treatment. Kozlowski (1999) reported that decreasing result of A_{net} in higher bulk density can caused by smaller leaf area as resulted in this study. As a result, reduced photosynthesis could limit the availability of sugar which provides metabolic energy to decrease growth.

Hydraulic conductance and water potential: Hydraulic conductance (K) showed the level of water smoothness across the membrane which influences the hydraulic movement from root to leaves. Analyses of this data indicated that no statistically significant treatment effected on hydraulic conductance (F (3, 29) =1.403, p = 0.345). A larger value indicates that, individual seedlings had longer time to move water molecule. In bulk density of 1.2 g cm^{-3}, seedlings have higher values of K compared to control. Meanwhile, in other treatments, pressure of seedling to recover water losing resulted in low rate of water movement.

Water use efficiency: Intrinsic Water Use Efficiency (WUE_i) and instantaneous water use efficiency (WUE_{inst}) were not significantly affected by bulk density treatment (F (3, 29) = 1.74, p>0.05) and (F (3, 29) = 2.052, p>0.05), respectively. In opposite by time, there are significant difference found for these parameters as shown in Table 2. Meanwhile, the pattern over experiment shows that productivity of WUE_i and WUE_{inst} is decreasing (Fig. 7).

Survival adaptation capability: The causes of death in higher bulk density have been clearly discussed from many studies. It was also included the cause of root growth restriction, very poor movement of air, inessential nutrients and lacking of microbial activity (Saqib *et al.*, 2004). It

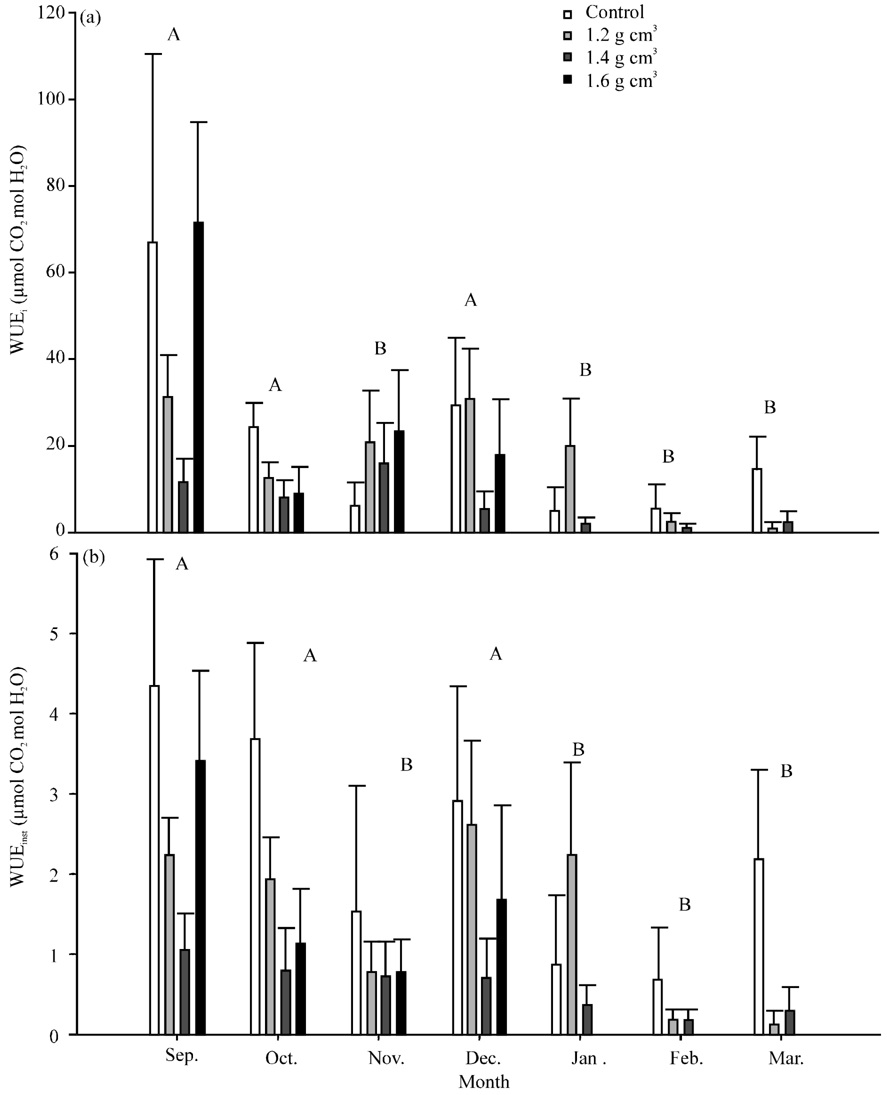

Fig. 7(a-b): Mean value over time of water use efficiency (a) WUE_i and (b) WUE_{inst} of *A. excelsa* seedlings planted at different soil bulk densities. Data is the mean of each treatment replicates±SEM, shown by vertical error bars. Uppercase letters indicate significant differences for all means comparisons with pairwise comparisons within different month

Table 2: Repeated measures ANOVA within and between subjects of the effects of soil bulk densities and time on the of intrinsic water use efficiency (WUE_i) and instantaneous water use efficiency (WUE_{inst})

SOV	MS	df	F
WUE_i			
Time	5.400	6,174	11.116***
Time*Treatment	0.661	18,174	1.36***
Treatment	5.188	3, 29	1.74^{ns}
WUE_{inst}			
Time	1.489	6,174	11.652***
Time*Treatment	0.203	18,174	1.592^{ns}
Treatment	1.829	3,29	2.052^{ns}

ns = non-significant,***p<0.001

was also having huge discussion in root adaptation as a major factor of tree damaged towards soil compaction, whereas changes occur in soil matrix which cause by oxygen and heat can be affected by losing the gradient from seedlings roots' membrane and their surroundings. Thus, sucking of nutrients and water was not occur and as a result, spiraling or some other root alteration will occur (Taylor and Brar, 1991). However, it was found that *A. excelsa* capable to tolerate by new sprouting which may due to releasing of ethylene as a respond to water shortage. The released ethylene was then accumulate of auxin which triggers response in surrounding cells and decreasing bud dominance (Van Bloem *et al.*, 2007).

Effects on morphology: From the growth perspective specifically diameter, common parameter of tree growth, it was tolerating by stopping growth in higher bulk density. It usually happen because of hormonal growth regulations in trees where increase in abscisic acid (ABA) and ethylene (Tardieu, 1994). Specifically, these hormones inhibit the primary growth where cell division from vascular cambium cannot form new xylem and phloem radially. In fact, two vascular tissues is most important parts affect physiological of seedlings. A study by Kamaluddin *et al.* (2005) found that early treatment give significant affect rather than soil compaction to decrease height growth of lodgepole pine and increase diameter growth of Douglas-fir. However, this study shows opposite result by showing height increment in early growth. Here, it can be seen that significant increment occur in height in treated seedlings in early of planting. It can be explained as the condition where root was shocked by the compacted soil which also supported by pattern of survival. Later, root surface stop uptaking nutrients which then causing no diameter increment as no encouragement for cells to divide in long vessels specifically in phloem. In contrary, storage of hormone for shoot growth, auxin was still spreading which was why seedlings can still have increment in height. Thus, the apical meristem cell which charge on dividing cells and elongate at the base of the bud to create upward growth in seedling.

The similar result of decreasing leaf area was also found by Grzesiak (2009) and Montagu *et al.* (2001). The stress of compacted soil condition has been cues for seedling to respond with less leaves surfaces areas. The principle of leaf growth as written by Van Volkenburgh (1999) stated that inhibition of growing leaf cells were influenced by photosynthetic activities. As result showed in photochemical efficiency, it was equivalent with the result in leaf area. Other than that, root response towards underground soil roughness occurred. Whereas, leaf size decreases when water is limited and is an important indicator of transplant stress (Parkhurst and Loucks, 1972). In more detailed physiologically, it was the consequences by hesitance of cell division which influenced by solutes, absorbing water, turgor pressure and extending the cell wall. Here again, it stated that reduction of leaf expansion growing on compacted soil were also correlated with endogenous hormone ethylene levels and ABA concentration (Hussain *et al.*, 1999).

Biomass allocation: Reduction in plant biomass and yield on compacted soil is related to the gas exchange rate (Masle and Passioura, 1987; Masle *et al.*, 2002). The same result of decreasing dry matter in shoots and roots were studied by Grzesiak (2009). Through this result, it can be correlated that plant regulator, auxin was exhibited during compaction as roles as cell enlargement in apical dominance and root initiation. Meanwhile, Hoffmann and Jungk (1995) suggested that under soil compaction, shoot growth was decreased when root growth was restricted and that both these traits were closely related, irrespective of the cause of root growth limitation by compaction. The biomass and morphological structures of seedlings' root system including root length, root surface and root volume were negatively related to soil compaction (Liu, 2011).

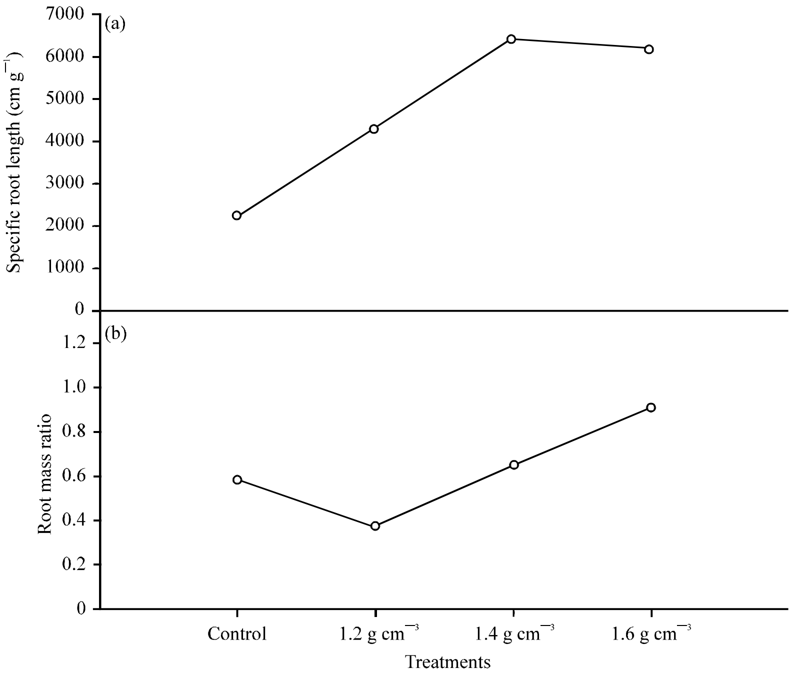

Fig. 8(a-b): (a) Specific root length and (b) Root mass ratio of *A. excelsa* seedlings planted at different levels of soil bulk densities

It was known that root grows by exploring through the pores of soil or moving soil aside. In the high bulk density, root of *A. excelsa* do not have the ability and also resist on doing the same thing (Saqib *et al.*, 2004). Thus root capacity decreased and affected the uptake of water and nutrient (Hussain *et al.*, 1999). In addition, soil-borne organisms include nematodes, smut and insects are absence in higher bulk density which also affected roots distribution. Coupled with the fact on compaction treatments had elevated levels of carbon dioxide compared to control with the highest level (0.62%) in the heavily compacted soil (Kuzovkina *et al.*, 2004). As consequence, regular task was disturbed in root part such as less cytokinin hormone produced which then resulted by in reduction of cell division (Miransari *et al.*, 2009). However, *A. excelsa* seedlings were making investment for producing more roots rather than other parts in order to overcome soil compaction delinquent (Fig. 8).

Physiological toleration rates in leaves: The common outcome for chlorophyll content was resulted by chlorosis issues, condition of insufficient chlorophyll on the leaves which can directly limit photosynthetic potential (Kobaissi *et al.*, 2013; Liu, 2011; Shang *et al.*, 2008; Yan *et al.*, 2009). The same result have been found by Smiley *et al.* (2006) by having significantly lower of chlorophyll content in compacted treatment than in uncompact/suspended pavement. They believed that soil pH (8.8-9.1) has contributed to the lower reading of SPAD values. The chlorosis influenced by percentage of leaf nitrogen (N) was associated with SPAD values which proved by majority of leaf N is contained within the chlorophyll molecules (Farrokh *et al.*, 2012; Weir and Cresswell, 1997). The N influences characteristic of individual trees including structure, vigour, as well as the ability to tolerate environmental stress. Consequently, lower chlorophyll content in treated seedlings may due to lowered uptake of N.

Theoretically, the values of F_v/F_m significantly decreased for all treated seedlings, 1.2, 1.4 and 1.6 g cm^{-3} due to occurrence of the photo-damage of PSII under severe soil stress condition while no photo-inhibitory or comparatively smaller photo-damage to the photosynthetic apparatus at control observed (Baker and Rosenqvist, 2004). A significantly decrease of F_v/F_m also suggest development of slowly relaxing quenching process of open PSII centres as well as an increase in energy dissipation as heat and increasing of photo-damage to the photosynthetic apparatus. The same results were found in study by Liu (2011) that photosynthetic physiological characteristics of *Michelia chapensis, Michelia maudiae, Ilex latifolia, Cinnamomum camphora, Photinia serrulata* and *Magnolia denudata* species decreased as the increasing of the soil compaction level. The research also found that diurnal maximum of photosynthetic physiological taking place time and value was varied under different soil compaction levels. In addition, another study suggested that a soil compaction of 180 MPa and more, would reduce the F_v of PS II, however did not affect the F_o and F_m values (Philip and Azlin, 2005).

Huang *et al.* (2013) study by Day (1999) revealed that mean F_v/F_m was higher in compacted fills without tree wells rather than with tree wells. Past research by Percival (2004) and Maki and Colombo (2001) indicated F_v/F_m values of 0.6 below which trees were affected in terms of reduced survival, height growth and foliar necrosis. Moreover, research by Weir and Cresswell (1997) found that quadratic regression models ranging between 0.71 and 0.88 R^2 values in all three tree species, *Acer pseudoplatanus, Fagus sylvatica* and *Quercus robur* indicate a foliar N content less than 1.5 is associated with reductions in F_v/F_m values of 0.8 (values associated with full photosynthetic functioning).

Gas exchange changes: Decrease of A_{net} can be attributed to the influence of soil compaction on soil aeration and reduction of air transmission in the root system. According to the results of Tu and Tan (1988) study the restriction of root growth may reduce demand for photosynthetic products from the roots. These substances build up in the leaves and feed back to the photosynthetic mechanism which then leads to a drop in A_{net}. Similarly, decrease of chlorophyll content as well as A_{net}, nitrogen play role as the major component of photosynthetic enzymes and its content can affect mesophyll conductance to CO_2 (Longstreth and Nobel, 1980). Stomatal conductance was found significantly decreased in high bulk density was adapted by *A. excelsa* as respond to compacted soil. It occurred due to limiting in transpiration and in maintaining leaf water potential. It also resulted in other physiology process involved such as decrease of capacity for electron transfer and chlorophyll content. It was also probably by the fact that prompt closing of stomata by stressed plants as consequence by early damage to photosystem II (Else *et al.*, 2009; Jackson, 2002). It was believe that to prevent serious damage in short period, adaptation on stomatal closure were usually found in most plants.

Intercellular CO_2 (C_i) decreased as consequence of biochemical processes CO_2 fixation. Low partial pressure of CO_2 inside of leaf part was supposedly to increase stomatal conductance (Tahery, 2012). As a preview, decrease in stomatal conductance lead to decrease in CO_2 uptake from air. Moreover, seedlings use efficient CO_2 uptake was most probably for energy usage against soil compaction for rooting survival. Other than that, gas exchange parameter of transpiration and vapour pressure deficit influenced by water which are lacking in high compaction. It was supported by research of Gollan *et al.* (1985) where *Nerium oleander* L., was found to have ultimate influence with soil water status rather than leaf water status. In depth, the decreasing gas exchange parameter was due to soil water status where compacted soil had low water hold capacity as compared to uncompacted soil.

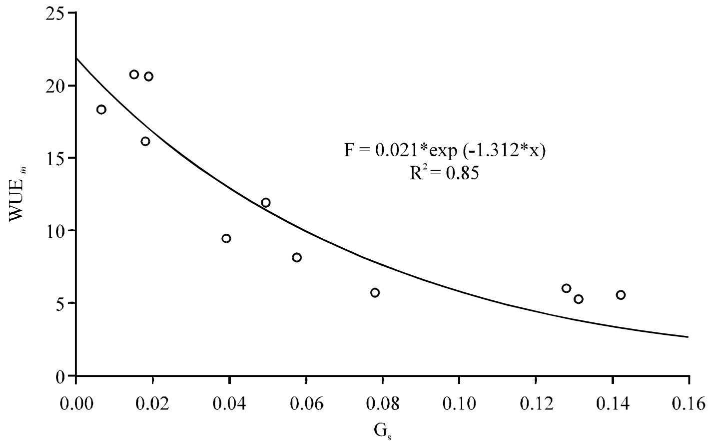

Fig. 9: Relationship between G_s and WUE_{inst} for *Azadirachta excelsa* seedlings in respond to soil compaction condition

Water relation affect: It was found that as the values of hydraulic architecture parameters decreased, the bulk density of soil compaction increased (Liu, 2011). As discussed earlier, lacking of water availability in the compacted soil leads to inadequate turgor in drive expansion of growing cells. This developed a situation as xylem channels collapse and also mesophyll and bundle sheath shrinks. Other than that, inhibition of leaf growth also may influence this result. It was stated that water uptake occur due to hormonal root signals which lead to stomatal closure causing decrease in transpiration thus, there are no hydraulic conductivity occur inside seedlings (Kozlowski, 1999). Briefly, water potential is a sensitive indicator for water stress status in a tree which is influenced by soil condition. Statistically, no difference was found in both $\Psi_{predawn}$ and Ψ_{midday}. In the month of December (middle of experiment) for $\Psi_{predawn}$, seedlings shows pattern of higher water stress in higher bulk density. It can be seen that, midday had higher values than the predawn which were caused by transpiration during the day. The same result was also found in water potential under compacted condition of *Lycopersicon esculentum* Mill. (Hussain *et al.*, 1999). It was caused by changes in the root system architecture where root growth constrained also lead to water deficit in leaves (Grzesiak *et al.*, 2013). In fact, this was supported by study of *Acacia confuse* and *Litsea glutinosa* which only experience slight changes in leaf water potential mean although decreased stomatal conductance (Liang *et al.*, 1996). Possible causes of the reduction in the leaf growth (number and area) is the limited supply of water with nutrients. Under low leaf water potential, stomata close in response to the decreased in leaf cell turgor or to the increased in ABA concentration in the transpiration stream (Pospisilova, 2003; Yordanov *et al.*, 2003).

WUE which considered A_{net}, E and G_s is the powerful regulation to any changes in environment. Decreasing pattern in gradual implementation of abiotic stress leads almost exclusively to the decreased in stomata conductance (Cornic and Fresneau, 2002). In addition, as stated before, due to low leaf water potential seedling will regulate stomata to be closed (Pospisilova, 2003; Yordanov *et al.*, 2003). Briefly, rather than soil water is lacking in compacted soil, it also occurred of increase in mechanism of water molecule pathway from shoot to root.

There was also minimum stomatal aperture found in this experiment in order to have maximum WUE_{in} (Fig. 9). Instructively, stomata adjusted evaporation by having optimal conductance is mechanism which has been done by seedlings in order to keep efficient assimilation to happen due to limited water uptake cause by compacted soil.

CONCLUSION

Growth of *A. excelsa* may be acknowledged as partially sensitive to different degrees of bulk densities. In correspond to the hypothesis, overall performance of this seedling towards compacted soil was getting lower in higher bulk density. The seedlings were seen struggling to survive by still sprouting new shoots even after showing dying characteristics. Significant decrease in diameter, height and leaf elongation also occurred in this experiment resulting from root damage thus initiated reducing physiology performance in leaf traits. It was noticed that, compacted soil effect greatly towards the plants' underground part, by keep allocated carbon invests near root parts rather than aboveground parts. Moreover, mechanical resistance is the main factor for root growth changes in leaf water potential. Meanwhile, the result of decreasing photosynthesis efficiency and strong relationship were found in WUE_{in} and Gs as adaptation to compaction in *A. excelsa* by maximizing water use at low stomatal conductance planting or allowing this seedling to grow in locations where such stresses have the probability to take place is plausible as the trees could still have chance to survive. Built on this understanding, we might gain some initial information to manage urban tree growth confronted with environmental stresses and to ensure the trees live prosper to benefit human in the long run.

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