

Ecophysiological screening of tree species for biomass production: trade-off between production and water use

DAN WANG,^{1,2,3,†} DAVID LeBAUER,^{2,3} GARY KLING,^{3,4} THOMAS VOIGT,^{3,4} AND MICHAEL C. DIETZE⁵

¹*International Center for Ecology, Meteorology and Environment, School of Applied Meteorology,
Nanjing University of Information Science and Technology, Nanjing 210044 China*

²*Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA*

³*Energy Biosciences Institute, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA*

⁴*Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA*

⁵*Department of Earth and Environment, Boston University, Boston, Massachusetts 02215 USA*

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Abstract. Trees are an important biomass source for cellulosic ethanol production. The ability to identify tree species that are efficient in balancing water loss and carbon uptake based on physiological traits associated with growth and water use is useful for screening candidate species and genotypes. We used a common-garden approach to evaluate the relationships between traits, productivity, and WUE among 21 tree species across two growing seasons. Species differed significantly in leaf-level gas exchange, $\Delta^{13}\text{C}$, phenology and growth. *Robinia pseudoacacia*, *Populus deltoides*, *Catalpa speciosa*, *Rhus copallina*, and *Acer saccharinum* had higher total height (Ht) and basal diameter (DB) growth than the other species. $\Delta^{13}\text{C}$ scaled positively with growth rate and negatively with intrinsic WUE, suggesting that $\Delta^{13}\text{C}$ could be an effective proxy for productivity and WUE for the species evaluated. Principle component analysis indicated that among the faster-growing species (high Ht and A_{net}), *Robinia pseudoacacia* distinguished itself by a higher $\Delta^{13}\text{C}$ and lower PNUE, while *Populus deltoides*, *Rhus copallina*, *Catalpa speciosa* and *Platanus occidentalis* had a higher PNUE and lower $\Delta^{13}\text{C}$, making it possible to select trees that could optimize the trade-off between carbon gain and water use and provide guidelines for policy making. Systematic measurements of gas exchange across the growing season are essential for validation of growth models and to elucidate the physiological basis for observed differences in productivity and WUE. Parameters such as A_{net} , SLA, N_{mass}, and $\Delta^{13}\text{C}$ give very useful information for future breeding programs having goals of improving woody species productivity and WUE.

Key words: $\Delta^{13}\text{C}$; growth rate; intrinsic water use efficiency; phenology; stable carbon isotope; water use efficiency.

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† E-mail: danwang2008@gmail.com

INTRODUCTION

Concerns over climate change and energy security have moved society towards an ecologically sustainable management of resources that

requires a reduction of the use of fossil fuels (Somerville et al. 2010). Renewable energy sources will play a key role in meeting CO₂ emission reduction objectives, given that renewable sources of energy have low net CO₂

Table 1. Abbreviations and descriptions of the variables used in the paper.

Abbreviation	Description	Units
A _{net}	net photosynthetic rate	μmol m ⁻² s ⁻¹
DB	basal diameter growth	mm
F _v '/F _m '	PSII efficiency in the light	%
g _m	mesophyll conductance	μmol m ⁻² s ⁻¹ Pa ⁻¹
g _s	stomatal conductance	mol m ⁻² s ⁻¹
Ht	total height growth	cm
iWUE	intrinsic water use efficiency (the ratio of A _{net} to g _s)	%
J _{max}	potential light saturated electron transport rate	μmol m ⁻² s ⁻¹
LAI	leaf area index	m ² m ⁻²
N _{mass}	leaf mass-based nitrogen concentration	%
PNUE	photosynthetic nitrogen use efficiency	μmol s ⁻¹
R _d	leaf dark respiration in the light	μmol m ⁻² s ⁻¹
SLA	specific leaf area	m ² kg ⁻¹
SRF	short rotation forestry	
V _{cmax}	maximum rate of rubisco activity	μmol m ⁻² s ⁻¹
WUE	water use efficiency	

emissions when substituted for fossil fuels (Kheshgi et al. 2000). In the US the Advanced Energy Initiative mandates that renewable bioethanol meet the 30 × 30 goal (30% of replacement of fossil fuels by biofuel by the year 2030; USDOE 2007). The cultivation of fast-growing woody species within short rotation forestry (SRF; Table 1) provides a potentially important source of alternative and renewable energy. At the same time, woody species reduce greenhouse gas emissions, nitrate leaching, and soil erosion while increasing belowground carbon storage (Davis et al. 2012, Wang et al. 2012). Compared to annual crops, woody species grown in a SRF have higher energy densities, lower transportation costs, and reduced need for annual inputs. These factors minimize the use of fossil fuels during production and thus improve the overall energy balance as a biofuel (Hill et al. 2006).

Woody species suitable for SRF should have common features such as wide, natural distribution range across regions, high initial growth rate, ease of vegetative propagation, and formation of coppice sprouts or suckers. Fast growing species of poplar (*Populus* spp.) and willow (*Salix* spp.) are two widely cultivated species in SRF, however, whether there are other woody species more suitable for regional climates and soils remains unclear. While some of the North American temperate species meeting these criteria have been previously evaluated for forestry production, there are only limited reports on evaluations of these plants, except for poplar, willow, sweetgum and black locust, in a short rotation coppicing system (Sartori and Lal 2006).

Trees of interest should be productive and easily managed while providing other ecosystem services including carbon storage reclamation of degraded lands. Thus, there is an urgent need for field trials or common garden studies to determine species productivity, make in situ measurements of plant performance in terms of water and nitrogen use efficiency, and to develop surveys for novel trait breeding.

Most terrestrial environments are subject to a limiting supply of water for plant transpiration, at least during some portion of the annual cycle (Cernusak et al. 2009). Thus, the efficiency with which terrestrial plants exchange water for CO₂ could have important implications for plant performance and production. On the other hand, the potential impacts of growing dedicated energy crops on the sustainability of water use must be assessed. There are two general hypotheses regarding the relationship between productivity and water use efficiency (WUE). One hypothesis is that more productive species have higher resource use efficiencies (including WUE) than the less productive ones (Gyenge et al. 2008). The other hypothesis is that high WUE results from low water use and consequently results in low productivity (Condon et al. 2004). Fast growth is usually associated with high water use (VanLoocke et al. 2012), and our objective is to identify species that are both productive and efficient with water use.

Commercial biomass species have been selected primarily based on criteria such as high productivity, adequate wood properties, and tolerance to pathogens such as foliar rusts.

Global climate change will produce an increased probability of drought or flood episodes and larger vapor pressure deficit (IPCC 2007). The counterbalancing effects of increasing water deficit and rising CO₂ concentration are the main factors expected to modify forest production (Calfapietra et al. 2010). High WUE allows species to assimilate more carbon per unit of water loss, which can be advantageous in water-limited conditions (Schwinning and Ehleringer 2001). The extension of forest cultivation to environments where soil water availability is subject to seasonal shortage would require species with greater WUE. WUE varies in different species and also in different leaf types and growth forms (Marshall and Zhang 1994). Thus, traits related to WUE provide useful criteria for woody species selection. Traits related to carbon assimilation and water loss at the leaf level are ideal targets for development of new genotypes (Tuberosa et al. 2007).

Through gas exchange measurements, WUE can be expressed as intrinsic WUE (iWUE, the ratio of net photosynthesis to stomatal conductance). Integrative WUE ($\Delta^{13}\text{C}$) can be assessed indirectly with measurements of the stable carbon isotope composition ($\delta^{13}\text{C}$) of leaves or other plant material. This latter method is based on the linear relationship between $\delta^{13}\text{C}$ and the ratio of the concentration of CO₂ inside and outside of the leaf (Farquhar et al. 1982). However, changes in mesophyll conductance or post-photosynthetic discrimination that affect $\delta^{13}\text{C}$ independently of C_i/C_a can result in positive or flat relationships between $\delta^{13}\text{C}$ and iWUE (Seibt et al. 2008). Using both gas exchange measurements (iWUE) and carbon isotope composition provides both instantaneous and integrated estimates of WUE. (Seibt et al. 2008). We will assess WUE by means of both gas exchange measurements (iWUE) and carbon isotope composition to get both the instantaneous and integrated estimates of WUE.

In this study, we measured growth, phenology, leaf-level gas exchange, iWUE, and leaf $\Delta^{13}\text{C}$ of 21 different woody species in order to assess the differences among species grown under common garden conditions. We aim to characterize the physiological and morphological characteristics underlying the observed differences in WUE and productivity. This will allow us to evaluate to

what extent leaf morphological and physiological traits are related to leaf WUE and whether $\Delta^{13}\text{C}$ can be used as an integrative ecological indicator for WUE when classifying species from different functional groups and native growing regions. In summary, the objectives of this study were (1) to screen 21 woody species for rapid growth rate and high WUE; (2) characterize phenological, physiological, and morphological traits as predictors of productivity; (3) investigate whether interspecific differences in iWUE were a result of stomatal control or changes in photosynthetic capacity and whether $\Delta^{13}\text{C}$ is a reliable indicator for iWUE when assessed across species and (4) quantify the relationship between growth, leaf traits, phenology, and WUE. We hypothesized that across 21 species, growth rate would be negatively correlated with WUE, with faster-growing species having lower WUE.

METHODS

Site characteristics

Replicated field trials comprising a total of 4.5 ha were established in the spring of 2010 at the Energy Biosciences Institute's Energy Farm (40.05° N, 88.18° W), Urbana, IL, USA, on a Flanagan series silt loam (Fine, smectitic, mesic Aquic Argiudolls). Before the experiment, the field was in a corn/soybean rotation with 2% soil organic carbon and 0.16% nitrogen was in the top 20 cm. We selected 21 woody species from the USDA Plants database (<http://plants.usda.gov>) that grow fast, are able to coppice, and are not considered being invasive in the region (detailed species information was given in Table 2). Throughout the manuscript species are referred to by their USDA acronyms (<http://plants.usda.gov>). The first coppicing treatment was applied at the conclusion of the 2011 growing season. The experimental design was a randomized complete block with 5 independent replications. Each block contains 21 plots (species) in a 2 × 11 arrangement and each plot contains 36 individual plants, with row spacing at 1.52 × 1.83 m (5 × 6 feet). For each block, there was an extra guard row on the outer row to minimize edge effects. The unusual drought and windy conditions in 2010 contributed substantially to the poor establishment of BENI, COAM3, ILDE, LIST2 and QUOCO2. In 2011, 958 individuals (out of 3780

Table 2. The full species name, common name, USDA acronym, source, growth form, initial height and seedling age for the species included in this study.

Species	Common name	USDA acronym	Source	Growth form	Initial height (in)	Seedling age (yr)
<i>Acer rubrum</i>	Red maple	ACRU	Elsberry, MO	Tree	24	2
<i>Acer saccharinum</i>	Silver maple	ACSA2	Elsberry, MO	Tree	36	2
<i>Alnus incana tenuifolia</i>	Thinleaf alder	ALINT	Bellingham, WA	Shrub	18–24	2
<i>Betula nigra</i> †	River birch	BENI	Plains, MT	Tree	24–36	2
<i>Castanea dentata × moll.</i>	Hybrid chestnut	CADE12	Kalamazoo, MI	Tree	12–18	2
<i>Catalpa speciosa</i>	Northern catalpa	CASP	McMinnville, TN	Tree	24	1
<i>Celtis occidentalis</i>	Common hackberry	CEO	McMinnville, TN	Tree	24	2
<i>Cornus sanguinea</i>	Bloodtwig dogwood	COSA81	Plains, MT	Shrub	18–24	2
<i>Corylus Americana</i> †	American filbert	COAM3	McMinnville, TN	Shrub	24	2
<i>Cotinus obovatus</i>	American smoketree	COOB2	Perry Hall, MD	Shrub	12–24	2
<i>Ilex decidua</i> †	Possumhaw	ILDE	McMinnville, TN	Shrub	12–18	2
<i>Liquidambar styraciflua</i>	American sweetgum	LIST2	McMinnville, TN	Tree	36	2
<i>Liriodendron tulipifera</i> †	Tuliptree	LITU	McMinnville, TN	Tree	36	2
<i>Maclura pomifera</i>	Osage-orange	MAPO	McMinnville, TN	Tree	24	2
<i>Platanus occidentalis</i>	Sycamore	PLOC	Elsberry, MO	Tree	24	1
<i>Populus deltoides</i>	Eastern cottonwood	PODE3	McMinnville, TN	Tree	10‡	2
<i>Prunus serotina</i>	Black cherry	PRSE2	McMinnville, TN	Tree	36	2
<i>Quercus coccinea</i> †	Scarlet oak	QUCO2	McMinnville, TN	Tree	24	2
<i>Rhus copallina</i>	Flameleaf sumac	RHCO	McMinnville, TN	Shrub	48	2
<i>Robinia pseudoacacia</i>	Black locust	ROPD	McMinnville, TN	Tree	36	2
<i>Salix × '9871-31'-Sherburne</i>	Hybrid willow	SALIX	SUNY, NY	Shrub	18–24	1

† Species not harvested in 2011 due to poor establishment in 2010.

‡ Unrooted cutting.

total individuals) for the poorly established species were replanted. Replanted individuals were not included in the growth estimates. Irrigation was only supplied during establishment and no fertilization was applied for the growth period. Alfalfa was used as a cover crop. In 2010 and 2011, Treflan was applied immediately after planting followed by Devrinol 50DF 2 weeks later and Devrinol another 8 weeks following the first Devrinol application. Liquid Fence, Deer and Rabbit Repellant, which contains putrescent egg solids (24.65%), garlic (2.96%), sodium lauryl sulfate (0.61%) and potassium sorbate (0.49%) was applied at a rate of 8 oz. per gallon in a CO₂ backpack sprayer one week after initial damage was documented and then at intervals of approximately every three weeks until the end of January.

Meteorological data were obtained from an eddy covariance tower located approximately a 100 m away (VanLoock et al. 2012). Monthly mean temperature was 11.4°C and 11.5°C in 2010 and 2011, respectively (Fig. 1). The annual precipitation was 930 mm in 2010 and 1000 mm in 2011. However, the growing season precipitation was 340 mm in 2010 and 540 mm in 2011. Growing season was defined from April (day of year [DOY] 106) to November (DOY 318) based

on our phenological survey. The annual daytime photosynthetic photon flux density (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$) averaged 467 and 427 in 2010; daytime VPD averaged 0.48 (95% CI: 0.026–1.16 kPa) and 0.44 (95% CI: 0.04–1.10 kPa) in 2010 and 2011, respectively.

Growth census

Total tree height and basal diameter were measured for each plant in June and September 2010 (3780 plants) and in September 2011 (2822 plants). We estimated leaf area index (LAI) in July 2010 and 2011 by counting the number of leaves on each tree and multiplying this by the species-specific average leaf area. Average leaf area for each tree was estimated each year from ten random samples using image analysis (ImageJ; Schneider et al. 2012). The LAI of each plot was estimated by dividing the total plot-level leaf area by the area of the plot. This approach will overestimate the effective LAI as it does not account for the leaf angle distribution, which was not measured.

Sixteen species (Table 2) were harvested in January 2012, leaving 5-cm stumps to resprout. For the harvested species, fresh and dry weight were determined for each individual plant and dry biomass per hectare was calculated accord-

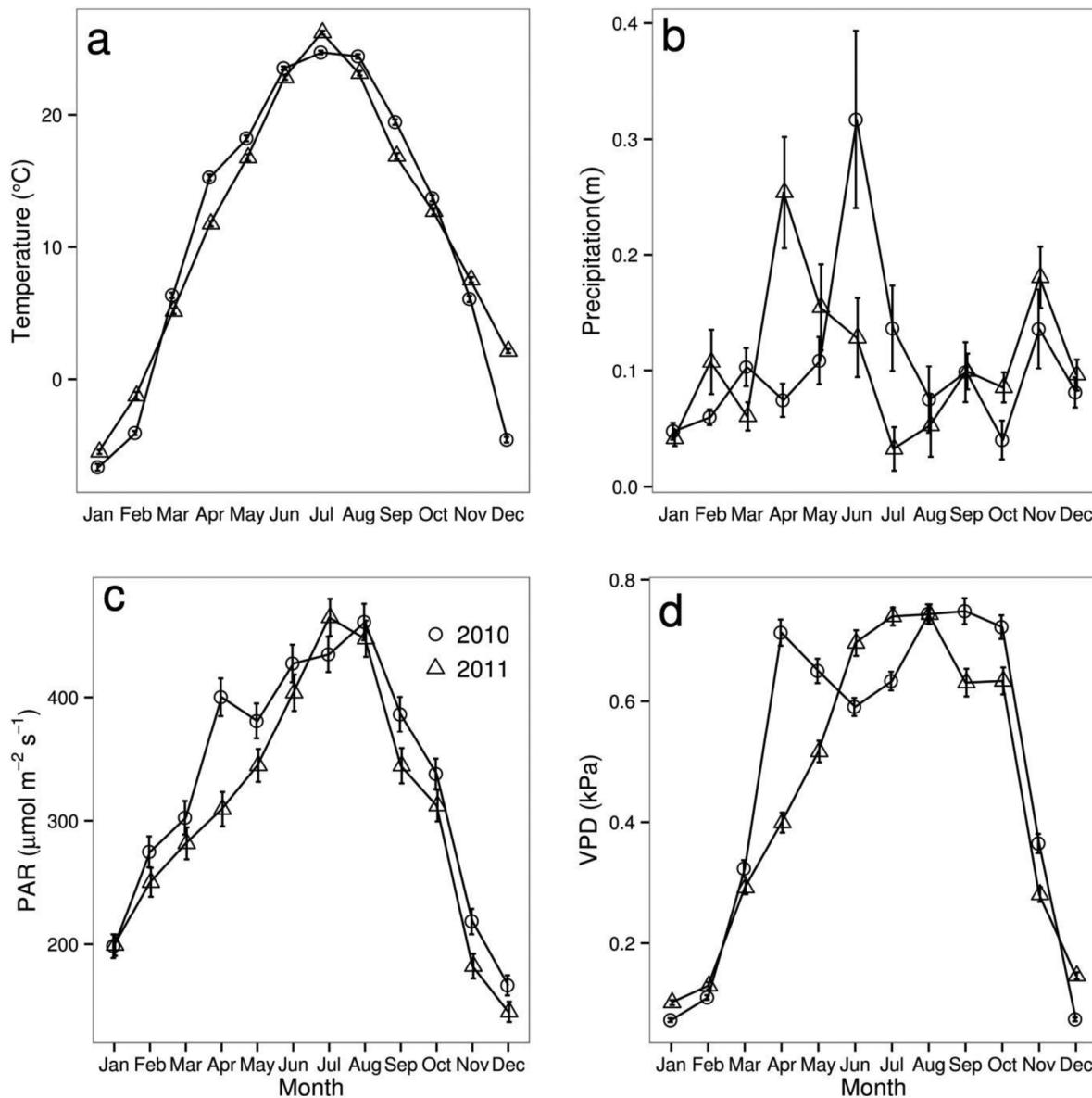


Fig. 1. Monthly mean (a) temperature, (b) precipitation, (c) daily photosynthetic active radiation (PAR) and (d) day-time vapor-pressure deficit (VPD), at the energy farm, Urbana, IL in 2010 and 2011.

ingly for each species.

Phenological survey

Phenology of all species was surveyed every two weeks in spring and fall in 2011, and the phenological stage was assessed on a scale of 0–4 (Richardson et al. 2006). We calculated the growing season as the time between stage 3 in the spring (when leaves are half of their fully expanded size) and stage 1 in the fall (when half

of the leaves had fallen).

Gas exchange and leaf trait measurements

Gas exchange (including net photosynthetic rate and stomatal conductance) was measured on fully expanded leaves at sunlit layers with a portable infrared gas analyzer (LI-COR 6400LCF; LI-COR, Lincoln, NE) from 10:00 am to 4:00 pm three times in each growing season. Intrinsic WUE was assessed through gas exchange mea-

surements conducted on 10 trees per species, with two leaves randomly selected from each block. During measurements, leaves were exposed to a CO₂ concentration of 370 μmol mol⁻¹, leaf temperature of 25°C, and airflow through the chamber of 250 μmol s⁻¹. Leaves were acclimated to a photosynthetic photon flux (PPFD; 1500 μmol m⁻² s⁻¹) until photosynthetic rates stabilized. The rate of photosynthesis at a PPFD of 1500 μmol m⁻² s⁻¹ was defined as the net photosynthetic rate (A_{net}). PSII efficiency in light-adapted leaves (F_v'/F_m') was also measured using a LICOR 6400-40 Leaf Chamber Fluorometer. The CO₂ response ($A - C_i$) curves were measured each year in the middle of the growing season (July or August). During measurement, leaves were acclimated for 30–60 minutes before adjusting the CO₂ concentrations. Thereafter, CO₂ concentration was decreased in 5 steps (400, 300, 200, 100 and 50 ppm CO₂) and then increased in 3 steps (400, 600 and 800 μmol mol⁻¹ CO₂). The $A - C_i$ curves were fit to the Farquhar-von Caemmerer-Berry (1980) model based on the methods developed by Miao et al. (2009). By using grid search and non-linear two-stage least square regression technique, the fitting model solves the $A - C_i$ parameters including maximum ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation rate (V_{cmax} , μmol m⁻² s⁻¹), potential light saturated electron transport rate (J_{max} , μmol m⁻² s⁻¹), leaf dark respiration in the light (R_d , μmol m⁻² s⁻¹), mesophyll conductance (g_m , μmol m⁻² s⁻¹ Pa⁻¹) and triose-phosphate utilization (TPU, μmol m⁻² s⁻¹), respectively.

Immediately following gas-exchange measurements, 10 0.5-cm² leaf punches from each leaf were taken and oven-dried at 65°C for two weeks for measurement of SLA. Leaf samples were then ground and N and C concentration were measured with a Perkin Elmer CHN Analyzer (Model 2400).

Integrated water use efficiency ($\Delta^{13}\text{C}$) and $\delta^{15}\text{N}$

Carbon and nitrogen isotope discrimination were measured on the 10 leaves on which gas exchange measurements were made in late August in 2010 or early September in 2011. Leaves were oven-dried at 65°C for two weeks, then ground to fine powder. Approximately 2 mg of homogenized leaves were weighed into tin

capsules and analyzed with an elemental analyzer coupled to an isotope ratio mass spectrometer (Elemental combustion system 4010, Costech instruments). Carbon isotope ratios are expressed in conventional δ notation and referenced to the Pee Dee Belemnite (PDB) standard for δ¹³C. Measurement error was less than 0.3‰ for δ¹³C and 0.4‰ for δ¹⁵N. The carbon isotope composition (δ¹³C) was calculated as the ratio (%):

$$\delta^{13}\text{C} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where R_{sample} and R_{standard} are the ¹³C:¹²C ratios in plant tissue and the standard, respectively.

The nitrogen isotope composition was calculated as the ratio (%):

$$\delta^{15}\text{N} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{atm}}} \right) - 1 \right] \times 1000$$

where R_{sample} and R_{atm} are the ratios in plant tissue and the atmosphere, respectively.

Carbon isotope ratio values were converted to discrimination values (Δ, ‰) by the equation (Farquhar et al. 1989):

$$\Delta = (\delta a - \delta p) / \left(1 + \frac{\delta p}{1000} \right)$$

where δa is the carbon isotope ratio of CO₂ in the atmosphere (assumed to be −8 pars per mil, Seibt et al. 2008) and δp is the measured carbon isotope ratio of the leaf tissue. Lower values of Δ indicate higher water use efficiency values.

Statistical analysis

Three-way analysis of variance (ANOVA) was used to test the fixed effects of year, month, species and their interaction on A_{net} , g_s , iWUE, SLA, N_{mass} and F_v'/F_m' . For DB, Ht, LAI, V_{cmax} , J_{max} , R_d , g_m , Δ¹³C and δ¹⁵N, the effects of species and year were tested with a two-way ANOVA. Species differences on phenological observations of leaf-on and leaf-off dates, season length, and yield were tested with a one-way ANOVA. A nesting effect of individual plant within plot was included in the two-way ANOVA for growth rate measurement. Due to our randomized complete block design, terms of block, block × year, and block × species were included as random effects for ANOVA. Post-hoc Tukey HSD tests were conducted on specific contrasts to examine significant treatment effects among groups. An

arcsine-square root transformation was used for F_v'/F_m' data, which normalizes proportional data (i.e., bounded by 0 and 1). General linear models (GLM) were used to assess the relationship between growth rate, $\Delta^{13}\text{C}$, iWUE and physiological parameters. Forward stepwise-based selection regression using R^2 as criteria was used to determine the best models to predict growth rate. For all tests, the normality of the residuals was tested using the Shapiro-Wilk test. A principal component analysis was performed on Ht, A_{net} , $\Delta^{13}\text{C}$, and PNUE in order to group species with similar resource use strategy and growth pattern. All statistical tests were considered significant at $P \leq 0.05$. Mean values of each variable were expressed with their standard error (SE). All analyses were conducted in R (R 2.14; <http://www.r-project.org/>) and the code is provided in the Supplement.

RESULTS

Tree growth rate

Tree growth in total height (Ht) and basal diameter (DB) differed among species and years (Fig. 2, Table 3). Species ranking remained stable between years for Ht and DB (Fig. 2a, b, Table 3). Averaged across two years, the five most rapid-growing species were ROPS, PODE3, CASP, PLOC, and ACSA2 based on Ht. According to DB, PODE3 was smaller than COSA81. COAM3, ILDE, LIST2 and QUCO2 were among the slowest-growing species based on both Ht and DB. Averaged across species, trees grew faster in 2011 than in 2010. Leaf area index was found to be highest for ROPS, PLOC and CASP in both years (Fig. 2c). The harvested yield was highest in ROPS ($11.35 \pm 0.17 \text{ Mg/ha}$), CASP ($3.94 \pm 0.51 \text{ Mg/ha}$), RHCO ($3.66 \pm 0.31 \text{ Mg/ha}$), ACSA2 ($3.46 \pm 0.28 \text{ Mg/ha}$) and PLOC ($2.54 \pm 0.23 \text{ Mg/ha}$) (Fig. 2d). Yields were significantly correlated with tree growth rate in accumulative Ht and DB across the two years (Fig. 2e, f).

Tree phenology

Species differed significantly in leaf-on and leaf-off dates and season length (Fig. 3, Table 4). Leaf-on date ranged from 106.0 ± 2.4 (day of year) for PODE3 to 129.0 ± 0 for PLOC. Leaf-off date was earliest for ALINT (283.8 ± 9.8) and latest for RHCO (318.0 ± 5.0). The longest

growing season length was found for LIST2 (195.4 ± 8.3 days) and the shortest for PLOC (164.2 ± 4.8 days).

Temporal patterns in leaf traits and photosynthesis

Gas exchange measurements were made on 21 species in 2010 and 20 species in 2011 (except ILDE). A significant species effect was detected for A_{net} , g_s , iWUE, V_{cmax} , J_{max} and R_d among 21 species and between years (Tables 3 and 4, Appendix: Fig. A1). A_{net} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was highest for MAPO (20.3 ± 1.0), PODE3 (19.3 ± 1.6), PLOC (17.3 ± 0.9) in 2010 and for RHCO (21.8 ± 1.0), ROPS (21.1 ± 0.9) and PODE3 (19.5 ± 1.0) in 2011. Stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) ranked highest for CASP (0.24 ± 0.02) in 2010 and RHCO (0.37 ± 0.02) in 2011 and lowest for ILDE (0.11 ± 0.02) in 2010 and ACSA2 (0.15 ± 0.01) in 2011. iWUE ranged from 116.1 ± 13.2 for BENI to 81.5 ± 3.8 for CEOC in 2010 and from 96.0 ± 7.4 for LIST2 to 62.2 ± 3.1 for RHCO in 2011. V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was highest for SALIX (118.8 ± 15.2), LIST2 (108.5 ± 14.1) and COSA81 (101.0 ± 30.7) in 2010 and for ALINT (101.4 ± 28.7), SALIX (94.3 ± 7.4) and LIST2 (85.5 ± 17.3) in 2011. J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was highest for ACRU in 2010 (210.9 ± 40.3) and 2011 (235.2 ± 69.7) and lowest for CADE in 2010 (114.2 ± 11.4) and QUCO2 in 2011 (98.7 ± 12.5). R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$) showed a marginal species effect, with a cross species mean of 2.2 ± 0.8 in 2010 and 3.2 ± 1.3 in 2011. g_m ($\mu\text{mol}^{-1} \text{m}^{-2} \text{s}^{-1} \text{pa}^{-1}$) showed no species effect, with cross species mean of 6.2 ± 1.2 in 2010 and 5.3 ± 0.8 in 2011. The ranking of F_v'/F_m' followed a similar trend as A_{net} in 2010. The highest F_v'/F_m' was achieved by BENI, SALIX, RHCO and ROPS in 2011.

There was significant species effect in SLA, N_{mass} , $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Tables 3 and 4; Appendix: Fig. A2). SLA ($\text{m}^2 \text{kg}^{-1}$) ranged from 11.6 ± 0.6 for SALIX to 16.0 ± 0.6 for ROPS in 2010 and from 10.5 ± 0.6 for SALIX to 19.2 ± 0.7 for PLOC in 2011. The legume species (ROPS) had the highest N_{mass} in 2010 ($3.3\% \pm 0.1\%$) and 2011 ($4.0\% \pm 0.1\%$). $\Delta^{13}\text{C}$ ranged from $18.5\% \pm 0.13\%$ for COSA81 to $21.37\% \pm 1.3\%$ for ILDE in 2010 and from $18.4\% \pm 0.38\%$ for QUCO2 to $22.3\% \pm 0.3\%$ for ROPS. The isotopic $\delta^{15}\text{N}$ was highest in CEOC ($5.88\% \pm 0.33\%$) in 2010 and COOB2 ($4.14\% \pm 0.51\%$) in 2011 and lowest in ACRU

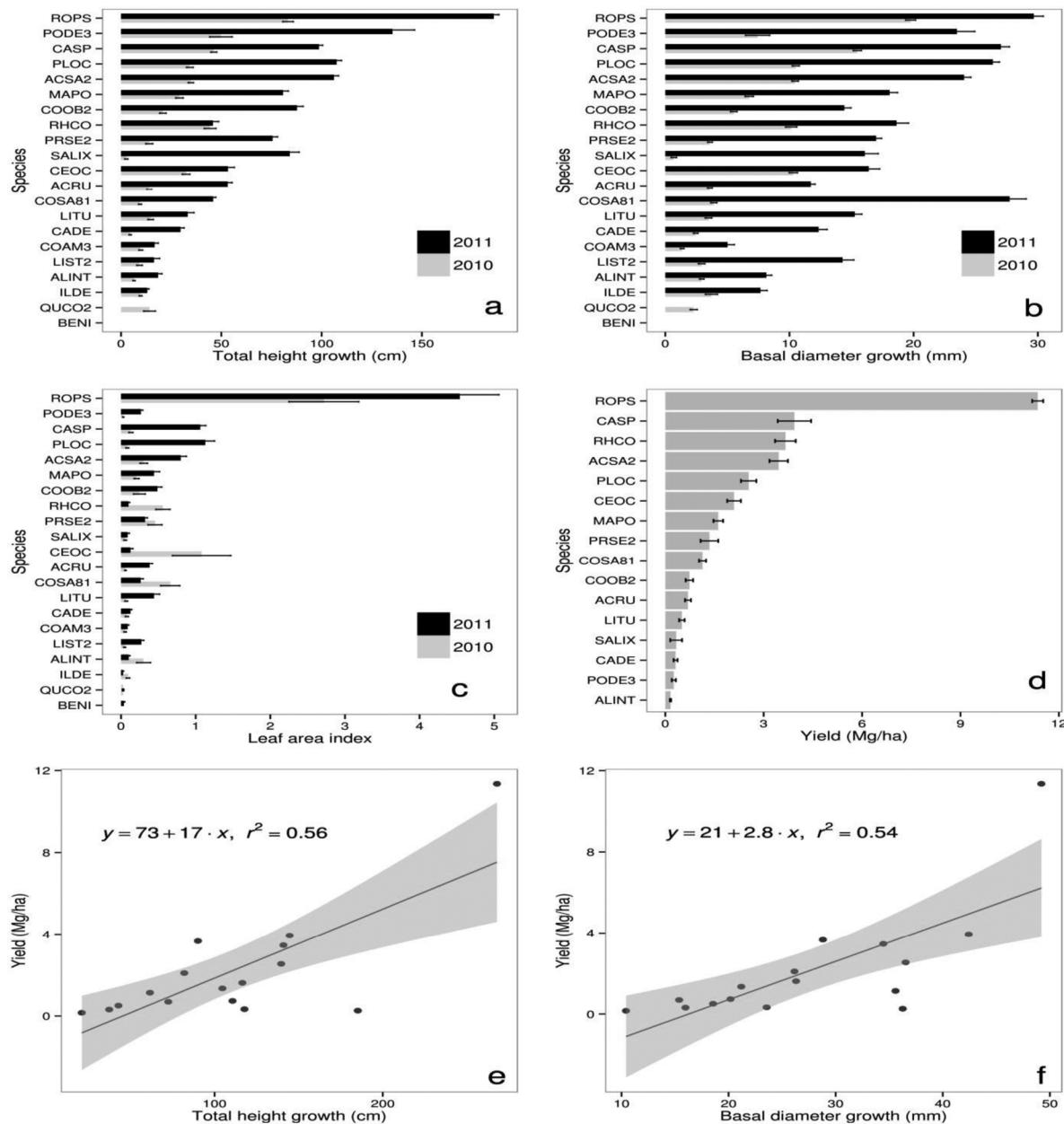


Fig. 2. (a) Total height (Ht) growth, (b) basal diameter (DB) growth, (c) leaf area index (LAI) for 20 species in 2010 and 19 species in 2011, and (d) yield measurement in 2011. Linear regression was conducted between (e) yield and Ht and (f) yield and DB. Each data point represents the mean (\pm SE) of 4–5 independent replicates. The ranking of the species was based on the height growth over two growing seasons. See Table 1 for full species name.

($2.39\% \pm 0.27\%$) in 2010 and ALINT ($0.93 \pm 0.21\%$) in 2011.

Averaged across species, A_{net} , g_s , and SLA were significantly higher in 2011 than in 2010;

iWUE, V_{cmax} , J_{max} , R_d , N_{mass} , $\Delta^{13}C$ and $\delta^{15}N$ were significantly higher in 2010 than in 2011 (Tables 3 and 4). The rate of F_v'/F_m' did not differ between years. A_{net} for most species peaked in

Table 3. ANOVA (F value) of the effects of species and year on maximal carboxylation rate (V_{cmax}), potential light saturated electron transport rate (J_{max}), leaf dark respiration in the light (R_d), mesophyll conductance (g_m), $\Delta^{13}\text{C}$, $\delta^{15}\text{N}$, growth in Ht and DB and leaf area index (LAI).

Source	V_{cmax}	J_{max}	R_d	g_m	$\Delta^{13}\text{C}$	$\delta^{15}\text{N}$	Ht	DB	LAI
Species	2.2**	2.7***	1.5†	1.1	18.5***	8.3***	426.5***	187.4***	152.4***
Year	5.8*	2.9†	10.0**	0.0	10.3**	16.1***	4060.1***	3150.1***	2569.8***
Species × year	0.5	0.5	0.3	1.3	4.5***	1.0	91.3***	30.4***	50.8***

*** P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05 and †P ≤ 0.1.

the late growing season in 2010 (day 259) and in the middle or late growing season in 2011 (days 209 and 238) (Appendix A: Fig. A3). The rate of g_s was generally highest, and iWUE lowest, in the middle of the growing season for both 2010 and 2011. SLA was generally lowest in the late growing season in 2010 and 2011 (Appendix: Fig. A3).

Contributions of leaf traits to iWUE, $\Delta^{13}\text{C}$, and growth

iWUE was marginally negatively correlated with A_{net} ($R^2 = 0.16$, $p = 0.08$) in 2011. iWUE formed a significantly negative relationship with g_s in both 2010 and 2011 (Fig. 4). iWUE was negatively correlated with SLA and F_v'/F_m' in 2010. Leaf-on date negatively correlated with iWUE in 2011.

$\Delta^{13}\text{C}$ had a positive relationship with A_{net} , g_s , and g_m and a negative linear relationship with iWUE in 2011 (Fig. 5), which is the expected direction based on sign conventions.

Ht growth scaled positively with A_{net} , N_{mass} , SLA, and PNUE in both years and with $\Delta^{13}\text{C}$ in 2011 (Fig. 6). The best parameter set for determining Ht was the combination of A_{net} and SLA ($R^2 = 0.56$ in 2010; $R^2 = 0.54$ in 2011).

Clustering on the basis of growth and physiology

Principal components analysis (PCA) revealed that the proportion of variance explained by PC1 and PC2 were 58.2% and 16.9%, respectively (Fig. 7, Table 5). All four parameters (Ht, $\Delta^{13}\text{C}$, A_{net} , and PNUE) loaded with similar weight on PC1 and PC2 was mostly loaded by $\Delta^{13}\text{C}$ and PNUE. Species with a positive PC1 had relatively higher growth rate and A_{net} while species loaded with a negative PC2 had a higher PNUE and lower $\Delta^{13}\text{C}$. Among the fast-growing species, ROPS distinguished itself by a higher Ht, A_{net} and $\Delta^{13}\text{C}$ and lower PNUE; whereas PODE3, RHCO, CASP and PLOC had a higher Ht, A_{net} and PNUE, but a lower $\Delta^{13}\text{C}$.

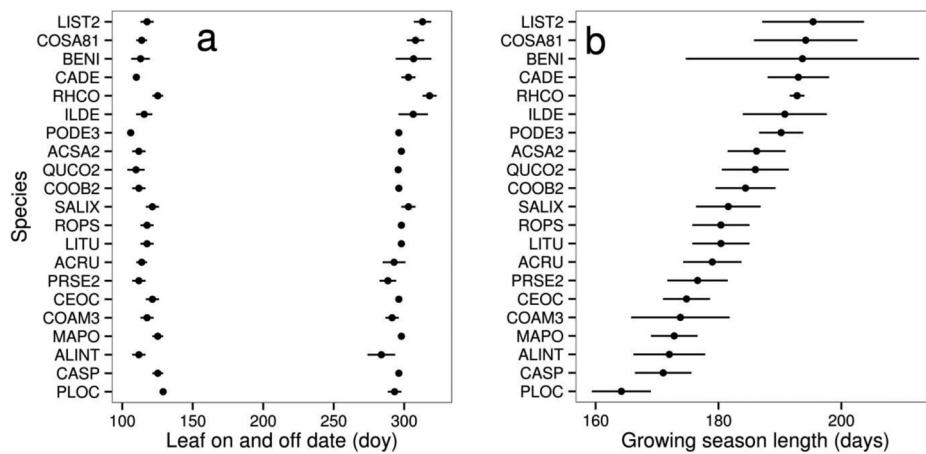


Fig. 3. Phenological survey of (a) leaf-on, leaf-off date and (b) season length for 21 species in 2011. Each data point represents the mean (\pm SE) of 4–5 independent replicates. The ranking of species was based on the season length.

Table 4. ANOVA (F value) of the effects of species, year, month and their interaction on net photosynthetic rate (A_{net}), stomatal conductance (g_s), instantaneous WUE (iWUE), specific leaf area (SLA), leaf nitrogen concentration (N_{mass}) and photosystem II efficiency (F_v'/F_m') measured during the two growing seasons; and the effect of species on season length, leaf-on and leaf-off date and yield.

Source	A_{net}	g_s	iWUE	SLA	N_{mass}	F_v'/F_m'	Season length	Leaf-on date	Leaf-off date	Yield
Species	22.7***	17.2***	4.0***	21.0***	32.4***	2.75***				
Year	10.4**	130.0***	80.3***	43.0***	6.6*	2.77†				
Month	60.1***	117.8***	68.2***	105.3***	23.5***	109.3***				
Species × Year	3.7***	5.4***	1.9*	4.8***	2.6***	1.53†				
Species × Month	2.6***	2.1***	1.2	2.4***	2.0***	1.7*				

*** P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05 and †P ≤ 0.1.

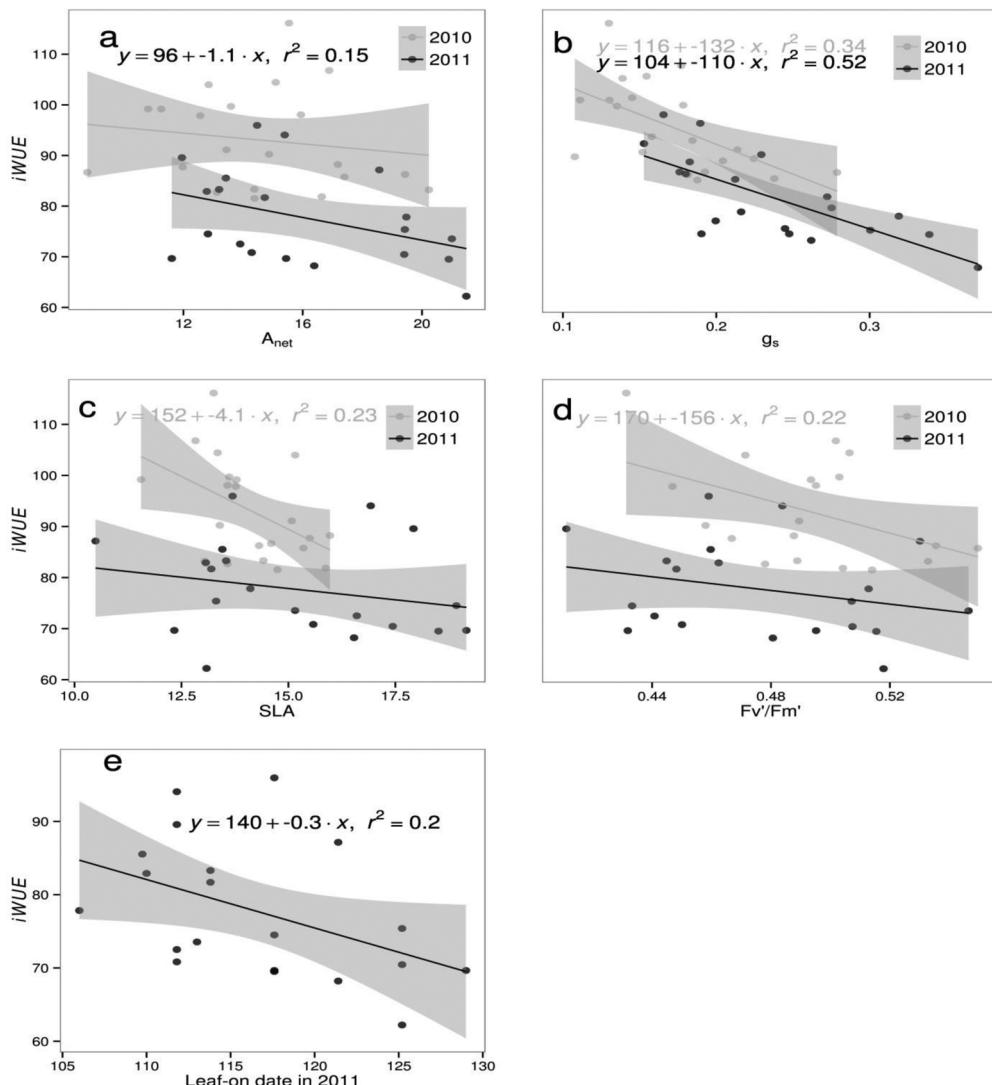


Fig. 4. Linear regression between intrinsic water use efficiency (iWUE) and (a) net photosynthetic rate (A_{net}); (b) stomatal conductance (g_s); (c) specific leaf area (SLA); (d) PSII efficiency in the light (F_v'/F_m'); and (e) leaf-on date. Shaded area indicated confidence interval of 0.95. Equations and r^2 are only shown for significant relations.

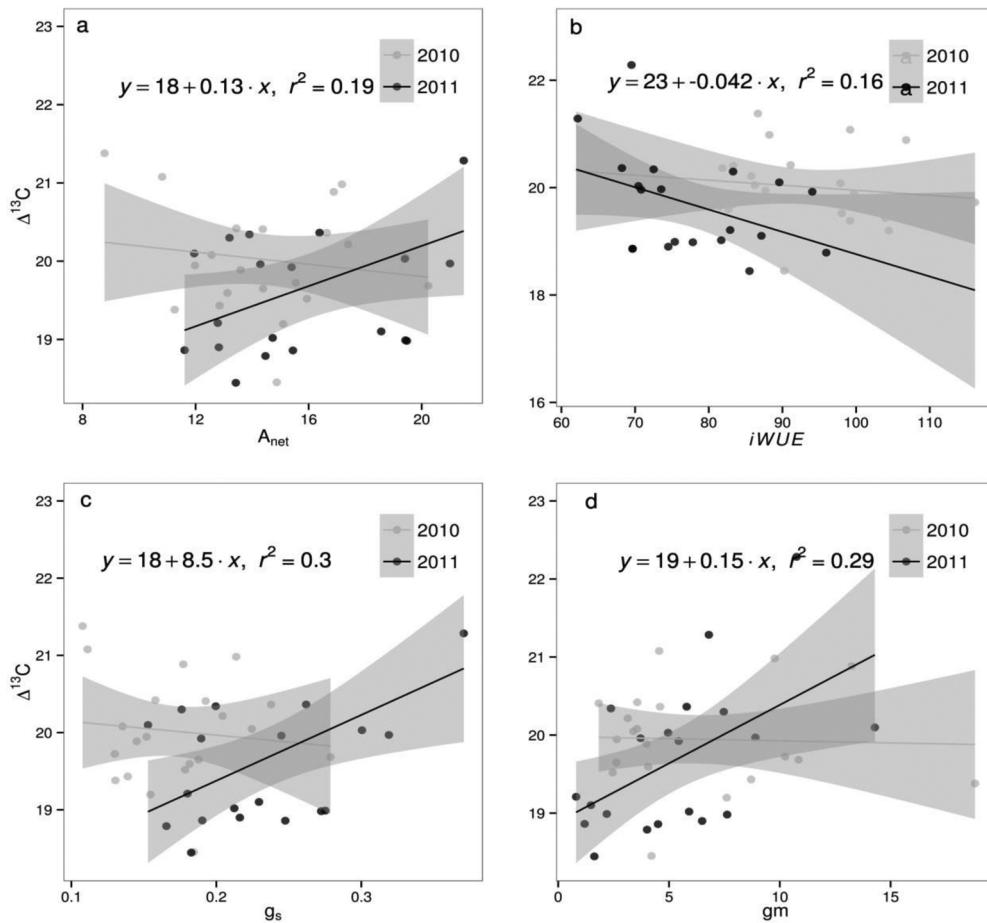


Fig. 5. Linear egression between $\Delta^{13}\text{C}$ and (a) net photosynthetic rate (A_{net}); (b) intrinsic water use efficiency (iWUE); (c) stomatal conductance (g_s); and (d) mesophyll conductance (g_m). Shaded area indicated confidence interval of 0.95. Equations and r^2 are only shown for significant relations.

DISCUSSION

In order to screen for suitable biomass feedstock and find out whether there is a trade-off between productivity and WUE, we assessed growth, phenology and leaf traits between 21 woody species in a common garden field site across two growing seasons. These observed relationships between leaf traits, WUE, phenology and growth facilitated the trait-based parameterization of dynamic simulation models (Wang et al. 2012; D. Wang et al., *unpublished manuscript*) in order to predict tree production on a regional scale. Systematic measurements of gas exchange and phenology across the growing season are essential for validation of growth and ecosystem models and to elucidate the physiological basis

for observed differences in productivity and WUE.

Species with rapid growth rate and high water use efficiency

One of our primary objectives was to understand the magnitude of variations in WUE and productivity across species growing in a common environment in order to screen for the fast-growing species with high WUE for biomass purposes. Several traits related to productivity and leaf WUE were tested among 21 species. Large species variability was detected during 2010 and 2011 for all traits. Although the values reported during 2010 differed markedly from those of 2011 for most of the variables, species variability and ranking remained comparable

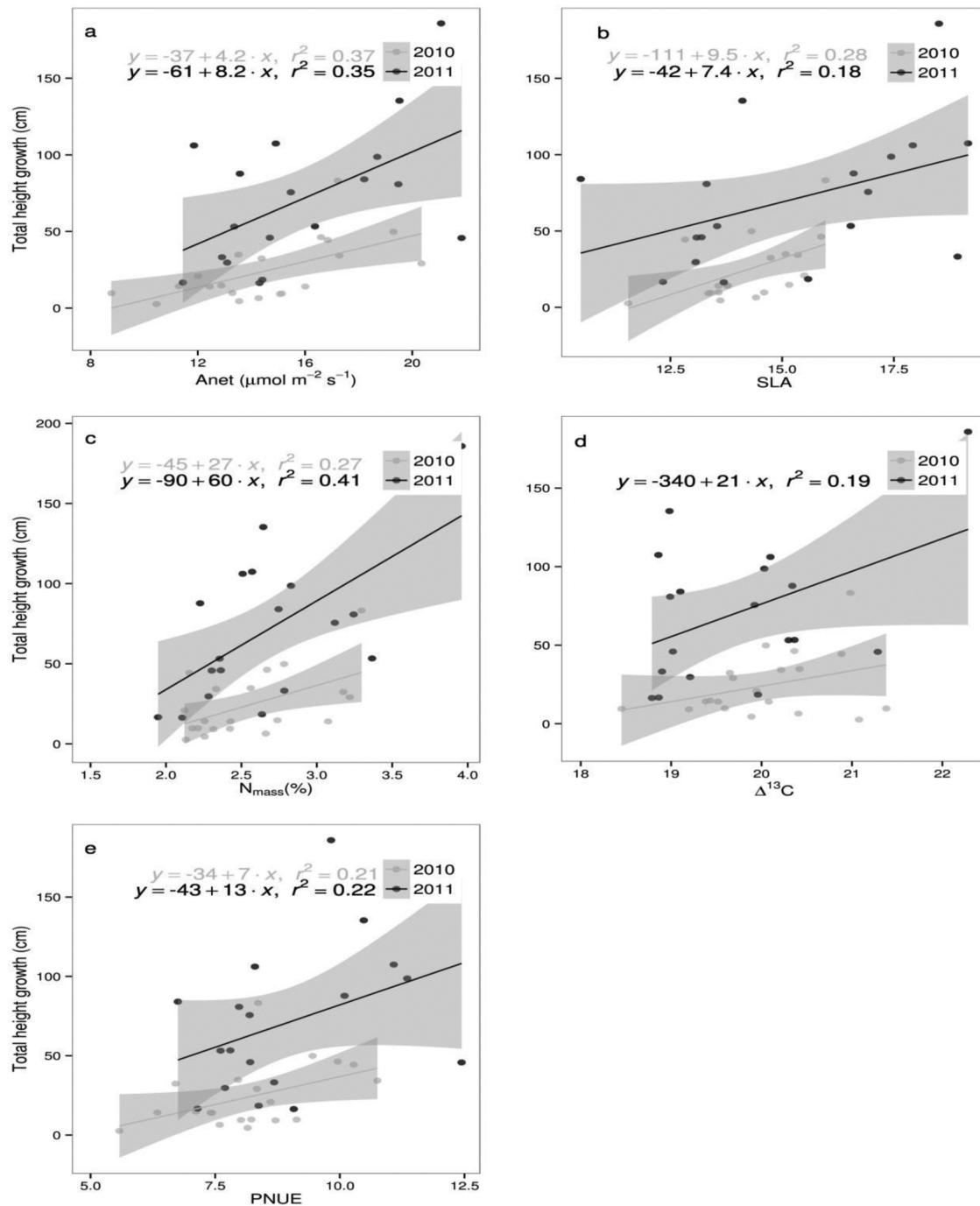


Fig. 6. Linear regression between total height (Ht) growth and (a) net photosynthetic rate (A_{net}); (b) specific leaf area (SLA); (c) leaf nitrogen content (N_{mass}); (d) $\Delta^{13}\text{C}$; and (e) photosynthetic nitrogen use efficiency (PNUE). Shaded area indicated confidence interval of 0.95. Equations and r^2 are only shown for significant relations.

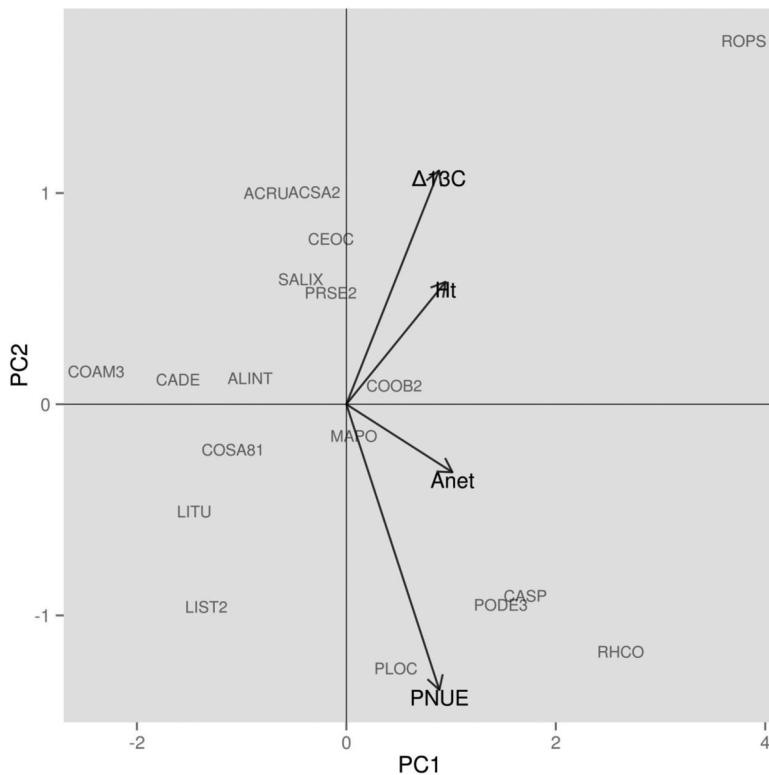


Fig. 7. Principal component analysis based on the correlation between total height growth, net photosynthetic rate (A_{net}), $\Delta^{13}\text{C}$ and photosynthetic nitrogen use efficiency (PNUE).

and stable from year to year. A positive relationship between productivity (Ht in Fig. 6; yield in Appendix: Fig. A4; DB not shown) and $\Delta^{13}\text{C}$ suggests that the most productive species have the least efficient water use, supporting our hypothesis that fast-growing species had a lower WUE. Similar results have been shown in Sonoran Desert winter annuals (Angert et al. 2007, 2009), which reported that relative growth rate was positively related to $\Delta^{13}\text{C}$. However, within 33 poplar genotypes from a *Populus deltoides* \times *Populus trichocarpa* F1 progeny, $\Delta^{13}\text{C}$ scaled negatively with productivity (Monclús et al. 2009). Among eight nonnative tree species including evergreen species in the Los Angeles Basin, there was no relationship between leaf $\delta^{13}\text{C}$ and leaf-level WUE, integrated WUE, or growth rate (McCarthy et al. 2011). Given the diverse results shown in different studies, caution must be taken when $\Delta^{13}\text{C}$ is being used as a proxy for WUE and growth rate depending on the group of species tested. According to the

PCA analysis, some fast-growing species, such as sumac (RHCO), catalpa (CASP), sycamore (PLOC) and cottonwood (PODE3), have relatively higher WUE compared to other species (Fig. 7). Thus, our study supports the idea that there is a potential for selecting a fast-growing species without sacrificing WUE (Binkley et al. 2004, Cernusak et al. 2007, Gyenge et al. 2008). While a positive relationship between Ht and $\Delta^{13}\text{C}$ was not observed in 2010, this might be due to the fact that $\Delta^{13}\text{C}$ is partly controlled by the isotopic signature of carbon assimilated before planting.

The positive relationship was found between SLA, A_{net} , LAI and Ht and the combination of SLA and A_{net} was the best model to predict growth, which is in agreement with our previous work suggesting the variability of productivity is driven by a combination of total leaf area and photosynthetic capacity (Wang 2010, 2013). Species that display a high growth rate allocate a large fraction of biomass to photosynthetic surfaces and have the ability to deploy large leaf

Table 5. Trait loadings, species scores, and percent variation explained by the first and second principle component of variation in functional traits.

Species or trait	PC1	PC2
Trait		
Ht	0.47	0.31
$\Delta^{13}\text{C}$	0.51	0.59
A_{net}	0.54	-0.17
PNUE	0.48	-0.72
Species		
ACRU	-0.77	1.00
ACSA2	-0.31	1.00
ALINT	-0.92	0.12
CADE	-1.61	0.12
CASP	1.71	-0.91
CEOc	-0.15	0.78
COAM3	-2.39	0.15
COOB2	0.46	0.09
COSA81	-1.08	-0.21
LIST2	-1.34	-0.96
LITU	-1.45	-0.51
MAPO	0.07	-0.15
PLOC	0.47	-1.25
PODE3	1.47	-0.95
PRSE2	-0.15	0.53
RHCO	2.62	-1.17
ROPS	3.79	1.72
SALIX	-0.44	0.59
Variation explained	58.2%	16.9%

area for light interception to maximize growth. The evaluation of the performance of 53 tree saplings in a rain forest indicated that SLA was a reasonable predictor of interspecific variation in height growth (Poorter and Bongers 2006). The results in the study showed that the relationship between growth rate and SLA was more significant in the first growing season than in the second growing season (Fig. 6), which suggests SLA may be more important for light interception of saplings, for which it determines to a large extent the exposed leaf area, but less so for larger trees, where light interception is largely determined by branching patterns, the number of meristems, and tree architecture (Sterck and Bongers 2001).

Height growth also scaled positively with N_{mass} and PNUE (Fig. 6). Plants with rapid growth rates are associated with high tissue resource concentrations, thus high photosynthetic capacity (Wright et al. 2004). Our results showed that the legume black locust (ROPS), the fastest-growing species, had a lower $\delta^{15}\text{N}$ in 2011 and a higher N_{mass} than the other, non-legume trees, which suggests that symbiotic nitrogen fixation might contribute to the fast

growth rate of black locust (Makarov 2009). However, the other N-fixing species, alder (ALINT), did not achieve high growth rate, possibly due to poor establishment.

Poplar and willow in the eastern US are considered potentially important biomass crops for the region (DOE 2006). Maximal annual yield ranged from 3.5 to 12.8 Mg ha⁻¹ yr⁻¹ for poplar (Strong and Hansen 1993, Netzer 2002) and reached 16.3 Mg ha⁻¹ yr⁻¹ for a willow clone (SV1) in its fifth growing season (Kopp et al. 2001) and 12.7 Mg ha⁻¹ yr⁻¹ for another willow clone (Sx-61) in the Great Lakes region (Randall et al. 2010). However, the performance of poplar (seedling material) and willow (*Salix* x '9871-31' – Sherburne) were not among the fastest-growing species in our study possibly due to the variety selection, as in a parallel experiment where these clones were among the slowest growing clones of more than 40 poplar and willow clones (G. Kling et al., *unpublished data*). As different clones differed in terms of growth, partitioning and leaf traits (Marron et al. 2007, Tyree et al. 2009) and responded differently to planting density (DeBell 1997, Strong and Hansen 1993), future studies would include more genotypes per species and management types to make the comparison more representative for each species. Further investigation is in progress regarding to test how early years establishment will affect the longer term productivity of these species.

Drivers for iWUE

Gas exchange measurements provided additional information about the processes involved in the inter-specific variability of iWUE (Fig. 4). iWUE is calculated as the ratio between assimilation rate and stomatal conductance and therefore systematic variation of both traits can change iWUE (Seibt et al. 2008). Higher iWUE may be achieved by a tight regulation of water losses through fine control of resistances to water loss (Galmes et al. 2007) and/or by presenting photosynthetic machinery more suited for water stressing conditions (Gilbert et al. 2011). Contrary to the expectation that high photosynthetic rate was related to high iWUE, there was a marginally negative relationship between A_{net} and iWUE in 2011 ($R^2 = 0.16$, $p = 0.08$), mainly because increases in the leaf resistances to gas exchange (mainly g_s) occurred at a higher

magnitude than increases in A_{net} . Therefore, variation in photosynthetic capacity did not seem to be the driver for variation in the estimates of iWUE. We conclude that variation in iWUE is primarily driven by variation in g_s , as observed in the temporal patterns of leaf traits (Appendix: Fig. A3). Despite the higher A_{net} and relatively lower growing season precipitation in 2011, the rate of iWUE was lower in 2011 than that in 2010 when averaged across all species, mainly due to the higher g_s in 2011. iWUE was lowest in the middle of the growing season for all the species in 2010 and 2011 due to higher rate of g_s (except for hackberry, tulip tree, oak and black locust in 2010) (Appendix: Fig. A4). It is widely stated that SLA influences iWUE by affecting photosynthetic performance (Wright et al. 2005, Poorter et al. 2009), which was supported by the negative correlation between SLA and iWUE in 2010. Species that produced leaves earlier in the season tended to have a lower iWUE. The relationship between leaf-on date and iWUE requires further investigation, but may relate to leaf aging or leaf morphological traits such as stomatal size and density.

Drivers for $\Delta^{13}\text{C}$

$\Delta^{13}\text{C}$ is considered to have an advantage over measurements of iWUE through photosynthetic capacity because it integrates WUE over the growing season. There is evidence for a negative linear relationship between iWUE and $\Delta^{13}\text{C}$ in cereals (Farquer et al. 1989), six genotypes of *Quercus robur* (Roussel et al. 2009), two sympatric oak species (Ponton et al. 2002) and monsoon forest species (Orchard et al. 2010). This relationship can be difficult to resolve because the two variables integrate plant response over different time spans: iWUE is an instantaneous measurement while $\Delta^{13}\text{C}$ is integrated over the growing season. Recent analyses have suggested that using $\Delta^{13}\text{C}$ as an indicator of variation in WUE could be less effective when applied across species (Warren and Adams 2006, Cernusak et al. 2009, Seibt et al. 2008). Relationships between $\Delta^{13}\text{C}$ and iWUE could be related to different leaf types and growth forms (Medrano et al. 2009, Orchard et al. 2010) included in different studies. Our study showed that the diversity of $\Delta^{13}\text{C}$ matched the independent gas exchange measurements of iWUE and suggests that across the

species tested (all deciduous) comparisons of leaf $\Delta^{13}\text{C}$ provided a robust indication of variation in WUE. The overall $\Delta^{13}\text{C}$ during carbon assimilation is dependent on the CO_2 concentration at the sites of carboxylation, which in turn is strongly dependent on mesophyll conductance (g_m). Many studies reported no significant relationship between $\Delta^{13}\text{C}$ and iWUE (Seibt et al. 2008, McCarthy et al. 2011), claiming mesophyll conductance contributed to the observed variability of $\Delta^{13}\text{C}$. The linear correlation between $\Delta^{13}\text{C}$ and g_m found in this study confirmed the dependence of $\Delta^{13}\text{C}$ on g_m , suggesting that lower $\Delta^{13}\text{C}$ was partly due to the limited capacity of the internal leaf tissue to diffuse CO_2 and confirming that CO_2 concentration at the sites of carboxylation varies systematically as a function of g_m (Warren and Adams 2006, Flexas et al. 2008). Plants can increase WUE by increasing the efficiency of carbon fixation inside the leaf, either by increasing the efficiency of light harvesting or carboxylation processes. $\Delta^{13}\text{C}$ was positively correlated with photosynthetic capacities (A_{net}), but not J_{max} or F_v'/F_m' in this study, suggesting that the variability in $\Delta^{13}\text{C}$ was not driven by light harvesting ability. The positive relationship between $\Delta^{13}\text{C}$ and A_{net} might be due to the same reason that increased rates of A_{net} were also associated with increases in g_s . Stomatal conductance appeared to be the main driver for both iWUE (Fig. 4) and $\Delta^{13}\text{C}$ (Fig. 5), which suggests that WUE could be informed successfully by comparing g_s measurements across species.

CONCLUSION

This study provides forest managers and policy makers with an ecophysiological approach to screen for suitable species with both rapid growth rate and high WUE for biomass purposes and ecosystem models with a systematic measurement of leaf traits across the growing season to predict ecosystem-level carbon and water balance. Among the species evaluated, there is a trade-off between growth rate and WUE associated with variability in the ecophysiological traits, phenology and leaf $\Delta^{13}\text{C}$. Species with rapid growth rate had lower WUE across 21 species. While species such as black locust had a higher growth rate and lower WUE, other faster-growing species such as sumac, catalpa and

sycamore were associated with intermediate-level WUE. This suggests that an understanding of plant growth, water use, and regional climate conditions can make it possible to select species which optimize the trade-off between carbon gain and water use. At situations where water resources are limited, species such as sumac, catalpa and sycamore, are more suitable. Species like black locust is more desirable where water resources are luxury to increase soil water storage capacity. $\Delta^{13}\text{C}$ was found to be an effective proxy for comparing WUE across the species we chose. A_{net} and SLA are useful predictors of tree growth, while g_s effectively predicts both intrinsic and integrated WUE. Parameters such as A_{net} , g_s , SLA, N_{mass} and $\Delta^{13}\text{C}$ linearly correlated with either growth or iWUE, and provide a suite of traits that can be used for breeding programs aimed at improving woody species productivity and WUE.

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SUPPLEMENTAL MATERIAL

APPENDIX

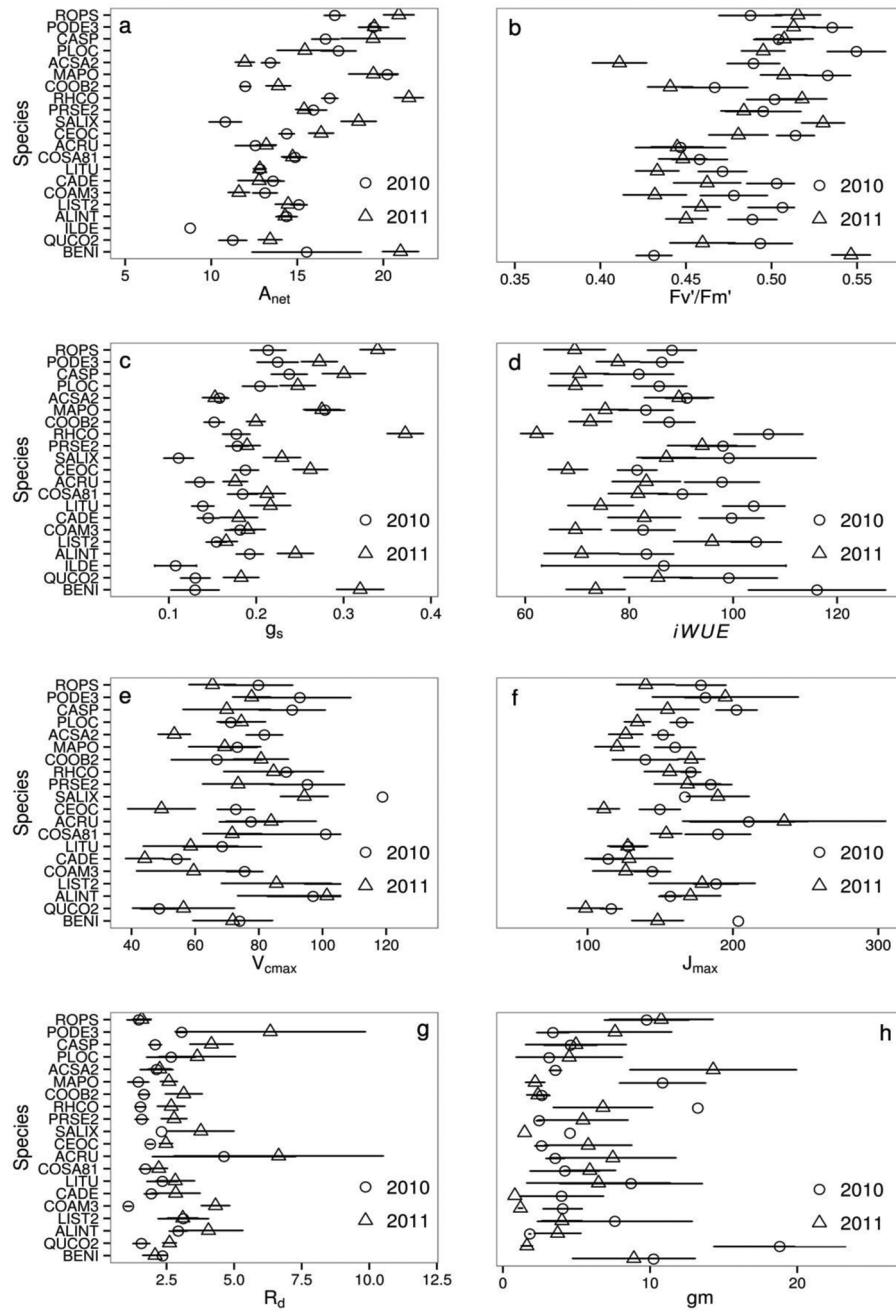


Fig. A1. Measurements of (a) net photosynthetic rate (A_{net}); (b) photosynthetic II efficiency in the light (F_v'/F_m'); (c) stomatal conductance (g_s), (d) intrinsic water use efficiency ($iWUE$); (e) maximal rubisco carboxylation rates (V_{cmax}); (f) potential light saturated electron transport rate (J_{max}); (g) dark respiration (R_d); and (h) mesophyll conductance (g_m) for 21 species averaged across three measurements in 2010 (days 175, 232 and 259) and 2011 (days 167, 209, and 238). Each data point represents the mean (\pm SE) of 4–5 independent replicates. The ranking of the species was based on the height growth over two growing seasons

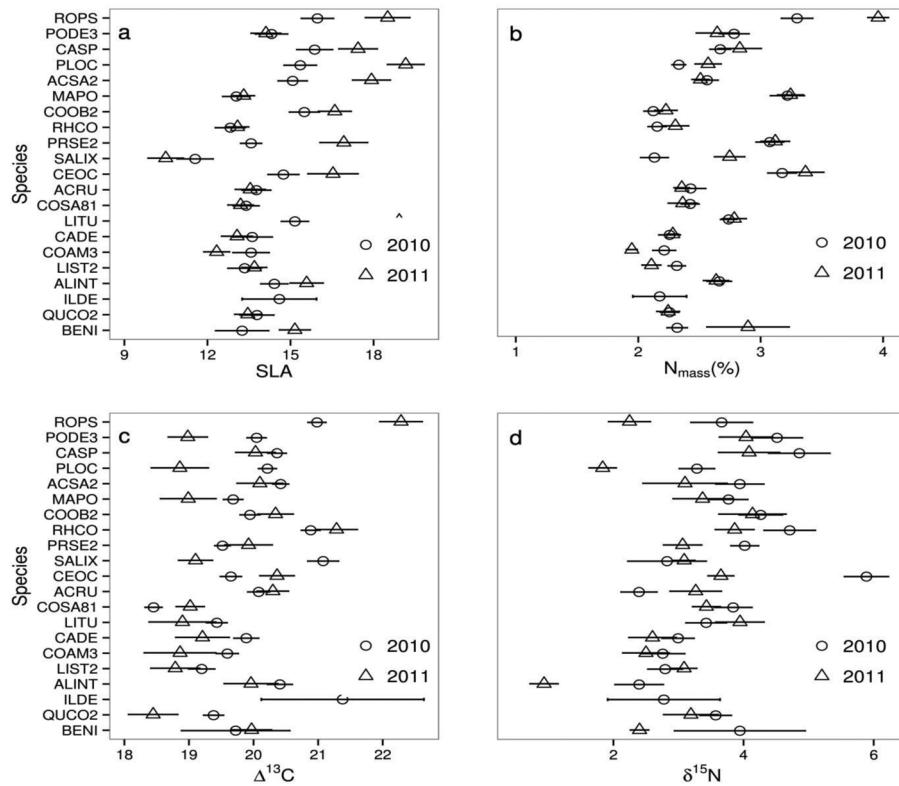


Fig. A2. Measurement of (a) specific leaf area (SLA); (b) leaf nitrogen content (N_{mass}), (c) $\Delta^{13}\text{C}$ and (d) $\delta^{15}\text{N}$ for 21 species in 2010 and 20 species measured in 2011. Each data point represents the mean ($\pm \text{SE}$) of 4–5 independent replicates. The ranking of the species was based on the height growth over two growing seasons.

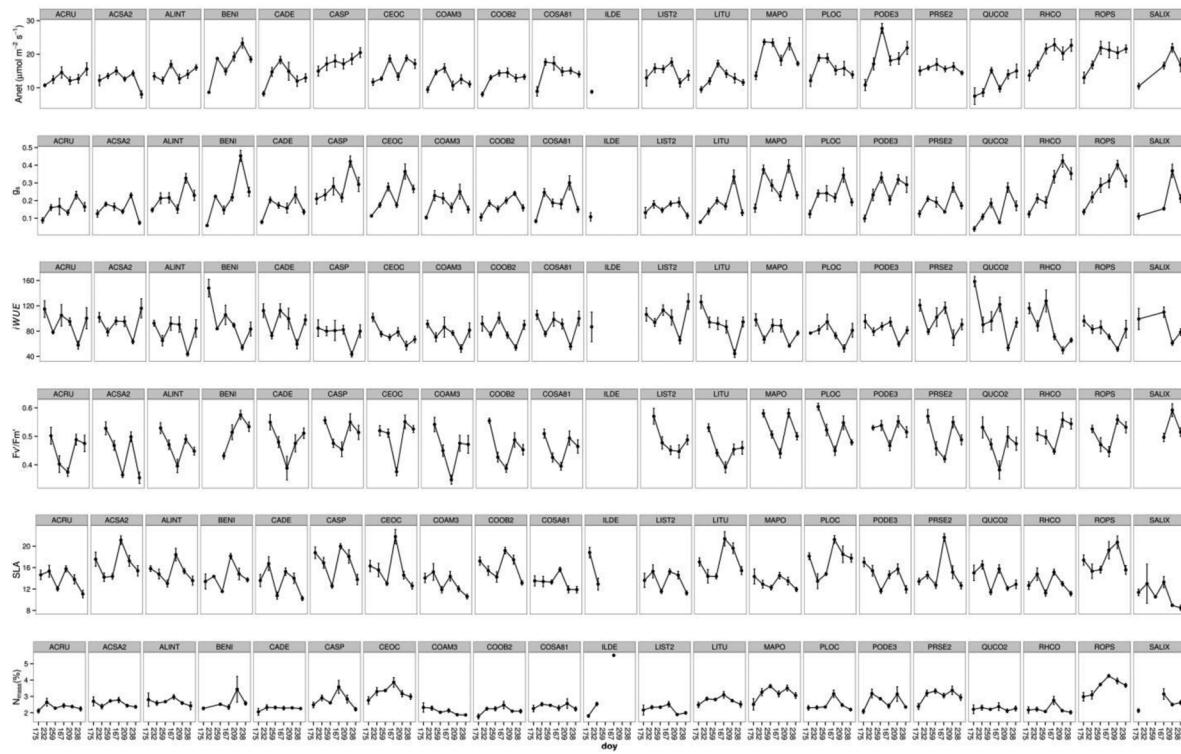


Fig. A3. Net photosynthetic rate (Anet), stomatal conductance (gs), instantaneous water use efficiency (iWUE), photosystem II efficiency in the light (Fv'/Fm'), specific leaf area (SLA) and leaf nitrogen content (Nmass) for 21 species measured in 2010 (days 175, 232 and 259) and 2011 (days 167, 209, and 238). The ranking of the species was based on the height growth over two growing seasons. Data for ILDE and SALIX were not complete due to poor establishment of the species.

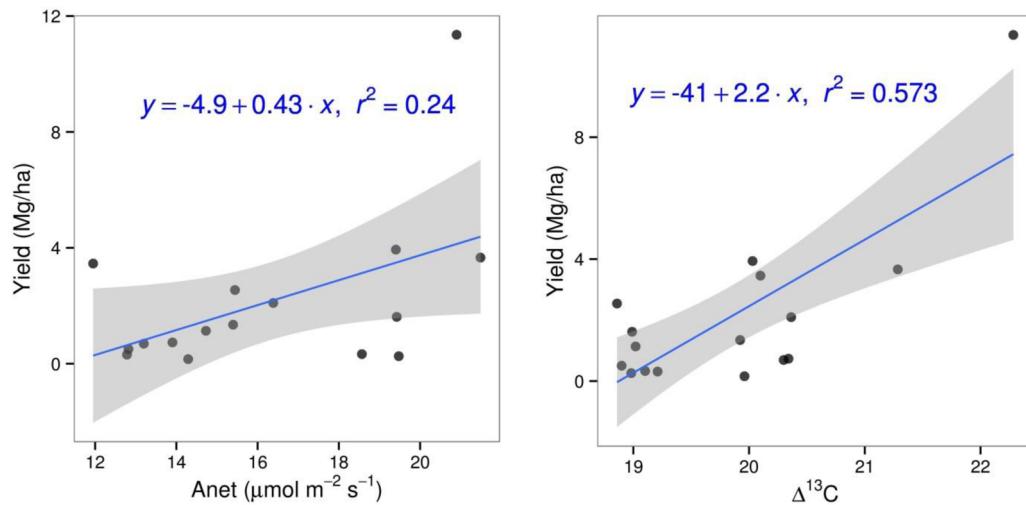


Fig. A4. Linear regression between yield and A_{net} and $\Delta^{13}\text{C}$.

SUPPLEMENT

R scripts for conducting statistical analysis described in the main text ([Ecological Archives C004-015-S1](#)).