Physiological Characteristics of Nitrogen-fixing Tree Species in Mt. Makiling and La Mesa Watershed, Philippines

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INTRODUCTION

Restoration and rehabilitation of degraded and secondary forest has become an important issue

Philippines: one of the countries in Asia with the highest rates of forest loss (1.5% per year)

2010: the remaining total forest cover of the country was around 7 million ha (26%)

Identify factors that influence plant's ability to survive in various conditions

INTRODUCTION

Nitrogen-fixing trees: well recognized for their contribution in restoring degraded areas and in improving the productivity of the land

INTRODUCTION

Only few or limited researches have been conducted with regard to the physiological characteristics of the stands

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Early Growth and Physiological Characteristics of Planted Seedlings in La Mesa Dam Watershed, Philippines

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Six-month-old native species of *Erythrina variegata* L., *Dracontomelon dao* (Blanco) Merrille et Rolfe, *Pterocarpus indicus* Willd. and *Bischofia javanica* Blume were planted to determine their growth and physiological characteristics. The study compared early growth performance among species and determined the suitable species in the area, compared growth performance of species in the flat and mountain areas, and determined the relationship between growth and physiological characteristics. A randomized block design of two blocks and three replicates per species was established at a spacing of 4 x 4 m between seedlings. Growth parameters used were height, root collar diameter, survival rate, fresh and dry weights, root-shoot ratio, root and shoot growth characteristics, leaf area and leaf anatomical characteristics. Physiological characteristics such as net photosynthesis, transpiration rate, water use efficiency, stomatal conductance, stomata size and number and chlorophyll content were determined and compared as well.

Results and analysis revealed that among the four species, *E. variegata* showed best growth performance as it grew fast and fixed nitrogen. Based on the results, *E. variegata* has a potential in the reforestation of many denuded areas in the country since it grows fast and is shade-intolerant. In terms of physiological characteristics, nitrogen-fixing trees, *E. variegata* and *P. indicus*, exhibited good performance. Generally, the growth and physiological characteristics of four species in the mountain area which has fertile and moist soil conditions were better than in the flat area with its dry soil condition.

Key words: early growth, physiological characteristics, seedlings

Abbreviations: asl – above sea level, CHLt - chlorophyll total, D – diameter, DI - diameter increment, DW - dry weight, E - transpiration rate, FW - fresh weight, g_s - stomatal conductance, H – height, HI - height increment, LA - leaf area, P_N - net photosynthesis, STN - stomata number, STS - stomata size, WUE - water use efficiency

INTRODUCTION

Among the terrestrial ecosystems, grasslands and degraded areas are the most neglected. In the Philippines, they are vast (ranging from about 1/6 to 1/3 of the country's landmass, according to Malvas 1995) as a result of illegal cutting, shifting cultivation and fire. As of 1982, uncontrolled logging and slash and burn farming have converted an estimated 5 million ha of forestlands to grasslands; about 1.4 million ha of these areas are located in critical watersheds (NEPC 1983) and are largely covered with *cogon* grass (*Imperata cylindrica*).

The traditional approach of the Philippine government in rehabilitating grassland areas is through reforestation

INTRODUCTION

Exotic/invasive species have higher growth rates than native species

INTRODUCTION

Ecophysiological traits that can increase resource capture ability and utilization efficiency

OBJECTIVES

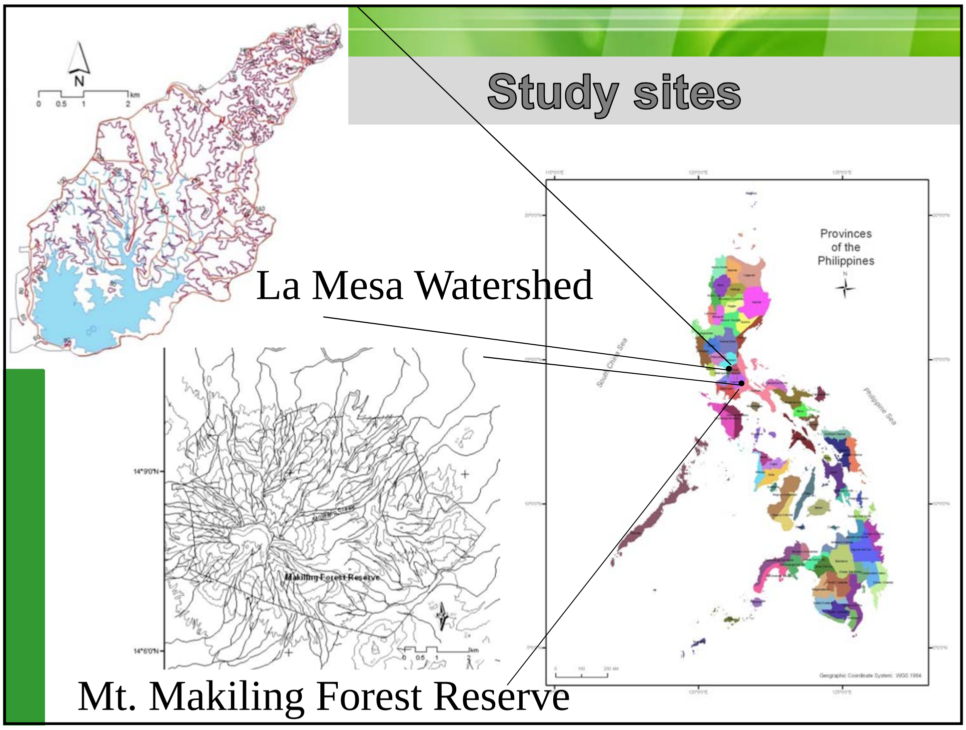
1. **To compare the aboveground biomass and productivity traits of the exotic species, *Acacia mangium* A. Cunn. ex Benth. and *Acacia auriculiformis* Willd., and native species, *Pterocarpus indicus* Willd.; and**

OBJECTIVES

2. To determine the different patterns of physiological response in the different age classes of *A. mangium*, *A. auriculiformis*, and *P. indicus* in the natural condition.

MATERIALS AND METHODS

Study sites



Study sites

	Mt. Makiling	La Mesa Watershed
Coordinates	14°08'N – 121°11' E	14°45' N – 121°05' E
Area	4,244 ha	2,700 ha
Altitude	~500 m asl	~113 m asl
Rainfall	2,397 mm	2,700 mm
Temperature	25.5 – 27.5°C	23.8 – 30°C
Relative humidity	~81.8%	69~84%

Tree species and age classes

Acacia auriculiformis	*Acacia mangium*	*Pterocarpus indicus*
• 2-yr-old	• 2-yr-old	• 2-yr-old
• 10-yr-old		• 10-yr-old
• 20-yr-old	• 20-yr-old	• 20-yr-old

Species

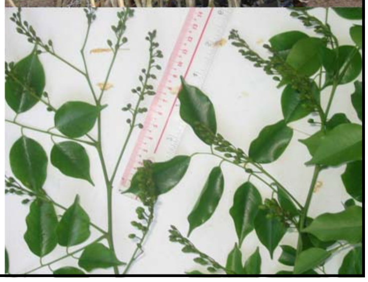
***Acacia* species**

- Have substantial nitrogen-fixation ability, have been widely used for afforestation and greening in other countries.
- *A. auriculiformis* (Auri) and *A. mangium* (Mangium) were introduced to degraded tropical and subtropical regions to establish forest communities
- Could improve soil nutrients

Species

Pterocarpus indicus

- One of the best-known trees in Southeast Asia; indigenous in the Philippines
- Known as Narra in the Philippines and favorite timber for the manufacture of fine furniture.



Field layout

La Mesa

Mt. Makiling

3 plots
(20 m x 20 m)
in each age
class

4 sub-plots
(2 m x 2 m)

2 m

2 m

I. Aboveground biomass and productivity of *Acacia auriculiformis*, *Acacia mangium* and *Pterocarpus indicus* stands

Stand characteristics

- Stand density (No. trees/ha)

- Basal area ($0.7854*D^2$)

- Importance value index (relative density, relative frequency, relative coverage)

- ANPP (sum of litterfall and biomass increment estimated from diameter increments over 6-month periods by biomass allometrics)

Aboveground biomass and carbon estimation

- Tree biomass and CWD (≥10 cm): allometric equation by Brown (1997)

$Y = EXP(-2.134 + 2.530 \times LN(D))$

where, y: aboveground biomass (kg); D : DBH (cm)

- Understory/herbaceous vegetation and litters: formula by Lasco *et al*., 2005):

$$TODW = \frac{TFW - (TFW * (SFW - SODW)]}{SFW}$$

where, TODW: total ovendry weight; TFW: total fresh weight; SFW: sample fresh weight; SODW: sample ovendry weight

Litterfall (January 2008-January 2009)

- Sorted into fractions: leaves, non-leaves (branches/twigs), and reproductive part (flowers and fruits).

- Dried at 65°C for approximately 48 hr and ground to pass a 1-mm sieve.

- Total litter was obtained by adding their weights.

4 litter traps (0.5 m x 0.5 m) in each plot

Foliage analysis

- Nutrient content (N, P, K, Ca, Mg, Zn, Cu, Mn and Fe)
- Specific leaf weight (SLW)
- Leaf area index (LAI)
- N productivity (ratio of ANPP on a year basis to the amount of foliage N)

Soil analysis

- Nutrient content (N, P, K, etc.)
- pH, CEC
- OM: Loss-on-ignition method

$$OM\ (\%) = \frac{Sample\ weight\ after\ ignition\ (g)}{Sample\ weight\ before\ ignition\ (g)}\ x\ 100$$

where, OM: organic matter

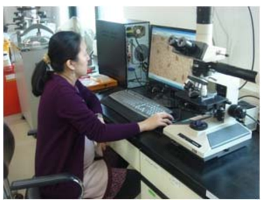
4 samples in each plot using soil auger at 0-30 cm depth

Weather data

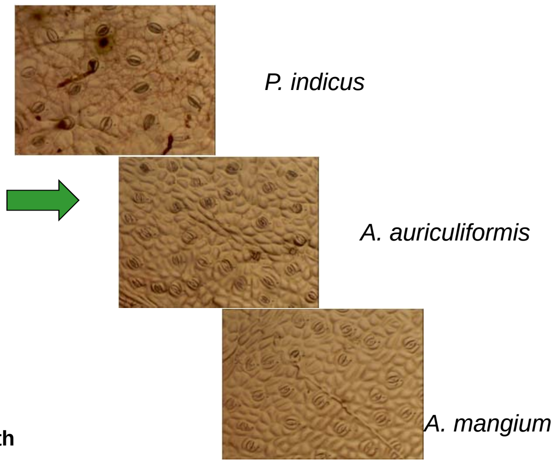
- Air temperature, relative humidity, and soil temperature were measured by HOBO from July 2007 up to February 2010 and were downloaded in the field every six months.

II. Physiological characteristics of *Acacia auriculiformis*, *Acacia mangium*, and *Pterocarpus indicus* stands

Stomata size and number



Light microscope equipped with Micropublisher 5.0 RTV (Q imaging) – 200 x magnification



Leaf anatomical characteristics

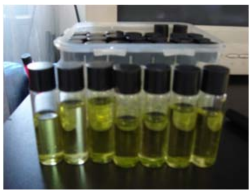
- Microtome sectioning (Histological paraffin technique)
- Total leaf thickness, epidermal leaf thickness, and palisade mesophyll thickness

Total leaf nitrogen and carbon concentrations

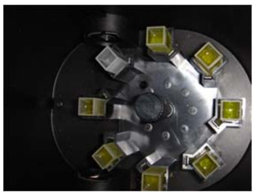
- Oven-dried samples at 60°C for 48 hr and finely ground.
- Leaf N and C

Chlorophyll content

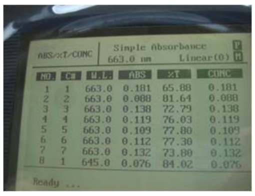
10 mL 80% acetone



Kept cool in the dark at 4°C



UV/VIS Spectrophotometer (Optizen 2120v model)



Arnon's equation (1949)

- Chlorophyll content (SPAD)

Net photosynthesis, transpiration and stomatal conductance

- Li-Cor 6400 Portable Photosynthesis System with the standard leaf chamber & CO_2 injection system (400 μmol CO_2 mol air^{-1}).

- RH near ambient levels (60-70%); average cuvette temperature -> 25^{o}C.

- PPFD used were 0, 20, 50, 80, 100, 300, 500, 1000, 1500 and 2000 μmol m^{-2} s^{-1}.

$$P_N = \frac{U_e(C_e - C_c)}{100s} - C_c E$$

- where, P_N: net photosynthesis; U_e: mole flow rate of air entering the leaf chamber; C_e: Mole fraction of CO_2 in the leaf chamber; C_c: mole fraction of CO_2 entering the leaf chamber; s: leaf area; E: transpiration

Photosynthetic nitrogen use efficiency (PNUE)

- Calculated as the ratio of light-saturated photosynthetic rate (μmol $m^{-2}s^{-1}$) and total leaf nitrogen content (g m^{-2}).

Water use efficiency (WUE)

- Calculated as net photosynthesis (P_N)/ transpiration rate (E) (Wang, 2001 and Ashraf *et al.*, 2002).

- Each measurement of P_N and E was taken at 1,200 μmol $m^{-2}s^{-1}$ light density.

A/C_i curve and derived parameters (V_{cmax} and J_{max})

- ***$A=kC_i + i$*** within 50-200 μmol^{-1} C_i
where, *k:* initial slope of the A/C_i curve described as *CE* (carboxylation efficiency); *–i/k:* Γ* in the absence of mitochondrial respiration (Laisk, 1977)

- ***V_{cmax}*, *R_d* and *J_{max}*** (Farquhar and Sharkey, 1982; Feng *et al.*, 2007): where, K_c and K_o: 404.9 μmol mol^{-1} and 278.4 mmol mol^{-1}; O: 210 mmol mol^{-1}

$$V_{cmax} = \{k \ x \ K_c \ x \ (1 + O/K_o)]^2/[\Gamma^* + \ K_c \ x \ (1 + O/K_o)]\}$$

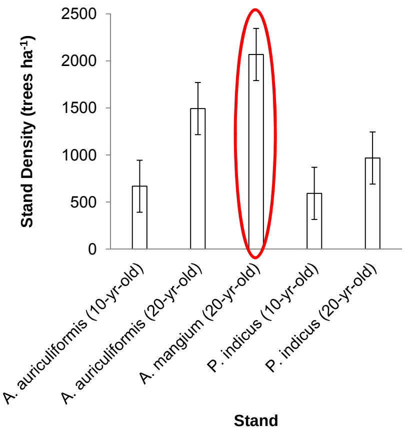
$$R_d = \{V_{cmax} \ x \ (C_i - \Gamma^*)/[C_i + \ K_c \ x \ (1 + O/K_c)] - (k \ x \ C_i + \ i)\}$$

$$J_{max} = \{[4 \ x \ (P'_{max} + \ R_d) \ x \ (C_i + \ 2 \ x \ \Gamma^*)]/ \ (C_i - \Gamma^*)\}$$

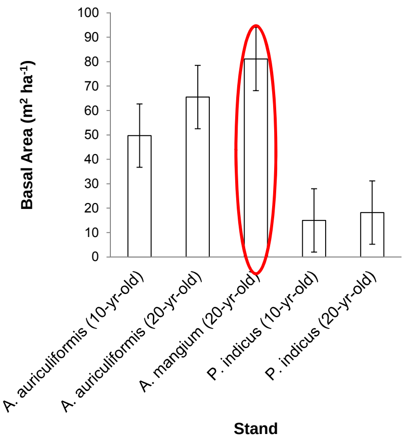
RESULTS AND DISCUSSION

I. Aboveground biomass and productivity of *Acacia auriculiformis*, *Acacia mangium* and *Pterocarpus indicus* stands

Stand characteristics



Stand density of species and age classes



Basal area of species and age classes

Aboveground biomass and carbon

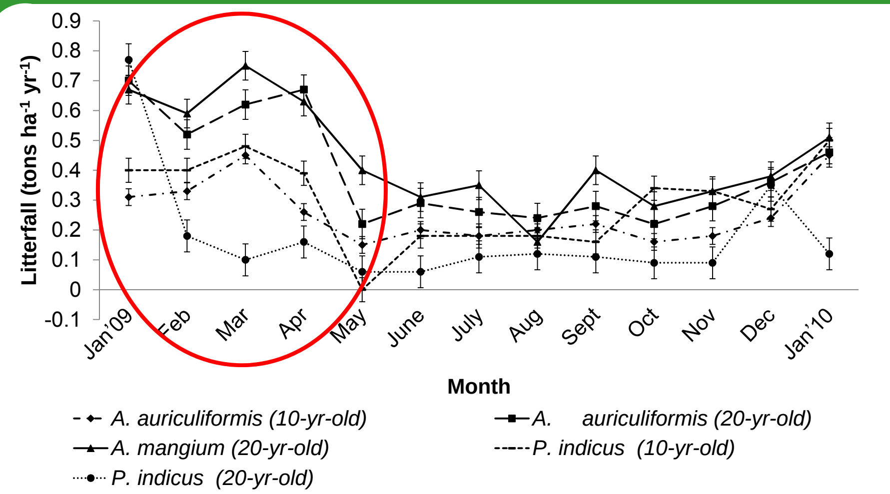
Aboveground biomass and carbon content of *Acacia auriculiformis*, *Acacia mangium* and *Pterocarpus indicus* stands

Sites	*A. auriculiformis* (10-yr-old)		*A. auriculiformis* (20-yr-old)		*A. mangium* (20-yr-old)		*P. indicus* (10-yr-old)		*P. indicus* (20-yr-old)	
	Biomass (tons ha^{-1})	Carbon content (tC ha^{-1})	Biomass (tons ha^{-1})	Carbon content (tC ha^{-1})	Biomass (tons ha^{-1})	Carbon content (tC ha^{-1})	Biomass (tons ha^{-1})	Carbon content (tC ha^{-1})	Biomass (tons ha^{-1})	Carbon content (tC ha^{-1})
Trees	64.10	29.50	149.25	68.65	198.84	91.47	88.20	40.60	122.5	56.10
Understory/ Herbaceous vegetation	1.70	0.70	0.72	0.29	1.08	0.43	2.40	1.00	2.00	0.80
Litter layer	5.00	2.10	3.87	1.63	3.90	1.64	1.20	0.50	1.70	0.60
CWD	0.04	0.02	0.07	0.03	0.39	0.15	0.05	0.02	0.06	0.02
Total	**70.84**	**32.34**	**149.25**	**70.60**	**204.21**	**93.69**	**91.80**	**42.10**	**126.30**	**57.50**

Litterfall

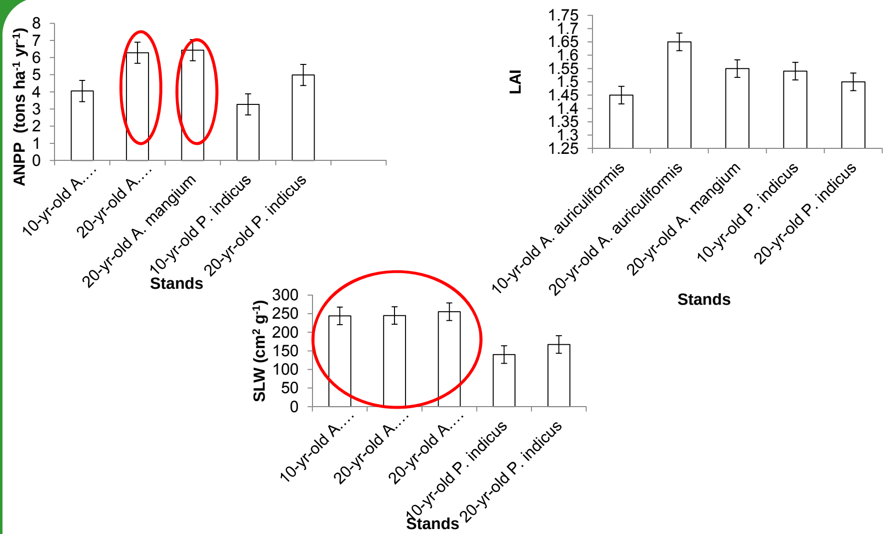
Litter production (tons ha^{-1} yr^{-1}) in *Acacia auriculiformis, Acacia mangium* and *Pterocarpus indicus* stands

	Foliar litter	Branches/ Twigs	Reproductive parts	Total litterfall
A. auriculiformis (10-yr-old)	2.55±0.2	0.43±0.1	0.34±0.1	3.31±0.3
A. auriculiformis (20-yr-old)	2.68±0.5	1.93±0.2	0.86±0.1	5.47±0.6
A. mangium (20-yr-old)	4.10±0.4	1.19±0.1	0.44±0.1	5.72±0.6
P. indicus (10-yr-old)	1.40±0.5	0.24±0.1	0.66±0.2	2.30±0.7
P. indicus (20-yr-old)	2.45±0.2	0.83±0.1	0.81±0.2	4.08±0.1



Monthly litterfall of the different stands from January 2009 to January 2010

Productivity, resource use and foliar nutrients



ANPP, leaf area index (LAI) , and specific leaf weight (SLW) of *A. auriculiformis, A. mangium* and *P. indicus* stands

Foliar nutrients and NP (nitrogen productivity) of *A. auriculiformis*, *A. mangium* and *P. indicus* stands

Species/Age class	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)	NP (kg kg⁻¹yr⁻¹)
Acacia auriculiformis										
10-yr-old	2.66bc	0.02c	0.24c	2.20bc	0.36c	32c	0.8c	124c	3409b	152.26b
20-yr-old	2.35c	0.06b	0.25c	2.44a	0.32c	44bc	1.1ab	208bc	332d	267.23a
Acacia mangium										
20-yr-old	2.90b	0.05bc	0.40a	1.85c	0.41bc	39c	1.3a	272a	1220c	221.72ab
Pterocarpus indicus										
10-yr-old	3.77a	0.08b	0.32b	2.41ab	0.59b	61b	0.7c	276ab	7313a	87.53c
20-yr-old	3.47ab	0.16a	0.35b	2.39b	0.65a	86a	0.9bc	233b	2985bc	143.80bc

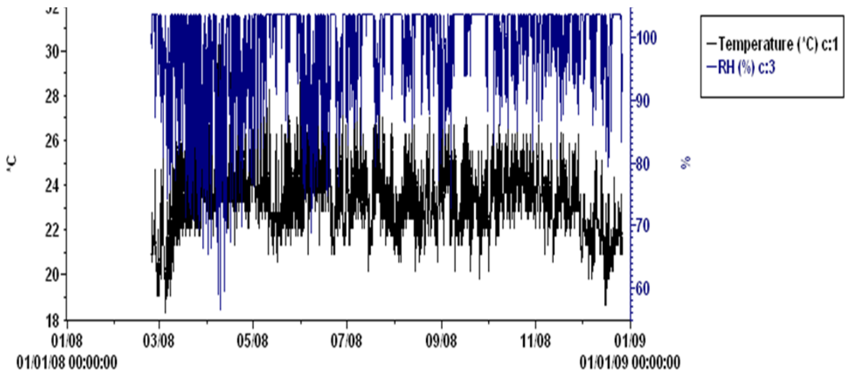
**Means within a column followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test (DMRT).*

Soil analysis

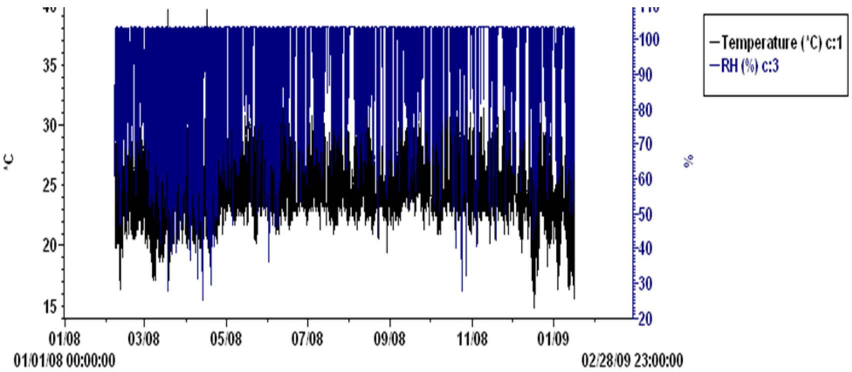
Soil characteristics of *A. auriculiformis* and *A. mangium* stands and *P. indicus* stands in Mt. Makiling Forest Reserve and La Mesa Watershed, Philippines

Age class	pH	OM (%)	N (%)	P (ppm)	K (cmol(+)/kg)	Ca (cmol (+)/kg)	Mg (cmol (+)/kg)	CEC (cmol (+)/kg)
Acacia auriculiformis								
10-yr-old	4.6	6.23	0.35	0.9	1.91	10.8	7.1	38.9
20-yr-old	4.9	6.25	0.35	0.6	2.53	10.7	5.2	38.3
Acacia mangium								
20-yr-old	4.6	5.79	0.33	1.3	2.03	8.9	4.7	32.3
Pterocarpus indicus								
10-yr-old	4.5	3.12	0.21	2.0	0.42	6.4	2.2	21.1
20-yr-old	4.7	3.55	0.25	3.1	0.35	7.9	2.8	13.6

Air temperature and relative humidity

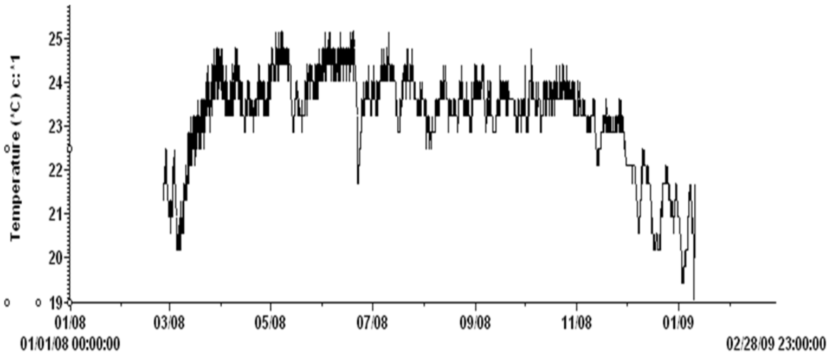


Air temperature and relative humidity in Mt. Makiling Forest Reserve from January 2008 to January 2009

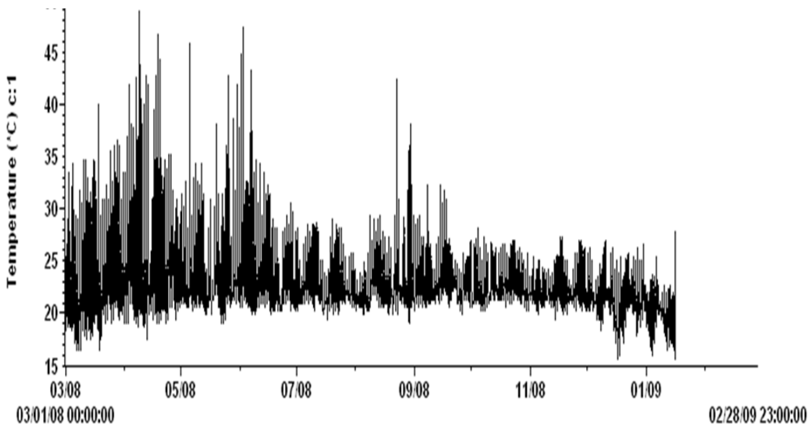


Air temperature and relative humidity in La Mesa Watershed from January 2008 to January 2009

Soil temperature



Soil temperature in Mt. Makiling Forest Reserve from March 2008 to January 2009



Soil temperature in La Mesa Watershed from March 2008 to January 2009

II. Physiological characteristics of *Acacia auriculiformis*, *Acacia mangium*, and *Pterocarpus indicus* stands

Stomata size and number

Stomata size and number of *Acacia auriculiformis* stands in the lower, middle and upper canopy

Age	Lower		Middle		Upper		Average	
	Size (μm^2)	No.	Size (μm^2)	No.	Size (μm^2)	No.	Size (μm^2)	No.
2-yr-old	268.8a	34ab	237.3a	36ab	228.8a	31ab	245.0a	34ab
10- yr-old	189.4b	37a	207.4b	36ab	228.8a	34ab	208.5b	36ab
20- yr-old	183.1b	38a	185.0c	41a	197.3b	37a	188.5c	39a

Means within a column followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test (DMRT).

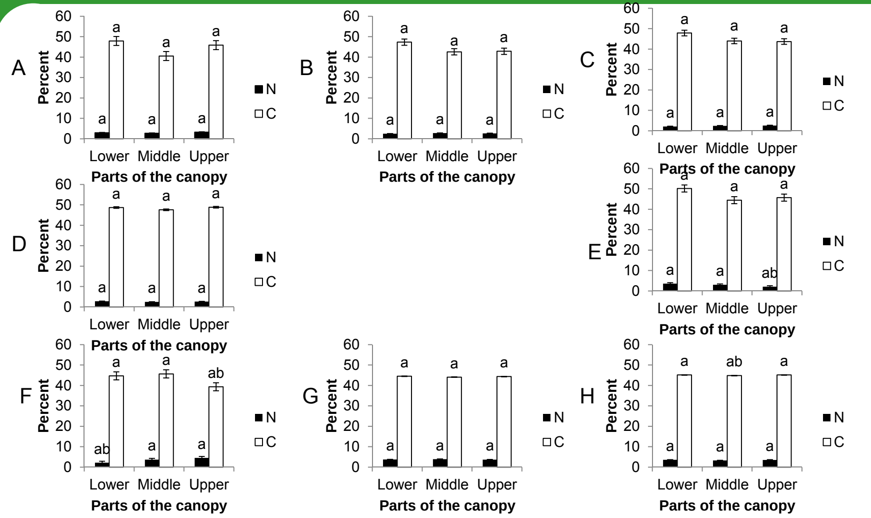
Leaf anatomical characteristics

Leaf anatomical features (µm) of *Acacia auriculiformis* stands in the lower, middle and upper canopy

Age	Lower			Middle			Upper			ALT
	PMT	ELT	TLT	PMT	EPT	TLT	PMT	EPT	TLT	
2-yr-old	60ab	23ab	161b	61ab	25a	163c	62ab	27ab	170b	165b
10- yr-old	64a	25a	165ab	66a	25a	170b	68a	27ab	182a	172ab
20-yr-old	65a	26a	169a	67a	26a	181a	69a	29a	183a	178a

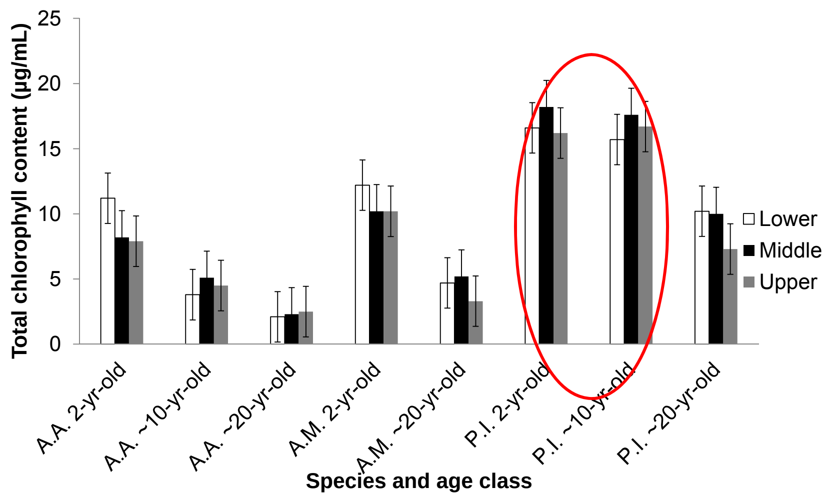
**Means within a column followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test (DMRT).*
Note: ALT - Average leaf thickness; ELT – Epidermal leaf thickness; PMT – Palisade mesophyll thickness; TLT – Total leaf thickness

Total leaf nitrogen and carbon



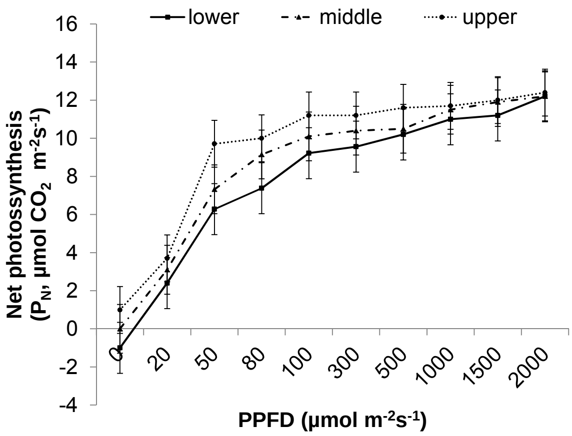
Total N and C of the 2-, 10 & 20-yr-old stands of *A. auriculiformis* (A-C), 2- & 20-yr-old stands of *A. mangium* (D-E); and 2-,10 & 20-yr-old stands of *Pterocarpus indicus* (F-H) in the lower, middle and upper portions of the canopy.

Chlorophyll content



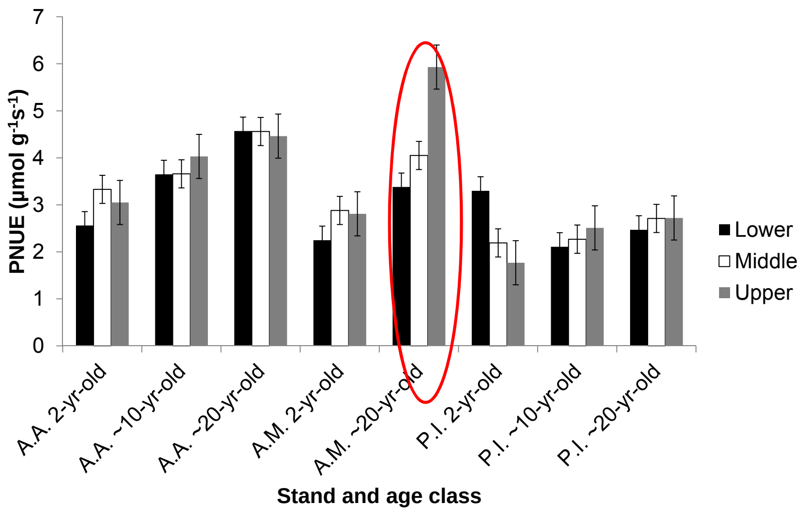
Chlorophyll content of *Acacia auriculiformis (AA)*, *Acacia mangium (AM)* and *Pterocarpus indicus* (*PI*) species in the lower, middle and upper portions of the canopy

Net photosynthesis



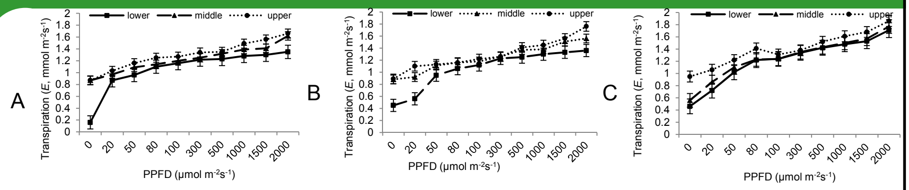
Net photosynthesis of the *Acacia mangium* 20-yr-old stand

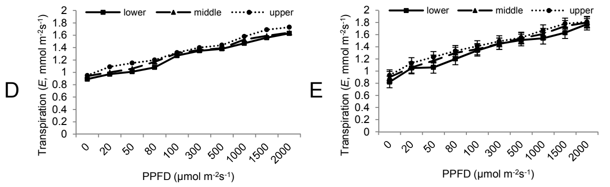
Photosynthetic nitrogen use efficiency (PNUE)



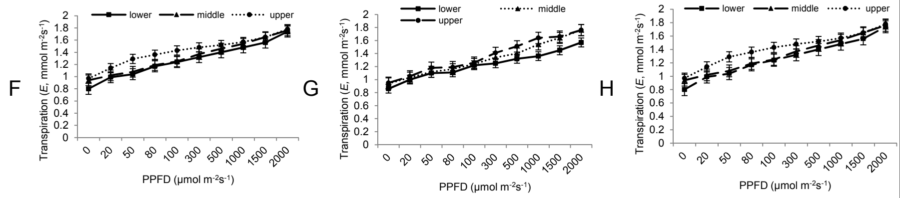
Photosynthetic nitrogen use efficiency of *Acacia auriculiformis, Acacia mangium* and *Pterocarpus indicus* in the lower, middle and upper portions of the canopy

Transpiration

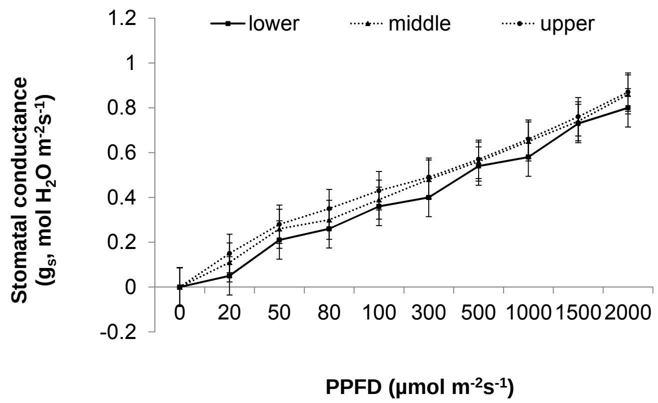




Transpiration of the 2-, 10 & 20-yr-old stands of *A. auriculiformis* (A-C), 2- & 20-yr-old stands of *A. mangium* (D&E); and 2-,10 & 20-yr-old stands of *Pterocarpus indicus* (F-H) in the lower, middle and upper portions of the canopy.

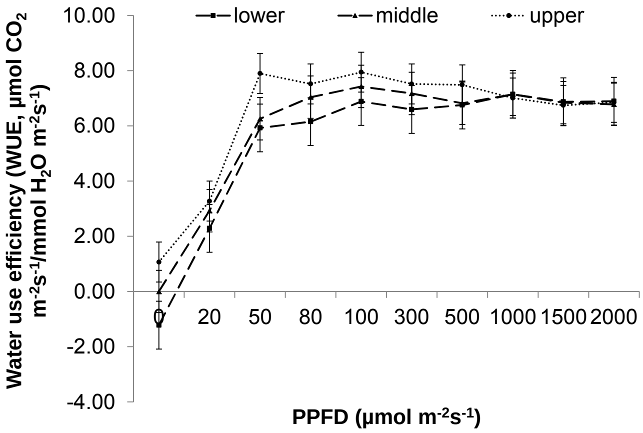


Stomatal conductance



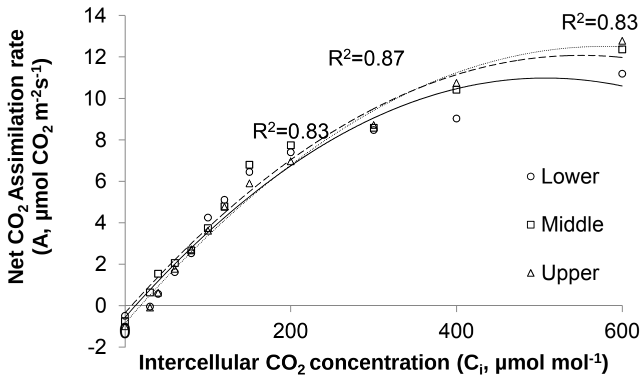
Stomatal conductance of the 20-yr-old stand of *A. mangium* in the lower, middle and upper portions of the canopy

Water use efficiency



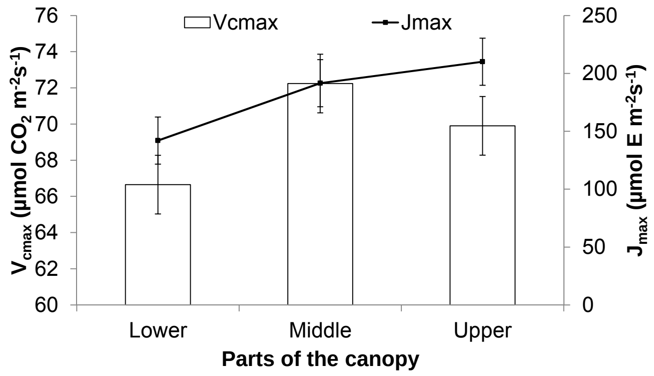
Water use efficiency of the 20-yr-old stand of *A. mangium* in the lower, middle and upper portions of the canopy

A/C_i curve



A/C_i curve of the 20-yr-old stand of *Acacia mangium* in the lower, middle and upper portions of the canopy

Derived parameters, V_{cmax} and J_{max}



V_{cmax} **and** ***J_{max}*** **of 20-yr-old stand of** ***Acacia mangium*** **in the lower, middle and upper portions of the canopy**

CONCLUSION

A. mangium and A. auriculiformis exhibited better performance in the field as shown by their productivity traits (stand density, basal area, ANPP, NP, etc.) and physiological characteristics (P_N, PNUE, WUE, stomatal conductance, V_{cmax} and J_{max})

A. mangium and A. auriculiformis proved to be suitable as reforestation species in the Philippines

Future research… by including a wide range of exotic/native species and age classes to understand the generality of the physiological patterns and to assess the ecological attributes afforded by these features.

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Thank you very much!