



RESEARCH ARTICLE

REVISED Eco-physiological adaptation of dominant tree species at two contrasting karst habitats in southwestern China [version 2; peer review: 2 approved, 1 approved with reservations]

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Abstract

The purpose of this study was to investigate the eco-physiological adaptation of indigenous woody species to their habitats in karst areas of southwestern China. Two contrasting forest habitats were studied: a degraded habitat in Daxiagu and a well-developed habitat in Tianlongshan, and the eco-physiological characteristics of the trees were measured for three growth seasons. Photosynthetic rate (Pn), stomatal conductance (gs), and transpiration rate (Tr) of the tree species in Daxiagu were 2-3 times higher than those in Tianlongshan under ambient conditions. However, this habitat effect was not significant when measurements were taken under controlled conditions. Under controlled conditions, Pn, gs, and Tr of the deciduous species were markedly higher than those for the evergreen species. Habitat had no significant effect on water use efficiency (WUE) or photochemical characteristics of PSII. The stomatal sensitivity of woody species in the degraded habitat was much higher than that in the well-developed habitat. Similarly, the leaf total nitrogen (N) and phosphorus (P) contents expressed on the basis of either dry mass or leaf area were also much higher in Daxiagu than they were in Tianlongshan. The mass-based leaf total N content of deciduous species was much higher than that of evergreen species, while leaf area-based total N and P contents of evergreens were significantly higher than those of deciduous species. The photosynthetic nitrogen- and phosphorus-use efficiencies (PNUE and PPUE) of deciduous species were much higher than those of evergreens. Further, the PPUE of the woody species in Tianlongshan was much higher than that of the woody species in Daxiagu.

Results from three growth seasons imply that the tree species were able to adapt well to their growth habitats. Furthermore, it seems that so-called "temporary drought stress" may not occur, or may not be severe for most woody plants in karst areas of southwestern China.

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Any reports and responses or comments on the article can be found at the end of the article.

Keywords

karst habitat; photosynthesis; chlorophyll fluorescence; stomatal sensitivity;
water potential

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REVISED Amendments from Version 1

We thank all reviewers for their comments and suggestions. In response to Prof. Reto Strasser's suggestions, we have made modifications for some expressions of the JIP test nomenclature used in this article.

- We have added three more parameters in this part. They are maximal trapping flux (TR_0) to the reaction center of PS II, maximal electron transport flux (ET_0) between PS II and PS I, and maximal heat dissipation flux (DI_0) of PSII per the light flux absorbed (ABS) by PS II antenna.
- We have added more information for definition of $PI(abs)$.
- We have added more information for expressions of $\Phi(P_0)$ and $\Phi(D_0)$ about the relationships between energy flux and fluorescence signal. We have changed Ψ as $\Psi(E_0)$ for avoiding of same expression Ψ as leaf water potential used in this article.
- The graphic frames of Figure 1 and 2 have been adjusted for clarity.

See referee reports

Introduction

Karst topography features soluble bedrock, which is usually carbonate rock such as limestone or dolomite¹. Karst topography is characterized by a very slow formation of soil from the carbonate rock, leading to low water retention capacity^{1,2}. China's karst topography is located mainly in the southwestern region, which is also characterized by diversified landscape types and a dense population. Deforestation was once one of the most serious environmental problems in China's karst region, and was mainly due to fuel wood production, agricultural expansion, and livestock husbandry. This land use has caused the ecosystem of the karst region to degrade beyond its already poor condition. As a result, many woodlands have degraded to rocky desert in many of the karst regions in southwest Guizhou Province. Karst ecosystems are very fragile. If destroyed, the soil regeneration process is slow, and thus karst ecosystems are only restored gradually, if at all. Precipitation is sufficient in this region, but the woody plants are supposedly subjected to temporary water stress. This water stress results from low soil water-holding capability, and the high leakiness of limestone rock^{2,3}. Numerous studies have simulated the above environments, and the data generated has shown that plants are suffering from conditions of temporary water-stress^{2,3}. In most of these studies, potted seedlings were used, and the experiments were conducted under controlled environments (e.g. greenhouses). It is risky to extrapolate the situation for mature tree species in their natural conditions from data from potted seedlings under controlled conditions. So far, reports on woody plant growth and physiological response to temporary water deficiency in the karst field areas have been sparse. This is particularly so for mature woody plants. Investigating how woody plants respond functionally to differently degraded habitats will help understand the adaptive mechanisms that these indigenous species have to their habitats. This will also help in optimizing the selection of tree species for forest ecosystem restoration in karst regions.

For our study, we selected two types of forests with contrasting karst habitats in the west and southwest of Guizhou Province: a well-developed secondary deciduous and evergreen broad-leaved mixed

forest at Tianlongshan Mountain, and a severely degraded forest dominated by sparse deciduous woody species at Daxiagu. We made *in situ* measurements of eco-physiological traits for the dominant tree species in three consecutive growth seasons (2007–2009) to address the following questions:

- 1) Compared with tree species in the well-developed forest habitat, are the eco-physiological traits down-regulated for the tree species in the degraded karst habitat?
- 2) Is there a difference in stomatal sensitivity between tree species in the two contrasting forests?
- 3) How do nutrients affect the eco-physiological characteristics of the tree species in the two contrasting forests?

Materials and methods

Study areas

For the purpose of making comparisons of how the tree species adapt to their own different karst habitats, we designed the experiment at two contrasting mature forest habitats. Tianlongshan, located in the west of Guizhou Province, has a relatively well-developed karst secondary forest. Daxiagu, located in the southwest of Guizhou Province, has a severely degraded karst forest.

Tianlongshan is located about 10 km south of Puding County in western Guizhou Province (26°15'N, 105°44'E) at an altitude of about 1200 m. This region is dominated by a humid monsoon climate. The mean annual temperature is 15.1°C⁴. The mean annual precipitation is 1398 mm, and 60–70% of the rainfall events occur during the growth season between May and October⁴. Tianlongshan has a well-developed secondary evergreen and deciduous broad-leaved mixed forest growing in lime yellow soil. Tree heights range from 3–7 m.

Daxiagu is located 20 km southwest of the town of Huajiang in southwestern Guizhou Province (25°42'N, 105°35'E) at an altitude of about 900 m. This area has a warm temperate climate and a mean annual temperature of 18.4°C⁴. The mean annual precipitation is 1100 mm, with 83% of this precipitation occurring during the growth season between May and October. Vegetation in this region is characterized by sparsely distributed secondary deciduous trees and shrubs on bare rocks.

Field investigations and collections of tree leaf samples in these two sites did not require specific permits. For the purposes of our work, collaboration with local (provincial) universities or research institutes was required. The locations of our field investigations are neither privately-owned nor protected lands. The tree species used in our investigations and sampling were not endangered or protected.

The dominant tree species in Tianlongshan and Daxiagu found in this study are listed in [Table 1](#).

In situ photosynthetic gas exchange and chlorophyll *a* fluorescence transient measurements

3–4 trees for each species were randomly (the nearest one every 20–30 m distance) selected from the study sites, and a detached branch from each tree was obtained from the top or middle sunny side (south-facing) of the canopy. Branches were detached using a

Table 1. Characteristics of the tree species examined in this study. For leaf phenology, D represents deciduous and E represents evergreen; For location, T represents Tianlongshan and Dx represents Daxiagu.

Species	Leaf phenology	Location	Measurement year
<i>Platycarya longipes</i>	D	T	2008, 2009
<i>Lithocarpus glabra</i>	E	T	2007, 2008, 2009
<i>Celtis sinensis</i>	D	T, Dx	2008, 2009
<i>Ligustrum lucidum</i>	E	T	2007, 2008, 2009
<i>Quercus aliena</i> var. <i>acutiserrata</i>	D	T	2007, 2008, 2009
<i>Lindera communis</i>	E	T	2007, 2008, 2009
<i>Daphniphyllum oldhami</i>	E	T	2008, 2009
<i>Stachyurus obovatus</i>	E	T	2007, 2008, 2009
<i>Carpinus pubescens</i>	D	T	2007, 2008, 2009
<i>Itea chinensis</i>	E	T	2007, 2008, 2009
<i>Zanthoxylum ovalifolium</i> var. <i>spinifolium</i>	D	T	2009
<i>Ilex chinensis</i>	E	T	2007
<i>Alangium chinense</i>	D	Dx	2008, 2009
<i>Rhus chinensis</i>	D	T, Dx	2008, 2009
<i>Picasma quassioides</i> (D. Don) Benn	D	Dx	2008, 2009
<i>Broussonetia papyrifera</i>	D	Dx	2008, 2009
<i>Mallotus japonicus</i> var. <i>floccosus</i>	D	Dx	2008, 2009
<i>Rhamnella franguloides</i>	D	Dx	2008, 2009
<i>Viburnum chinshanense</i>	D	Dx	2008, 2009
<i>Sapium sebiferum</i>	D	Dx	2007, 2008, 2009
<i>Ficus benguetensis</i>	E	Dx	2007, 2008, 2009
<i>Melia azedarach</i> Linn.	D	Dx	2007
<i>Solanum verbascifolium</i>	D	Dx	2007
<i>Mallotus barbatus</i>	D	Dx	2007
<i>Vernicia fordii</i>	D	Dx	2007
<i>Alchornea davidii</i>	D	Dx	2007
<i>Mallotus philippensis</i>	E	Dx	2007
<i>Flemingia philippinensis</i>	D	Dx	2007

pair of pruning shears mounted on a 5 m pole. The detached branch was immediately immersed in a water-filled bucket. The end of each branch was re-cut twice under water, ensuring continuity of the xylem conduit. Photosynthetic rate (Pn), stomatal conductance (g_s), transpiration rate (Tr), and instantaneous water use efficiency (WUE) were measured with new, fully expanded leaves from the detached branches using a LI-6400 photosynthesis system (LI-COR Inc., Lincoln Nebraska, USA). Measurements were conducted around 8:30–13:00 (Beijing Standard Time). Measurement conditions were set at a PAR (photosynthetically active radiation) level of $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$. Leaf temperature and humidity were at their ambient conditions during the measurement period of June–July 2007 and 2008. During this time, the leaf temperatures were 22–25°C in Tianlongshan and 30–33°C in Daxiagu.

Plant stomatal sensitivity was obtained using Lohammar's hyperbolic function method^{5–7}. We measured g_s -VPD (leaf-air vapor pressure deficiency) curves in July 2009, in which the VPD was manually set from saturated vapor [over 85% of relative humidity (RH)] to the

driest vapor (about 5% of RH). Leaf temperature was held at 30°C, and PAR at $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$. The g_s -VPD curves were fitted using the modified Lohammar's function: $g_s = -m \times \ln D + b$, to estimate stomatal sensitivity (m)⁸. In this equation, g_s and D stand for stomatal conductance and VPD, respectively, and m and b are parameters generated in a least square regression analysis.

Chlorophyll *a* fluorescence transients were measured in the leaves from the branches collected as detailed above for photosynthetic gas exchange measurements using a Handy-PEA portable fluorometer (Hansatech Instruments Ltd., Norfolk, UK). Before measurements were taken, the leaves were darkened for at least 30 min using leaf clips (Hansatech Instruments Ltd., Norfolk, UK). The polyphasic chlorophyll *a* fluorescence transients OJIP [fluorescence levels O: F_0 (50 μs); J: F_j (2 ms); I: F_i (30 ms), and P: $F_p = F_m$ (tF_{max})] were analyzed according to the JIP test procedure^{9,10}. The JIP test procedure has been widely used in studies of eco-physiology and stress physiology¹⁰. Several parameters can be derived from the following fluorescence values: 50 μs (F_0 , step O), 100 μs (F_{100}), 300 μs

(F300), 2 ms (step J), 30 ms (step I), and the maximum (Fm, step P), using Biolyzer software (version 3.0.7.2 and available by contacting Dr. Reto Strasser at Reto.Strasser@unige.ch). According to Strasser *et al.*^{9,10}, the definition of the JIP test parameters used in this study can be summarized as follows: As flux ratios or quantum yields expressed as maximal trapping flux (TRo) to the reaction center of PS II, maximal electron transport flux (ETo) between PS II and PS I, and maximal heat dissipation flux (DIO) of PSII per the light flux absorbed (ABS) by PS II antenna. $\Phi(Po)$, maximum quantum yield of primary photochemistry; $\Phi(Eo)$, quantum yield of electron transport, probability that an absorbed photon will move an electron into electron transport further than Q_A ; $\Phi(Do)$, quantum yield of dissipation; PI(abs), performance index on the basis of light energy absorption, which is responsible for the performance of the electron transport from water to plastoquinone.

$\Phi(Po) = 1 - (Fo/Fm) = Fv/Fm = TRo/ABS$; $Fo = F50$ μ s, minimal fluorescence at O-step (50 μ s); $Fm = FP$, maximal fluorescence at P-step;

$\Phi(Eo) = (Fv/Fm) \times \Psi(Eo)$; $\Psi(Eo) = (1 - V_j)$, $V_j = (F_j - Fo)/(Fm - Fo)$, $F_j = F2$ ms, fluorescence at J-step (2 ms) of O-J-I-P;

$\Phi(Do) = 1 - \Phi(Po) = Fo/Fm = DIO/ABS$;

$PI(abs) = (RC/ABS) \times [\Phi(Po)/(1 - \Phi(Po))] \times [\Psi(Eo)/(1 - \Psi(Eo))]$; RC=reaction center, ABS=absorption flux.

Leaf water potential

The midday leaf water potential (Ψ) was determined for each species at the two habitats using a PSYPRO Water Potential System (Wescor, Inc., USA). The measurements were carried out between 11:00 and 14:00 hours (Beijing Standard Time) in August 2009 on leaves from the same detached branches from which the photosynthesis/fluorescence measurements were taken.

Leaf nutrient assay

Leaf total N content and soil total organic N content (%) were determined using the Kjeldahl acid-digestion method (Kjeltec 2200 Auto Distillation Unit, Foss, Denmark). Leaf total phosphorus (P) content and soil total P content (%) were analyzed colorimetrically (UV-visible spectrophotometer, UV-2550, Shimadzu Corporation, Japan).

Statistical analysis

Before the Analysis of Variance (ANOVA) was carried out, all data was examined graphically for the normality of distribution (probability plots for residual analysis), and the homogeneity of variance (scatter plots) using Data Desk (version 6.01, Data Description, Inc., 1996). After examination using the above methods, all data except leaf water potential satisfied the assumption for ANOVA for normal distribution and homogeneity of variance. The data for leaf water potential were log-transformed, and the transformed data satisfied the normal distribution assumption for ANOVA. The effects of habitat (i.e. the severely degraded karst habitat vs. well-developed secondary forest habitat) and life form (i.e. deciduous vs. evergreen) were tested using the two-way ANOVA procedure in Data Desk.

Results

Photosynthetic gas exchange

When measured under their respective ambient conditions in 2007 and 2008 (i.e. ambient temperature and humidity, and PAR set at 1000 μ mol $m^{-2}s^{-1}$), the photosynthetic rate (Pn) across all investigated tree species was much higher in Daxiagu (around 2–3 times, $P < 0.001$) than in Tianlongshan (Figure 1). The g_s and Tr of the investigated tree species in Daxiagu were also much higher ($P < 0.001$) than those in Tianlongshan (Figure 1). However, when measurements were taken under controlled conditions in 2009 (i.e. PAR was set at 1000 μ mol $m^{-2}s^{-1}$, leaf temperature at 30°C, and VPD at 1 KPa), there was no significant habitat effect on Pn (Figure 1). There was a significant difference in Pn between deciduous and evergreen tree species across all investigated tree species in the two habitats. The Pn of deciduous tree species was significantly higher ($P < 0.01$) than the Pn of evergreen tree species (Figure 1). The g_s and Tr of the deciduous tree species were also significantly higher than the g_s and Tr of evergreen tree species ($P < 0.05$ and 0.01, respectively). The habitat effect on g_s and Tr was not statistically significant (Figure 1). The effects of leaf phenology (deciduous vs. evergreen) and habitat were not significant for instantaneous water use efficiency (WUE) under either ambient or controlled measurement conditions in either habitat.

Photosynthesis data

6 Data Files

<http://dx.doi.org/10.6084/m9.figshare.670620>

Chlorophyll fluorescence

Leaf phenology and habitat had no significant ($P > 0.05$) effect on chlorophyll fluorescence parameters in 2007 (Figure 2). However, the effect of leaf phenology became significant ($P < 0.05$) for maximal PSII efficiency ($\Phi(Po)$), quantum yield of PSII electron transport ($\Phi(Eo)$), quantum yield of dissipation ($\Phi(Do)$), and the comprehensive parameter for assessing plant's vitality: performance index (PI(abs)), for all investigated tree species in 2008 and 2009 (Figure 2). The $\Phi(Po)$, $\Phi(Eo)$, and PI(abs) of the evergreen tree species were significantly higher than those of the deciduous tree species, while the opposite effect of leaf phenology was seen in $\Phi(Do)$.

Chlorophyll fluorescence data

6 Data Files

<http://dx.doi.org/10.6084/m9.figshare.670617>

Stomatal sensitivity, leaf water potential, and specific leaf area

Habitat had a significant effect on stomatal sensitivity (m) ($P < 0.05$), and the stomatal sensitivity (m) was much higher in Daxiagu than in Tianlongshan (Figure 3). There was an interactive effect of habitat and leaf phenology on leaf water potential, and the leaf water potential of deciduous tree species in Daxiagu was significantly lower than the leaf water potential in Tianlongshan (Figure 3). Leaf phenology had a significant effect on the specific leaf area (SLA): the SLA of deciduous species was significantly ($P < 0.05$) higher than that of evergreen species (Figure 3).

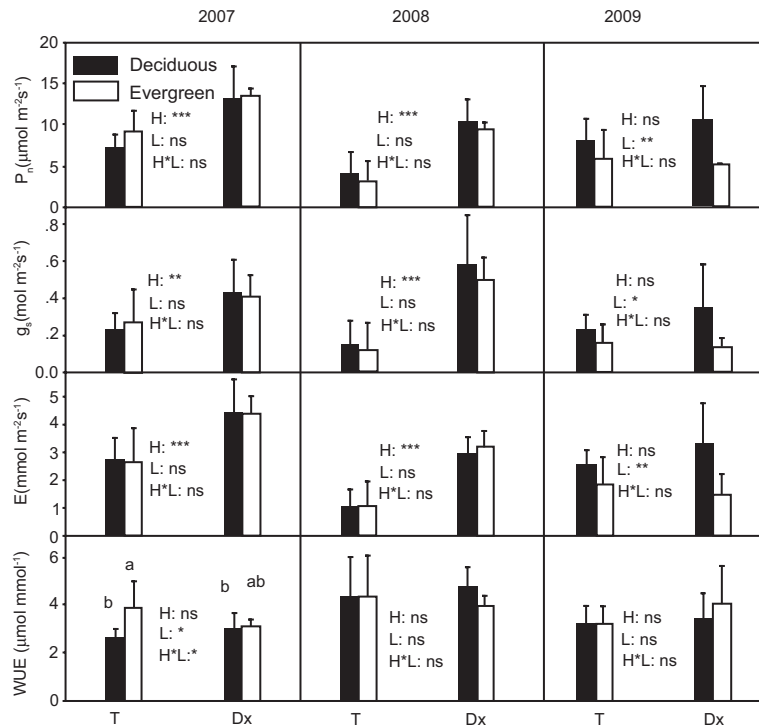


Figure 1. Photosynthetic gas exchange characteristics. Effect of habitat (H), leaf phenology (L), and their interaction (H × L), on P_n , g_s , E , and WUE (mean ± SD, $n = 3-5$) of the tree species in two habitats. T and Dx represent the two study sites Tianlongshan and Daxiagu, respectively. Measurements were conducted under ambient conditions in 2007 and 2008, and under controlled conditions in 2009. The significance levels (*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, and ns = $P > 0.05$) were based on ANOVA results.

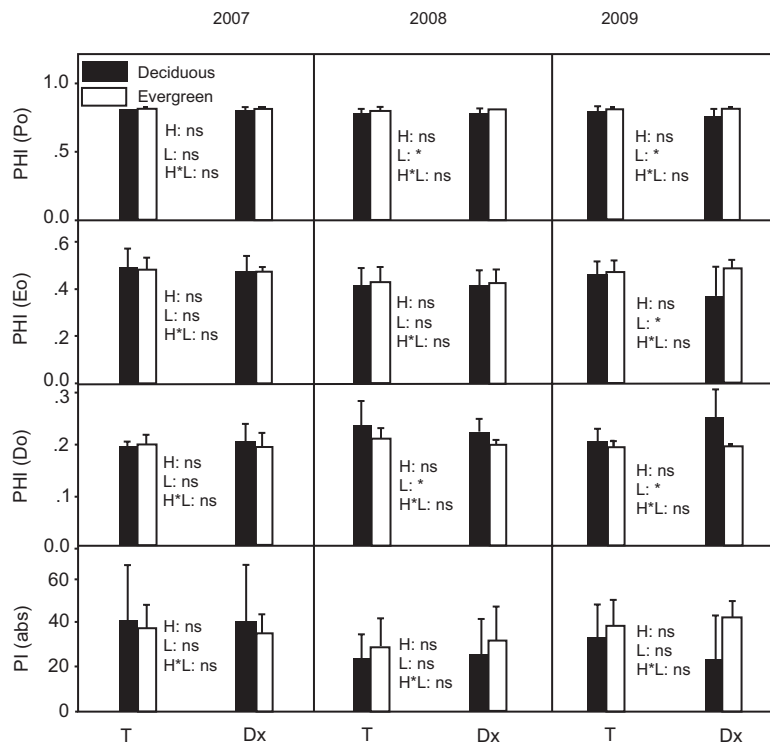


Figure 2. Photochemical characteristics. Effects of habitat (H), leaf phenology (L), and their interaction (H × L), on maximal PSII efficiency ($\Phi(P_o)$), quantum yield of PSII electron transport ($\Phi(E_o)$), quantum yield of dissipation $\Phi(D_o)$, and performance index ($PI(\text{abs})$) (mean ± SD, $n = 3-5$) of the tree species in two habitats. See Figure 1 for other explanations.

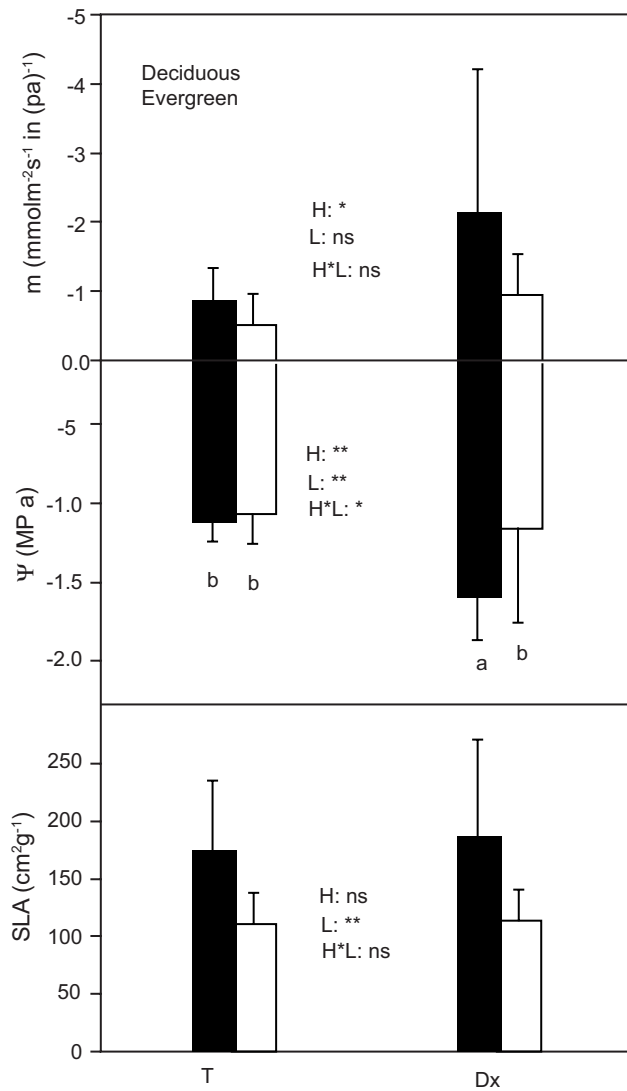


Figure 3. Leaf hydro-physiological and morphological characteristics. Effects of habitat (H), leaf phenology (L), and their interaction (H × L), on stomatal sensitivity (m), leaf water potential (Ψ) and specific leaf area (SLA) (mean \pm SD, n = 3–5) of the tree species in two habitats. The ANOVA for Ψ is based on log-transformed data. See Figure 1 for other explanations.

Leaf nutrients and their efficiencies for photosynthesis

Habitat had significant effects on leaf total N content expressed on the basis of either dry mass or leaf area ($P < 0.01$) (Figure 4). Leaf total nitrogen content in Daxiagu was much higher than leaf total nitrogen content in Tianlongshan. Leaf phenology also had a significant effect on leaf total nitrogen content expressed on the basis of either dry mass or leaf area ($P < 0.01$ and $P < 0.05$, respectively). The mass-based leaf total N content of the deciduous species was much higher than that of the evergreen species. However, when leaf total nitrogen content was expressed on the basis of leaf area, the leaf total N content of the evergreen species was significantly higher than the leaf total N content of the deciduous species. The habitat had a significant effect on leaf total phosphorus content expressed on the basis of either dry mass or leaf area ($P < 0.001$).

The leaf total phosphorus content in Daxiagu was much higher than leaf total phosphorus content in Tianlongshan (Figure 4). However, when leaf total phosphorus content was expressed on basis of leaf area, the values of the evergreen species were much higher than those of deciduous species, especially in Daxiagu. The habitat also had a significant effect on the ratio of leaf total N to P (N:P) for all measured tree species. The N:P ratio in Tianlongshan was significantly ($P < 0.001$) higher than the N:P ratio in Daxiagu (Figure 4). Leaf phenology had a significant ($P < 0.001$) effect on photosynthetic nitrogen use efficiency (PNUE), and the PNUE of deciduous species was much higher than that of evergreen species (Figure 5). Both habitat and leaf phenology had significant ($P < 0.001$) effects on photosynthetic phosphorus use efficiency (PPUE). The PPUE of the woody species in Tianlongshan was much higher than that in Daxiagu ($P < 0.001$), and the PPUE of deciduous species was much higher than that of evergreen species ($P < 0.001$) (Figure 5).

Water potential data

2 Data Files

<http://dx.doi.org/10.6084/m9.figshare.670621>

Leaf nutrient and specific leaf area data

2 Data Files

<http://dx.doi.org/10.6084/m9.figshare.670623>

Discussion

Plants in karst regions are thought to be subject to temporary drought stress in their growth seasons due to the poor water holding capacity of the soil. This is thought to be particularly so in severely degraded karst habitat^{2,3}. However, the data from this study for chlorophyll fluorescence (e.g. $\Phi(Po)$, $\Phi(Eo)$, and $PI(abs)$) in the three consecutive growth seasons suggests that drought stress does not occur in all of the investigated tree species during their growth seasons. The chlorophyll fluorescence technique has been used as a powerful tool to assess plant vitality in response to environmental stresses¹⁰. So-called “temporary drought stress” may not occur at all. Further, if such temporary stress really does occur, then it may not be severe for most woody plants in the karst areas of western and southwestern Guizhou Province. Further, the indigenous/native trees adapt well to their habitats, including plants in the most severely degraded karst habitat (Daxiagu in this study). This might be due to karst plants’ adaptive structural strategies (e.g. absorbing water from deep soil through roots penetrating into rock crevasses, according to Zhu²), or the plants’ adaptive functions (e.g. stomatal sensitivity to changed environments). It may also be due to the ample precipitation found in the studied areas (annually around 1000 mm, mainly occurring during the growth season). Liu *et al.*¹¹ did find that soil water stress (withholding water) affected photosynthesis and growth, and re-watering could remove or alleviate these effects in potted tree seedlings from almost the same karst area as in our study. However, the water stress treatment (withholding water) period in Liu *et al.*’s experiment was as long as 20 days. According to Zhu *et al.*¹², during the growth season, the most common period between two precipitation events in this region is less than 20 days.

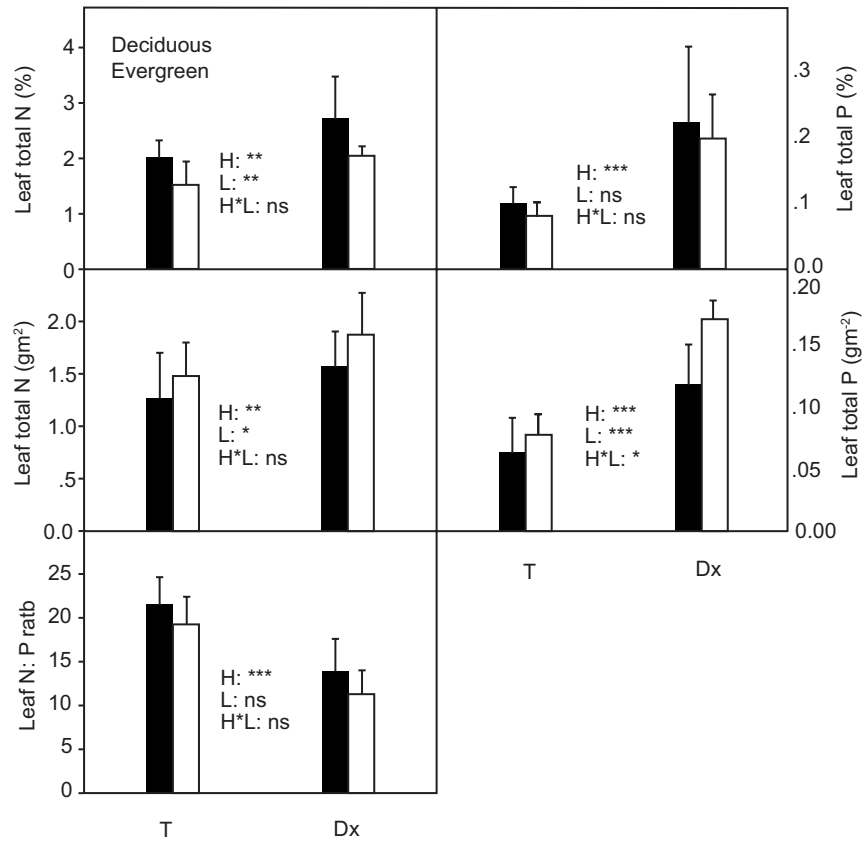


Figure 4. Leaf nutrient status. Effects of habitat (H), leaf phenology (L), and their interaction (H × L), on leaf area based- and mass-based total nitrogen (N) and phosphorus (P) (mean ± SD, n = 3–5) of the tree species in two habitats. See Figure 1 for other explanations.

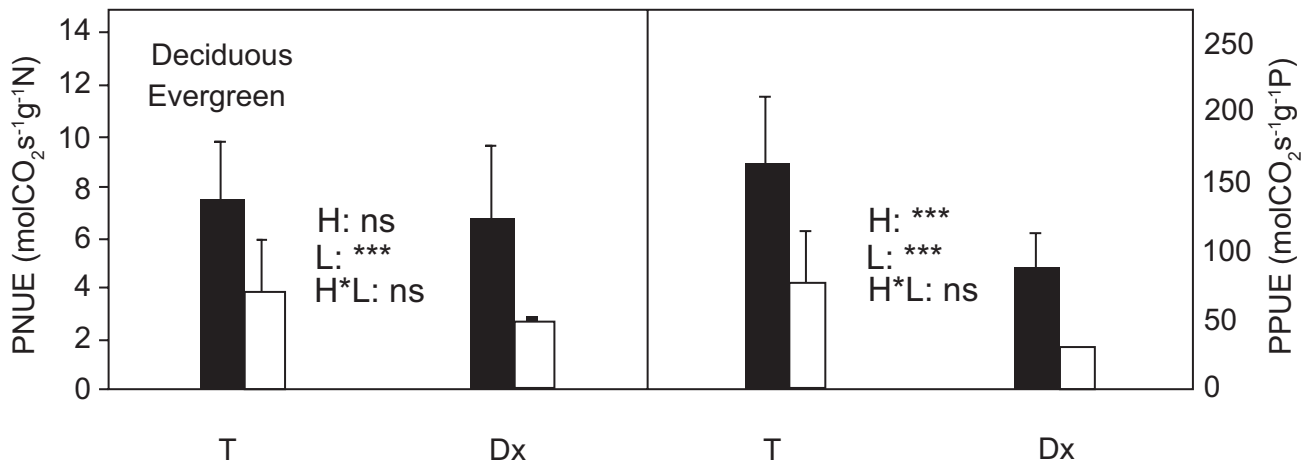


Figure 5. Photosynthetic N and P use efficiency characteristics. Effects of habitat (H), leaf phenology (L), and their interaction (H × L), on photosynthetic N and P use efficiency (PNUE and PPUE, respectively) (mean ± SD, n = 3–5) of the tree species in two habitats. See Figure 1 for other explanations.

Further, the available soil water from a heavy rain event could meet tree transpiration needs for 7–14 days¹³. This implies that in most cases, temporary soil water stress events would not occur during the growth season of this region. The data for leaf $\delta^{13}\text{C}$ value from Yang *et al.*¹⁴ and Fan *et al.*¹⁵ also confirms the above conclusions based on chlorophyll fluorescence analyses. They report that the average leaf $\delta^{13}\text{C}$ values of more than 50 tree species in three karst sites across Guizhou are -27.63%¹⁴, and -28.14%¹⁵. These values are only higher than the values of the tree species of tropic rain forests in Yunnan Province (e.g. -33.11% reported by Qu *et al.*¹⁶), and mostly lower than other areas of China (e.g. -26.24% of temperate forest¹⁷ and -27.00% of desert vegetation¹⁸). This indicates that the average long-term WUE in tree species in the karst area is low, and that they do not experience severe long-term drought stress.

Nevertheless, we did find a difference in photochemical traits of PSII (i.e. $\Phi(\text{Po})$, $\Phi(\text{Eo})$, and $\text{PI}(\text{abs})$) between the deciduous and evergreen tree species in the 2009 growth season. This is consistent with the results of midday leaf water potential (Figure 3), suggesting that the capacity for maintaining leaf hydro-physiological function in evergreen tree species was higher than that in deciduous trees. Fan *et al.*¹⁵ also confirmed that evergreen tree species had higher water stress tolerance for maintaining branch hydraulic conductivity than did deciduous trees species. This is based on data for the branch's hydraulic characteristics for roughly the same tree species in the two habitats. In addition, there was a much lower Ψ_{50} (xylem tensions at 50% of loss in hydraulic conductivity) in evergreen tree species than in deciduous tree species¹⁵.

WUE is an intrinsic trait that indicates plant strategies for environmental adaptation, and is understood in terms of a trade-off between carbon gain and water loss. WUE is also a reliable indicator for determining plant survivorship in arid areas¹⁹. To adapt to changed environments, in comparison with plants with low VPD, plants with high leaf-atmosphere VPD will generally increase their WUE^{20,21}. That there is no significant variation in the effects of habitat and leaf phenology on WUE also partly confirms that plants adapt well to their environments, even to severely degraded karst habitat. Furthermore, our data on photosynthetic gas exchange showed that the photosynthetic rates (P_n) of the tree species in Daxiagu (the severely degraded habitat) are nearly 2–3 times higher than those of the tree species in Tianlongshan (the well developed secondary forest habitat) under ambient conditions. This indicates that to determine the CO_2 assimilation of plants in these areas, it would be more important to take light and temperature into consideration than it would be to consider precipitation. The Daxiagu habitat has much less vegetation coverage than the Tianlongshan habitat. The tree species in Daxiagu are distributed sparsely and most tree canopies receive almost full sunlight. The tree canopies in Tianlongshan are closed and the light environment is lower for the middle and lower canopy leaves. The temperature in Daxiagu in the growth season is clearly higher than in Tianlongshan (with a difference of about 3–5°C). Many studies confirm that leaves exposed to sunlight have higher Rubisco activity, chlorophyll *a/b* ratio, maximum photosynthetic rate, and light saturation points than do shaded-leaves^{22–25}. In other words, when precipitation (water supply for soil) is guaranteed, full sunlight and higher temperatures improve the photosynthetic capacity of the plant.

In China, soil phosphorus deficiency occurs more commonly in the southern region than in the northern region. Furthermore, soil total phosphorus density in tropical and subtropical areas is much lower than in other areas²⁶. Soil phosphorus deficiency has a negative effect on plant absorption of nitrogen from the soil; thus phosphorus limitation inhibits plant growth. According to the stoichiometric relationship between N and P, the biomass N:P ratios could be effective indicators of the status of nitrogen and phosphorus in a plant^{27–29}. The average N:P ratio for terrestrial plant species in their natural field habitats is 12–13^{30–32}. Koerselman and Meuleman²⁸ suggest that phosphorus deficiency occurs when the N:P ratio is higher than the critical value of 16. The much higher leaf N:P ratio in Tianlongshan (mean = 20.32) as compared to Daxiagu (mean = 12.26) suggests that there was severe phosphorus limitation in Tianlongshan. Because phosphorus deficiency has negative effects on nitrogen absorption^{33,34}, phosphorus limitation could partly explain why the P_n was lower in Tianlongshan than in Daxiagu, while PPUE was more stimulated in Tianlongshan than in Daxiagu. There is also a marked difference in tree species composition between the two habitats: more than 90% of the tree species measured in Daxiagu are deciduous, while evergreen tree species account for 50% of the measured tree species in Tianlongshan. A great deal of data has shown that the photosynthetic capacity of deciduous species is higher than the photosynthetic capacity of evergreen species²⁵.

The stomatal sensitivity of woody species in open habitats (i.e. Daxiagu) is much higher than in closed habitats (i.e. Tianlongshan), again suggesting that the light, ambient humidity, and temperature conditions are much more important than precipitation in shaping the stomatal response of woody plants to changed leaf-air VPD. The study of the sensitivity of stomata to changed leaf-air VPD conditions in *Ligustrum sinense* also confirmed that the relationship between stomatal conductance and stomatal aperture for high-light leaves was more significant than that of low-light leaves³⁵. Higher stomatal sensitivity will help maintain the physiologically required water status of a plant³⁵. This could explain the difference in the stomatal sensitivity of the woody species between the two habitats. This could also explain why there was no difference in photosynthetic instantaneous WUE between the two habitats, although there was a significant difference in water consumption through transpiration in the woody species.

Author contributions

SZ and DF conceived and designed the experiment; SZ, DF, QW, HY and XX conducted the field measurements; SZ analyzed the data and wrote the manuscript. DF revised the manuscript.

Competing interests

No relevant competing interests were disclosed.

Grant information

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Jessica Gurevitch

Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, USA

This is a very comprehensive ecophysiological study carried out on a wide range of woody species in two contrasting karst sites over the course of three years. The extent and duration of the study is far greater than most comparable field studies, and the paper provides a great deal of valuable information on the physiological responses of woody species in these sites. The differences found between the degraded and intact karst forest must be considered as hypotheses rather than conclusive demonstration of differences between degraded and intact forest, because of the limitations of the experimental design, as discussed in [my review of the previous version](#). Because of the way the study was set up, a more accurate title would be: Ecophysiological responses of dominant tree species in two karst forest sites in southwestern China. I understand that this paper represents an enormous amount of work; ideally, the experimental design could have been thought through with respect to being able to test the factors most of interest before initiating all of that excellent work. In addition, the study design is not a factorial ANOVA; it is a hierarchical design with a nested structure and thus should have been analyzed as such (thereby changing the F tests and P levels); the reported values for the ANOVA are therefore inaccurate.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 17 Jul 2014

Shouren Zhang, Institute of Botany, the Chinese Academy of Sciences, Beijing, China

We sincerely thank Prof. Jessica Gurevitch for her new comments on our revised version. We would like to accept Prof. Jessica Gurevitch's advice and pay attention on the experimental design in our future work. In response to Prof. Jessica Gurevitch's comments on statistical issues, we will

check our data and calculations, and may update the statistical results later. Regarding the article title, Prof. Jessica Gurevitch suggested to replace eco-physiological adaptation with eco-physiological response. The trees we used for measurements were mature ones which had been naturally grown there more than 20 years old, so the differences in trees' eco-physiological traits between the two types of karst forests could reflect the variations of the trees' adaptation to their local habitats. Anyway, we would like to change our mind in this regard if more convincing reasons are kindly provided.

Again, we thank Prof. Jessica Gurevitch for her new comments and suggestions.

Competing Interests: No competing interests were disclosed.

Reviewer Report 09 December 2013

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Reto Strasser

Professor Emeritus for Bioenergetics, University of Geneva, Geneva, Switzerland

This revised version is in my opinion fully acceptable. The authors have followed the recommendations of the reviewers, and I've no additional comments.

Competing Interests: Whilst not directly connected to this article, Reto Strasser was the original developer of the techniques and equations used in the data analysis software Biolyzer, which was used in this study.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 13 November 2013

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Reto Strasser

Professor Emeritus for Bioenergetics, University of Geneva, Geneva, Switzerland

I read this manuscript several times with great interest. Immediately the reader gets the impression of a carefully conducted and complex investigation. The topic is a case study of ecophysiology as you find it *in vivo, in situ* in nature.

The title alone informs the reader that a complex multiparametric ecological experiment is presented. The areas of this investigation were forests in southwestern China with contrasting habitats, in the Guizhou Province called Tianlongshan and Daxiagu.

Some of the main contrasts are listed and compared below:

- **Altitude above sea level:** 1200m (Tianlongshan) and 900m (Daxiagu)
- **Karst:** Well-developed secondary forest with a humid monsoon climate (Tianlongshan) and severely degraded mixed forest with warm temperatures (Daxiagu)
- **Precipitation:** 1398 mm/year (Tianlongshan) and 1100mm/year (Daxiagu)
- **Rain during growth season:** 60% to 70% (Tianlongshan) and 83% (Daxiagu)
- **Vegetation:** Well developed secondary evergreen and deciduous (Tianlongshan) and secondary deciduous trees and shrubs (Daxiagu)

Branches were cut from both places and used for measurements such as gas exchanges (CO_2), stomatal conductance, transpiration and water use efficiency (WUE), on new fully expanded leaves. Many *in vivo in situ* fast fluorescence transients were collected and analyzed according to the equations of the JIP test, which reveals the bioenergetics constellation of the samples.

It is seldom that a research team is able to use so many quite different techniques on the same plant material within a short time span and outside in the forest. All techniques are precisely presented in the manuscript and the execution of the measurements appears to have been done very carefully.

Several years ago I had the opportunity to accompany a research group of the same institute as a guest for another project, to make such measurements in the forest. I became aware of the high professionalism of experimentation which was invested to get the experimental data recorded. E.g. black socks or sock-shaped towels were used to slip over branches on the trees to guarantee dark adaptation in a non-invasive way during the daytime.

In short, I can say that the investigation is technically very complex, however has been executed with a lot of skill and imagination.

I have seen the comments of the previous two reviewers. I fully agree with the scientific statements given by the reviewers who have pointed out the high quality of the data. For some logically understandable reasons one reviewer has given the article a 'Not approved' rating. Formally that reviewer is right, that some comparisons bypass strict rigor due to the chosen forest constellation which we cannot change. Most probably there is no better place available for performing these experiments in that area. For ecological investigations we are often forced to select a good site, but even so not all parameters may be optimal. Nevertheless the challenge is to make valid deductions of a non-complete or non-perfect data set. My saying is: A Scientist is allowed to do whatever he likes, as long he is saying what he is doing and how he is doing it. Often even incomplete information provides good hints for a better understanding of the topic. With many repetitions of the measurements and the testing of many samples the authors take care regarding the heterogeneity of the plant material, and minimize the risk of making important errors. My impression is that in the given situation the authors are fully aware about the strong and less strong results or conclusions they are giving in the manuscript.

It needs courage, skill and perseverance to do these experiments and to present the experimental data in the form given in this article. It's an excellent investigation, written in a well balanced form, based on solid

data. All these facts together are arguments to conclude that the manuscript deserves to be approved but the authors should take into account some modifications proposed by the reviewers.

For better understanding I'm proposing some minor changes at the end of the materials and methods section:

- The JIP test parameters used in this study can be summarized as follows:
 1. As flux ratios or quantum yields expressed as maximal trapping flux (TR_o) to the reaction center of PS II, or as maximal electron transport flux (ET_o) between PS II and PS I, or as maximal heat dissipation flux (DI_o) of PSII per the light flux absorbed (**ABS**) by PS II antenna.
 2. As partial and cumulated performances (or so called driving forces $DF = \log$ of the performances derived in analogy to the redox potentials.).
- Please make the frame of Figure 1 taller, so that the labeling of the ordinates on the graphs is better readable and not squashed.
- The partial yields or probabilities or performances are based on the functional flux-ratios and can be correlated to the following experimental signals:

$$F_{P_o} = TR_o / ABS = 1 - F_o / F_m = F_v / F_m$$

$y_{E_o} = ET_o / TR_o = (1 - V_J)$ where $V_J = (F_J - F_o) / (F_m - F_o)$ is the relative variable fluorescence at the J-step, and the structural ratio:

$$g_{RC} = Chl_{RC} / Chl_{total} = Chl_{RC} / Chl_{(RC+Antenna)} \text{ or}$$

$$g_{RC} / (1 - g_{RC}) = Chl_{RC} / Chl_{Antenna} = RC / ABS = 1 / \text{Antenna size of PS II}$$

Quantum yield (this means: per absorption ABS) for primary photochemistry:

$$F_{P_o} = TR_o / ABS$$

Quantum yield for electron transport:

$F_{E_o} = F_{P_o} * y_{E_o} = ET_o / ABS$ as the probability that a photon absorbed by PS II will move an electron into the electron transport chain further than Qa^- .

Quantum yield for heat dissipation by PS II:

$F_{D_o} = 1 - F_{P_o} = DI_o / ABS$ (please use y_{E_o} to distinguish it from y , which you are using for the midday leaf water potential.)

The performance index PI_{ABS} , which is responsible for the performance of the electron transport from water to plastoquinone is written as:

$$PI_{ABS} = (g_{RC} / (1 - g_{RC})) * (F_{P_o} / (1 - F_{P_o})) * (y_{E_o} / (1 - y_{E_o}))$$

The multi parametric approach to this realistic field study is appreciated and proves that the authors have a good ecological feeling and understanding.

Competing Interests: Whilst not directly connected to this article, Reto Strasser was the original developer of the techniques and equations used in the data analysis software Biolyzer, which was used in this study.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 26 June 2013

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Jessica Gurevitch

Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, USA

Scientific study of the ecologically interesting karst forests of southwestern China has been limited, particularly in the English-language literature, making this study potentially valuable. The comparison of *in situ* with common environment performance was excellent. However, major design flaws make it impossible to reach scientifically valid conclusions from this study. Most importantly, the study compares one degraded forest with one more-intact forest in order to reach conclusions about the ecophysiology of trees in degraded karst forests in comparison to intact forests. This is not possible with one forest of each type; the fallacy in this kind of comparison has been highlighted in the ecological literature for decades (sometimes called “pseudoreplication”). One cannot reach any valid conclusions from comparing trees in a single forest of one type with those of a single forest of a different type. The anova also fails to account for the nested design structure, a very common problem in the ecological literature.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 09 Jul 2013

Shouren Zhang, Institute of Botany, the Chinese Academy of Sciences, Beijing, China

We appreciate the invaluable comments from Prof. Jessica Gurevitch. We agree with Prof. Gurevitch’s comments on the experimental design in our study. However, at least two factors prevented us from selecting more sites for each forest type. Firstly, field acquisition of physiological parameters, such as the photosynthetic rate and maximum photochemical efficiency of PSII, is very time-consuming. Our ability to make the simultaneous measurements needed to compare different sites is therefore limited by resource constraints. Secondly, since these physiological parameters vary significantly throughout the day, they need to be measured at similar times of day to make sense of the comparison. For example, in this study we measured photosynthetic gas exchange and chlorophyll fluorescence from late-morning to noon, which is thought to be the daily based maximal photosynthetic functional period for the sampled trees. These factors make physiological investigations under controlled conditions on multiple sites unpractical. We note, also, that although we compared only two sites, the trees sampled within one site in our study were distributed over a large area (roughly 1 km²), and therefore could be regarded as growing on

different sub-sites with quite different local environmental situations. Most importantly, however, the physiological data in our study were collected over three consecutive years in field. Such long-term investigations of physiological processes in field are very scarce in eco-physiological literature.

Competing Interests: No competing interests were disclosed.

Reviewer Report 29 May 2013

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Jian Rang Wang

Faculty of Natural Resources Management, Lakehead University, Thunder Bay, Canada

The title is appropriate for the content of the article. The authors used two contrasting habitats (degraded versus well developed) and leaf phenology (deciduous versus evergreen) across many major tree species to address the ecophysiological adaptation of different tree species to habitat change. The research was well designed and relatively long-term (three year measurements) for ecophysiological study. The information presented in the study will provide significant insight to our understanding of tree species adaptation to climate change and habitat degradation. The conclusions from the study will provide useful guidance for biodiversity management and restoration of degraded sites.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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