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WATER STORAGE CAPACITY OF MULBERRY LEAVES AS AFFECTED BY FOLIAR SHAPE AND NITROGEN DEPOSITION

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Key words: Mulberry, Rainfall interception, Leaf water storage, Nitrogen

Abstract

An experiment was conducted to study the processes of water absorption and adsorption of mulberry (*Morus rubra* L.) leaves with different shapes under varied nitrogen (N) deposition by indoor simulation. Maximum water storage capacity of mulberry leaves ranged from 7.5 to 10.6 mg/cm^2, and the variations were mostly attributed to leaf shapes instead of wet N deposition. It indicated that even doubled N concentration in current rainfall (from 4 to 8 mg/kg) would have minor bearing on the maximum water storage capacity of mulberry leaf. On the other hand, mulberry leaves could uptake considerable water content when submersed in simulated rainfall within two hours, and the absorbed water was about 40% of the total leaf water content. Dynamics of leaf water content could be modelled well by exponential equation and leaf water retention was enhanced by N addition in simulated rainfall. It was concluded that mulberry with high leaf area index and fast growth would be preferred in afforestation due to its economic value and potential ecohydrological functions.

Introduction

Rainfall interception is the process where gross rainfall falling onto vegetative surfaces is subsequently redistributed. This gross rainfall on the canopy is finally separated into four parts including interception, evaporation, throughfall and stemfall (Crockford and Richardson 2000). Therefore, interception of rainfall by vegetation is an important hydrological and ecological process that affects the rate, total depth and spatial distribution of water available for other processes like infiltration, runoff, evaporation, transpiration and even climate moderation (Gomez *et al.* 2001). Some water is retained on the aboveground parts of the vegetation and its evaporation enhances water loss, resulting in less water available to the plant community. Generally, precipitation interception by forests and single tree canopy are greater than that of shrubs and other herbaceous plants (Pitman 1989).

Many factors may affect the amount of rainfall interception. These include leaf size, shape and orientation (Armstrong and Mitchell 1987), leaf hydrophobicity (Holder 2013, Wang *et al.* 2014), leaf area index, plant height and density, rainfall intensity, drop size, wind speed, air temperature, relative humidity and so on (Crockford and Richardson 2000). Furthermore, canopy storage capacity, the amount of water held on the canopy at the end of a rainfall event, plays a key role in the control of rainfall interception and other ecohydrological processes with plants (Garcia-Estringana *et al.* 2010). Although it is a function of rainfall intensity, leaf area, leaf configuration, liquid precipitation viscosity and mechanical activity (e.g., wind), the maximum water storage capacity of different plants varied greatly (Holder 2013, Keim *et al.* 2006). In addition, plant leaf

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can absorb water during rainfall interception and evaporation processes as well as nitrogen (N) in wet deposition (Adriaenssens *et al.* 2011). And these processes are all affected by leaf water storage capacity, the basis of canopy water storage (Boyce *et al.* 1991).

Mulberry (*Morus rubra* L.) is a perennial and economically important plant in the sericulture industry and has traditionally been used to feed the silkworm for thousand years in China, India, Japan and other sericulturally important countries (Vijayan and Chatterjee 2003). Presently, cultivation of mulberry has been expanded to many warm and moist climatic zones between 50ºN latitude and 10ºS latitude. This expansion of mulberry indicates increasing economic and ecological functions in sand prevention and control, stony desertification treatment, soil and water conservation, saline and alkaline land treatment, and returning farmland to forests (Qin *et al.* 2010). In addition, atmospheric N deposition has sharply increased mainly due to human activities such as consumption of fossil fuels, emission of industrial waste gas and excessive application of fertilizers during the last few decades (Vitousek *et al.* 1997). Forest ecosystems are sinks for inorganic N deposition (Pregitzer *et al.* 2008); however, it is hardly known whether increasing N deposition in rainfall affects leaf water storage capacity in either natural forest or artificial woodland such as mulberry wood (Holder 2013, Keim *et al.* 2006). China has the largest mulberry woodlands (Qin *et al.* 2010) and faces an increasing N deposition (Liu *et al.* 2013). The present investigation aimed at studying the processes of water absorption and adsorption of mulberry leaves with different shapes under varied nitrogen deposition in indoor simulation experiments.

Materials and Methods

The experiments were conducted at National Breeding Station of Mulberry of Southwest University (29º48′41″N, 106º24′35″E, 246 m) located at the Sichuan basin in south-western China. Based on 50-year (1975-2015) meteorological records of the weather station, the study site receives on an average 1105 mm rainfall, of which 78% occur between April and September, and the average annual temperature is 18.3°C. In study area, N deposition occurred mainly as wet N deposition (Lue and Tian 2007). According to previous study, N in wet deposition of this region varied with season with annual average of 4 mg/l including NH_4^+-N, NO_3^--N and dissolved organic N (Yuan *et al.* 2009).

Three mulberry leaf shapes were selected in this study (Fig. 1), because they were the most prevalent shapes. Leaf sampling was conducted in July and August, 2014. For each time, about 200 fully-developed and undamaged leaves for each leaf shape were collected in above mentioned experimental station. These samples were transported to the laboratory in a cool box as soon as possible.

Leaf water holding capacity was determined as the difference in weight between leaves before and after artificial wetting, expressed on a hemi-surface area basis (Wohlfahrt *et al.* 2006). Two factors were considered in this study: one was N concentration in simulated rainfall (0, 4 and 8 mg/l prepared by NH_4NO_3 in distilled water, recorded as N0, N4 and N8) and the other was measurement method. Therefore, six treatments were included (3 N rates × 2 methods) for each leaf shape. When leaves were shifted to laboratory, these collected leaves were randomly separated into 6 groups containing 15 leaves in each group. Their fresh ‘dry’ weights were recorded using a balance (JA5002, Jingtian Electronic Instrument, China). Then, mulberry leaves were moistened by submersing them in N solution for 10 seconds (submersing method) and by spraying N solution on them until saturation was reached (spraying method). Until all surplus water to drip-off (usually less than 10 seconds), leaves were re-weighed. The whole process was finished within 1 min to minimize water loss by transpiration and evaporation. After re-weighing, leaves were scanned by scanner (Epson, Japan) and hemi-surface leaf areas were determined by software (RHIZO 4b, Australia). Then, the leaves were dried at 70°C in an oven for 72 hrs and finally weighed as the dry weight (DW). Specific leaf areas (SLA, ratio of hemi-surface area to dry weight) were then calculated.

Measurement and modeling of leaf water absorption in simulated rainfall with different N rates (0, 4 and 8) was conducted by following the previous procedure (Liang *et al.* 2009). The randomly grouped leaves were weighed immediately and recorded as the initial weight. They were then soaked in distilled water or N solution. During absorption, the leaves were weighed after time intervals of 10, 30, 50, 70, 90, 120, 150, 180, 210, 240, 270 and 300 min (ensuring that there were no more changes in the leaf weight). Each successive weight was recorded as the current weight, and the final weight as the saturated weight. During each measurement, the leaves were first wiped using tissue paper to remove adhered water and then weighed with an electronic balance under the indoor temperatures around 25°C and about 60 ± 5% relative humidity. At last measurement, the leaves were picked up and weighted after allowing all surplus water to drip-off (the difference between initial and the values defined as total amount of leaf water holding), and then were wiped and weighed (defined as saturated leaf water content). Afterwards, leaves were scanned to record leaf areas and then dried to record dry weight for calculating specific leaf areas. Dynamics of leaf water content and increment of leaf water absorption (difference between the saturated leaf water content and the initial leaf water content) were modelled by exponential equation via SigmaPlot (Systat software, USA).

One-factor ANOVA procedure in SAS software (SAS 8.0, USA) was used for statistical analysis. Under each set by either N rate or measured method, means were separated by Fisher's protected least significance difference (LSD) test at $p < 0.05$.

Results and Discussion

Difference in leaf shape among these three kinds of mulberry was obvious (Fig. 1). On average, heart-shape leaf (HS) had leaf area of 198.5 cm^2/leaf, which was 88 and 62% higher than dragon-paw leaf (DP) and compound leaf (CL), respectively. In addition, the incised edges in leaf shapes had also great difference. Both properties indicated the genetic variations during evolution and domestication (Orhan *et al.* 2007).

Converse to leaf area, HS leaves showed the least value of maximum water storage capacity among three shapes (Fig. 2). Across the three N rates in simulated rainfall, maximum water storage capacity of mulberry leaves ranged from 7.5 to 10.6 mg/cm^2. Such values were in the scope of previous studies from grassland with abundant rainfall (Michel *et al.* 2013, Wohlfahrt *et al.* 2006); they were generally higher than that of xerophytic shrubs in north-western China (Wang *et al.* 2012). These variations were mostly attributed to morphological structures related with leaf roughness, surface free energy and especially hydrophobicity (Holder 2013, Wang *et al.* 2014). By minimizing the maximum water storage capacity of leaf, plant may benefit from it via decreased interception of water by canopy ensuring more water reaching the soil as well as improved plant water balance in arid or semi-arid environments (Holder 2013). In the present study, mulberries with big and rough leaves (Fig. 1) might be an adaptation to such ecological zones with abundant rainfall in southern China.

Regarding N rate in simulated rainfall, varied wet N deposition had little effect on maximum water storage capacity of mulberry leaves in all three shapes (Fig. 2). This indicated that even doubled N concentration (8 mg/kg) in current status (Yuan *et al.* 2009) would have minor effect on maximum water storage capacity of mulberry leaf. It indicated that change of ductility induced by N increase in rainfall is not strong enough to decrease the surface free energy and thereafter increased leaf water drop adhesion (Holder 2013, Wang *et al.* 2014). In addition, wetting method resulted in difference in maximum water storage capacity of mulberry leaves, but these extents were varied with leaf shapes (Fig. 2). Overall, spraying method got higher value than that with submersing, which was consistent with previous study (Wohlfahrt *et al.* 2006). At leaf scale, HS leaves had larger maximum water storage in both wetting methods (Fig. 3). Considering the economic benefit, eco-hydrological functions, high leaf area index and growth rate, mulberry with HS leaves would be preferred in afforestation (Qin *et al.* 2010).

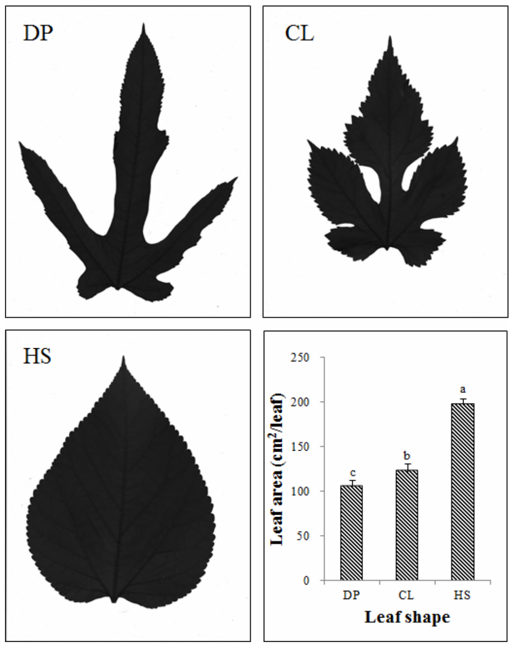

Fig. 1. Leaf shapes of mulberry used in this study and their leaf areas. DP, dragon-paw leaf; CL, compound leaf; HS, heart-shape leaf. Columns with different letters indicate significant difference in leaf area. The bar on each column indicates standard error as same as below.

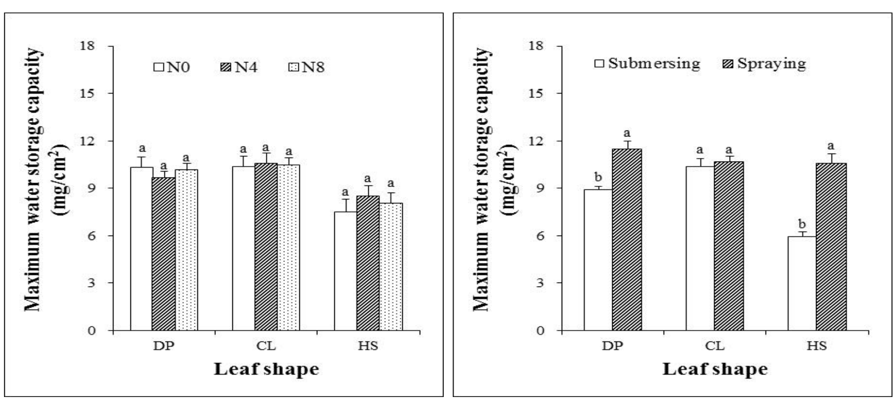

Fig. 2. Effect of N rates in simulated rainfall and study methods on maximum water storage capacity of mulberry leaves with different shapes. In each set, columns with different letters indicate significant difference.

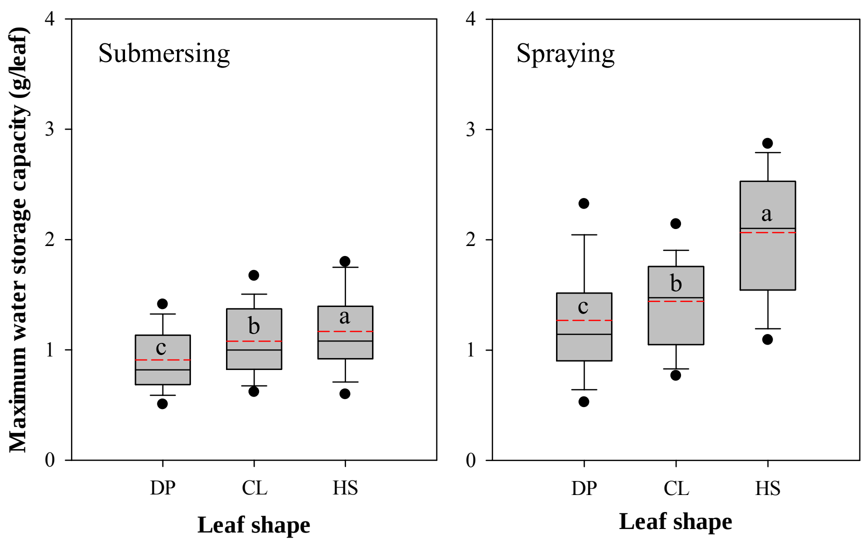

Fig. 3. The effect of leaf shapes on maximum water storage capacity of mulberry at leaf scale. Range of solid and red dashed lines in this figure indicate median and mean. Box boundaries indicate the 75 and 25% quartiles, the whisker caps indicate 90th and 10th percentiles, and the circles indicate the 95th and 5th percentiles. Columns with different letters indicate significant difference by group t test at $p < 0.05$.

Leaf water absorbing capacity was important part of leaf water storage in water environment such as rainfall and dew (Leuschner 2002). As shown in Fig. 4, mulberry leaves with all shapes could uptake water when leaves were submersed in N solution. The dynamics showed that leaf water content increased fast at the beginning and then got slow down when close to saturated leaf water content. Regression showed that the following exponential equation with 3 parameters can be well matched with experimental data ($p < 0.01$):

$$C = C_0 + \Delta C_{max} (1 - \exp^{-kt}) \quad (1)$$

where C is leaf water content, C_0 is the initial leaf water content, ΔC_{max} is maximum difference between the saturated leaf water content and the initial leaf water content, which represents the leaf water absorbing capacity (Liang *et al.* 2009). From Eq. (1), incremental amount of leaf water absorption (ΔC, Fig. 5) can be alternatively shown as follow:

$$\Delta C = \Delta C_{max} (1 - \exp^{-kt}) \quad (2)$$

The parameters of these equations showed that the initial leaf water contents among leaf shapes were different with highest in HS leaves and lowest in DP leaves (Table 1). Furthermore, these leaf water absorption functions varied among the three leaf shapes. Leaves with CL shape had largest ΔC_{max} than that of other leaf shapes, indicating that mulberry leaves with CL shape had stronger leaf water absorbing capacity. In the present study, the exponential factor *k* ranged from 0.019 to 0.028, which was far smaller than the previous study (Liang *et al.* 2009). This indicated that mulberry leaf absorbed water much slower than turf grasses. This may reflect the difference in stomata regulation, cuticle resistance, the amount of wax, leaf curl, and other morphological characteristics (Ma *et al.* 1998).

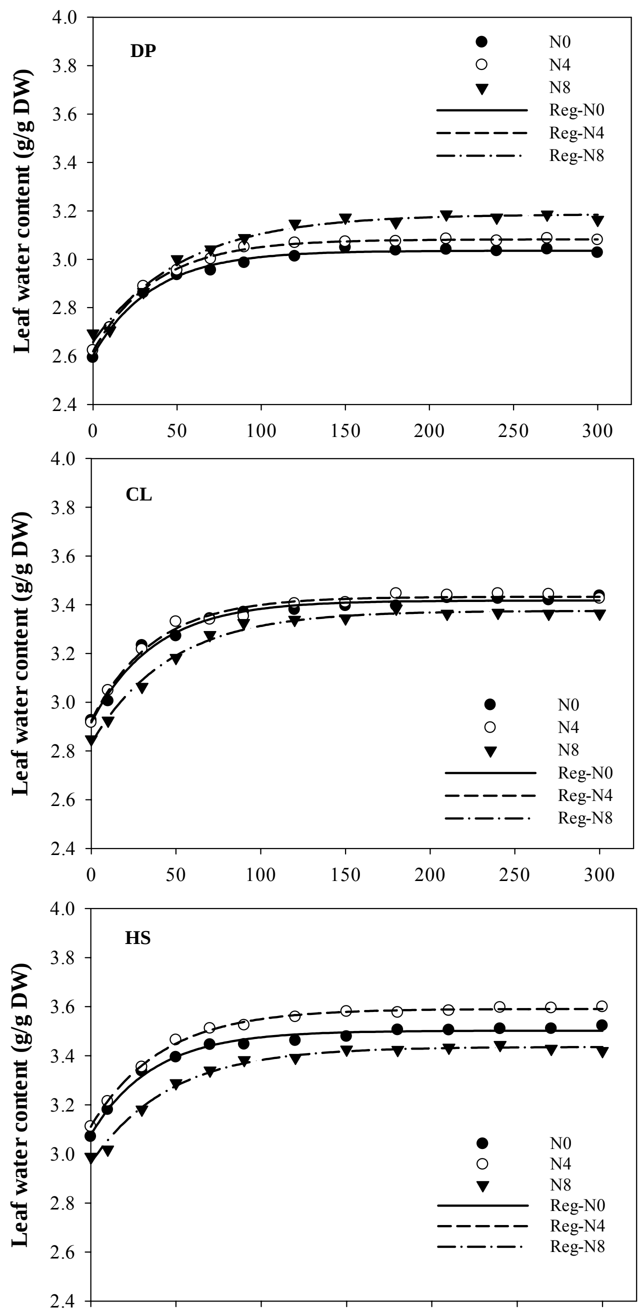

Fig. 4. Dynamics of leaf water content of mulberry leaves with different shapes as affected by N rates in simulated rainfall.

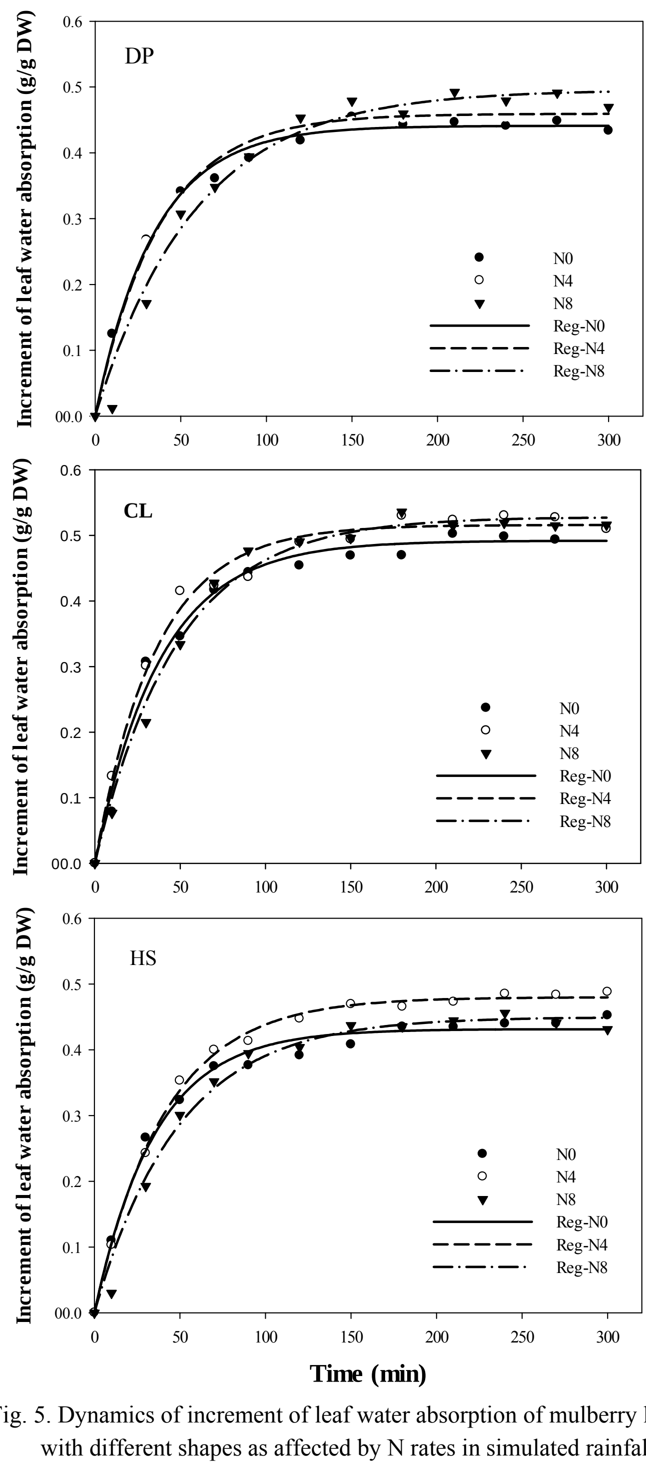

Fig. 5. Dynamics of increment of leaf water absorption of mulberry leaves with different shapes as affected by N rates in simulated rainfall.

Results also showed that ΔC_{max} was increased by N addition (N4 and N8) in simulated rainfall than that without N addition (N0), indicating that N deposition could enhance leaf water absorbing capacity. While, on the other side, the exponential factor *k* had a declining trend with increasing N rates in simulated rainfall (Table 1). The underlying reason is still unknown, but may be connective with change of osmotic pressure induced by leaf N absorption (Milla *et al.* 2007).

Table 1. The parameters of exponential equation for dynamics of leaf water content of mulberry as affected by leaf shapes and N rate in simulated rainfall.

Parameter	Leaf shape and N rate								
	DP			CL			HS		
	N0	N4	N8	N0	N4	N8	N0	N4	N8
C_0	2.6	2.6	2.7	2.9	2.9	2.8	3.1	3.1	3.0
ΔC_{max}	0.43	0.46	0.53	0.50	0.51	0.54	0.43	0.48	0.47
k	0.028	0.027	0.019	0.027	0.027	0.022	0.028	0.024	0.022

DP, dragon-paw leaf; CL, compound leaf and HS, heart-shape leaf.

In addition, the impact of specific leaf area (SLA) on leaf water absorption was also studied. Larger specific leaf area indicates that the leaves of the same dry weight have larger areas. SLA is one of the most important traits in determining plant ecological functions, and it is influenced by many structural and anatomical traits, including leaf dry matter concentration, leaf thickness, leaf water content, the proportion of vascular and sclerenchyma tissues, and the proportion of cell wall components (Sugiyama 2005). Larger SLA indicates a thicker leaf that may have a greater photosynthetic capacity than thinner leaves. Saturated leaf water content and SLA has a positive

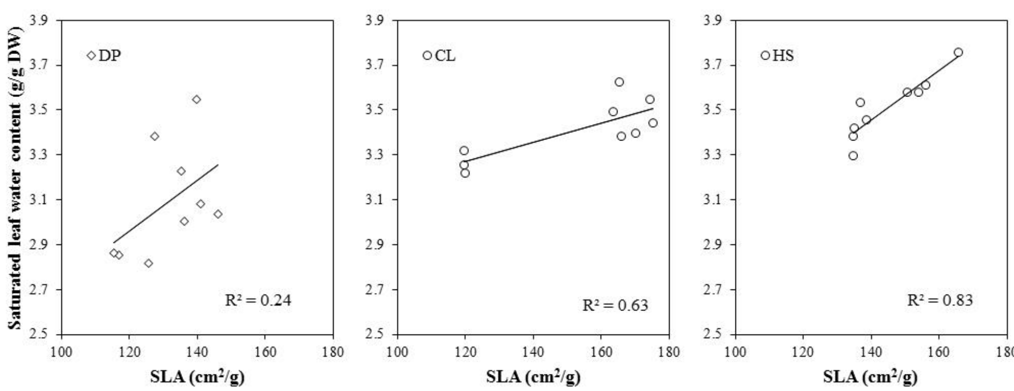

Fig. 6. Correlation between specific leaf area (SLA) and saturated leaf water content of mulberry leaves with different shapes.

and significant linear relationship for CL and HS leaves, but not for DP leaves (Fig. 6). Similar study was also reported in other plants (Liang *et al.* 2009). Even so, the coefficients of linear regression indicated that thicker leaves with HS shape would benefit in increasing saturated leaf water content most among these three shapes.

Total leaf water absorption can make up 37-46% of total amount of leaf water holding after emerging a simulated water environment (Fig. 7). While N rates in solution had minor effect on this proportion. This indicated the importance of leaf water absorption on leaf water holding especially under rain with long time or dew event (Liang *et al.* 2009, Wohlfahrt *et al.* 2006).

Up to canopy and watershed scale, artificial mulberry woods are expected to intercept considerable amount of water from rainfall or dew, presenting important ecohydrological functions including watershed hydrology, storm water management and runoff control in humid subtropical areas (Crockford and Richardson 2000). Rainfall interception by mulberry canopy can delay the buildup of soil moisture, and facilitate vertical water movement that may increase infiltration and drainage (Martello *et al.* 2015). All these processes are essential for watershed runoff control and storm water management. Thus, planting mulberry woods deserve preferential consideration by policy makers and managers except high economic value of mulberry woods.

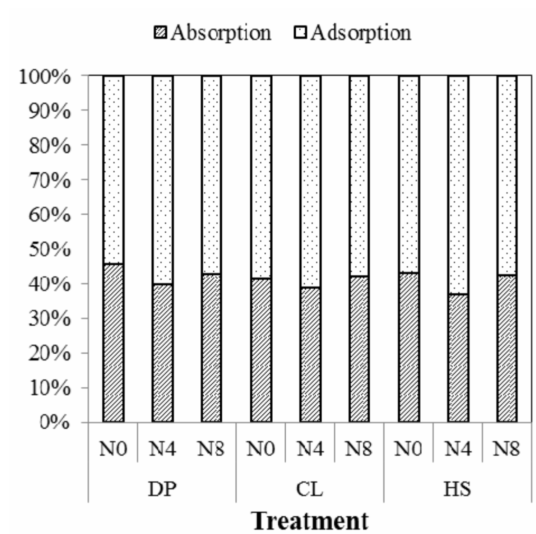

Fig. 7. Proportion of leaf water absorption and adsorption (leaf water storage) on total amount of leaf water holding of mulberry leaves with different shapes as affected by N rates in simulated rainfall.

In conclusion, mulberry leaves had strong water storage capacity which was affected mostly by leaf shapes. Wet N deposition even under high N concentration in rainfall is less effective. While, mulberry leaves can also uptake considerable water within 2 hrs when submersed in simulated rainfall. This process can be simulated by exponential model and was indeed enhanced by N addition in simulated rainfall. Therefore, mulberry artificial woodlands are expected to play a significant ecohydrological function except for the well-known economic value. However, the amount of rainfall interception by mulberry trees is still not quantified and modelled at canopy and watershed scale which deserves in future study.

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