

Nitrogen allocation, partitioning and use efficiency in three invasive plant species in comparison with their native congeners

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Abstract In this study, we hypothesized that invasive species may allocate a higher fraction of leaf nitrogen (N) to photosynthesis than phylogenetically related native species. To test this hypothesis, we determined N allocation and other ecophysiological traits of three invasive species in comparison with their respective native congeners by measuring response curves of photosynthesis to intercellular CO₂ concentration. The invasive species of *Peperomia* and *Piper* indeed allocated a higher fraction of leaf N to photosynthesis and were more efficient in photosynthetic N (N_P) partitioning than their native congeners. The two invasive species partitioned a higher fraction of N_P to carboxylation and showed a higher use efficiency of N_P , while their native congeners partitioned a higher fraction of N_P to light-harvesting components. The higher N allocation to photosynthesis and the higher N_P partitioning to carboxylation in the two invaders were associated with their higher specific leaf area. Nitrogen allocation and partitioning were the most important factors in explaining the differences in light-saturated photosynthetic rate and photosynthetic N use efficiency (PNUE) between the two invasive species and their native congeners. The differences in

N allocation-related variables between the invasive and native species of *Amaranthus* could not be evaluated in this study due to the method. Except PNUE, resource capture- and use-related traits were not always higher in all three invasive species compared to their native congeners, indicating that different invasive species may have different syndrome of traits associated with its invasiveness.

Keywords Construction cost · Invasiveness · Leaf area ratio · Nitrogen allocation and partitioning · Nitrogen use efficiency · Photosynthesis · Specific leaf area · Trade-off

Abbreviations

C_i	Intercellular CO ₂ concentration
CC	Leaf construction cost
Chl	Leaf chlorophyll content
G_s	Stomatal conductance
J_{max}	Maximum electron transport rate
LAR	Leaf area ratio
LMF	Leaf mass fraction
N_A	Area-based leaf nitrogen content
N_B	Nitrogen content in bioenergetics
N_B/N_P	The fraction of the photosynthetic nitrogen partitioned to bioenergetics
N_C	Nitrogen content in carboxylation
N_C/N_P	The fraction of the photosynthetic nitrogen partitioned to carboxylation

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N_{C+B}	$N_C + N_B$
N_L	Nitrogen content in light-harvesting components
N_L/N_P	The fraction of the photosynthetic nitrogen partitioned to light-harvesting components
N_M	Mass-based leaf nitrogen content
N_P	Nitrogen content in all components of the photosynthetic apparatus
P_B	The fraction of leaf nitrogen allocated to bioenergetics
P_C	The fraction of leaf nitrogen allocated to carboxylation
P_{C+B}	$P_C + P_B$
P_L	The fraction of leaf nitrogen allocated to light-harvesting components
P_{\max}	Light-saturated photosynthetic rate
P_{\max}/N_P	Photosynthetic use efficiency of the photosynthetic nitrogen
P_n	Net photosynthetic rate
P_T	The fraction of leaf nitrogen allocated to all components of the photosynthetic apparatus
PNUE	Photosynthetic nitrogen use efficiency
PPFD	Photosynthetic photon flux density
R_d	Dark respiration rate
SLA	Specific leaf area
$V_{c\max}$	Maximum carboxylation rate

Introduction

Invasive alien species are known to alter species composition, structure and function of invaded ecosystems, and often cause significant environmental damage and huge economic loss worldwide (D'Antonio and Kark 2002). Preventing the introduction of potentially invasive species is the most efficient and economical method of controlling biological invasions. To better predict potentially invasive species before introduction and control existing invasive species, it is necessary to identify the traits that are associated with invasiveness of alien species. However, an universal syndrome of traits that characterize all invasive species appears simplistic (Goodwin et al. 1999; Daehler 2003; Feng et al. 2007a, b); instead different traits may promote invasiveness of different species.

Growth is an important trait for plants because both survival and reproduction depend on plant size and, therefore, on growth rate (Shipley 2006). A higher growth rate may give invasive plants a competitive advantage over native plants due to its pivotal role in capturing available resources (Davis et al. 2000; Grotkopp and Rejmánek 2007). Using the effective approach of comparing multiple species pairs of invasive and phylogenetically related native or noninvasive alien plants, it has been demonstrated that invasive plants have higher growth rate (Grotkopp et al. 2002; Burns 2004, 2006; Grotkopp and Rejmánek 2007).

Specific leaf area (SLA) is strongly and positively correlated with growth rate, especially at low irradiance (Reich et al. 1997; Poorter 1999; Shipley 2006). The higher growth rate of invasive species indicates that they may have higher SLA than native species. Several studies have confirmed this prediction (Pattison et al. 1998; Grotkopp et al. 2002; McDowell 2002; Hamilton et al. 2005; Burns 2006; Grotkopp and Rejmánek 2007). The fraction of cell mass in cell walls decreases with increasing SLA. Cell walls are an important nitrogen (N) sink (Lambers and Poorter 1992), and 5–10% of primary cell wall mass is protein (Loomis 1997). Thus, invasive species with a higher SLA may allocate a lower fraction of leaf N to cell walls, leaving more N for photosynthesis. Compared with low-SLA species, high-SLA species allocate a higher fraction of leaf N to photosynthesis, partition a higher fraction of the photosynthetic N (N in photosynthesis) to bioenergetics (photosynthetic electron transport-related components) and Rubisco, but a lower fraction to light-harvesting components (i.e., more efficient in photosynthetic N partitioning) and have a higher photosynthetic N use efficiency (PNUE) (Poorter and Evans 1998).

In addition, the absence of natural enemies (Mitchell and Power 2003) may favor invasive species to reduce N allocation to defense in introduced range, leading to increased N allocation to photosynthesis as a trade-off between N allocation to photosynthesis and to defense has been documented (Onoda et al. 2004; Goodger et al. 2006). While higher PNUE has been documented for several invasive species (McDowell 2002; Niinemets et al. 2003; Xu et al. 2007), these studies did not measure N allocation and could not give mechanistic

explanations to the higher PNUE. In a recent study, we found that invasive *Buddleja davidii* allocated a higher fraction of leaf N to photosynthesis than native species (Feng et al. 2007c), while in another study, the higher N allocation to photosynthesis was not recorded for invasive species (Feng et al. 2007b). In addition, similar or even lower PNUE (Ewe and Sternberg 2003; Feng et al. 2007b) and SLA (Daehler 2003; Feng et al. 2007a) were also demonstrated for invasive species. Failing to take into account phylogenetic relatedness of invasive and native species while making such comparison may be the reason for the inconsistent results obtained in the above-mentioned studies. Though it is well known that comparisons of sympatric, closely related invasive and native congeners can provide more insight into traits that contribute to invasiveness of a species (McDowell 2002; Burns 2004), this approach has been seldom used in ecophysiological comparisons (but see McDowell 2002).

Thus, in this study, we compared ecophysiological traits of three invasive species with their respective native congeners at partially shady sites. We hypothesized that compared to its native congener each invasive species (1) allocates a higher fraction of leaf N to photosynthesis, (2) is more efficient in photosynthetic N partitioning, and (3) has higher PNUE and SLA. Particular attention was paid to the physiological and ecological consequences of N allocation and partitioning. In fact, this study is probably the first that compares ecophysiological traits of multiple pairs of invasive and native congeners and hence would pave way for more critical evaluation of the role of ecophysiological traits in facilitating alien plant invasion.

Materials and methods

Study area and species

This study was carried out in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (21°56' N, 101°15' E, 600 m above sea level), which is located in the southern part of Yunnan Province, southwest China. Here the mean annual temperature is 21.7°C, with a mean of 25.3°C in the hottest month (July) and 15.6°C in the coolest month (January); the mean annual precipitation is 1,557 mm; the prevalent soil type is Latosol.

We compared the three invasive species, *Peperomia pellucida* (Linn.) Kunth (Piperaceae), *Piper hispidinervium* C. DC. (Piperaceae) and *Amaranthus spinosus* Linn. (Amaranthaceae), with their native congeners, *Peperomia tetraphylla* (Forst. F.) Hook. et Arn, *Piper sarmentosum* Roxb., and *A. lividus* Linn., respectively. Although other alien invasives were present in the area, only the above three invasives have native congeners in the studied area. *Peperomia pellucida* and *A. spinosus* are invasive annual forbs in the subtropics and tropics of many countries (Li and Xie 2002). They invade crop field, orchard, plantation, wasteland and roadside. *Piper hispidinervium*, a perennial shrub, was introduced into China as an aromatic plant in 1979. Now it has been successfully naturalized and has formed dense thickets in some habitats, impacting local biodiversity. All three invasive species are native to tropical America and propagate through seeds. In addition, *Peperomia pellucida* can propagate asexually, and *Piper hispidinervium* can regenerate through new sprouts. The three native species are widely distributed in China, and *Peperomia tetraphylla* is a perennial forb, *A. lividus* an annual forb, and *Piper sarmentosum* a perennial subshrub.

Irradiance influences plant growth, morphology, biomass allocation, and physiology, including N allocation (Feng et al. 2007a, b). Thus, we compared each invasive and native congeneric species pair at same irradiance to exclude the confusing effects of irradiance. It was not necessary to choose all six studied species of the three genera at the same irradiance because we did not compare the differences among different genera. In fact, it was impossible to select the six species at the same irradiance. Soil conditions and plant size were also similar for the invasive and native species of each genus. In October 2006, 11 individuals for *Peperomia pellucida* and only 6 for *Peperomia tetraphylla* were chosen at 25% irradiance (75% shade) in a *Murraya exotica*-dominated community; 11 seedlings for both *Piper hispidinervium* and *Piper sarmentosum* were chosen at 20% irradiance in a rubber tree plantation; 10 individuals for *A. spinosus* and 11 for *A. lividus* were chosen at 40% irradiance in a grapefruit plantation. Light intensity above each sample individual and in an open site was simultaneously recorded in cloudy mornings with quantum sensors and Li-1400 (Li-Cor, Lincoln, NE). Relative irradiance above each sample

individual was calculated as the ratio of the light intensity above it to that in the open site.

Measurements

Photosynthetic response to intercellular CO₂ concentration (C_i) was determined on fully expanded leaves of the field-grown plants with a Li-6400 Portable Photosynthesis System (Li-Cor, Lincoln, NE). Under saturated photosynthetic photon flux density (PPFD) determined by preliminary experiments, net photosynthetic rate (P_n) was measured at 380, 300, 260, 220, 180, 140, 110, 80, 50 and 0 $\mu\text{mol mol}^{-1} \text{CO}_2$ in the reference chamber. Relative humidity of the air in the leaf chamber was controlled at 55% and leaf temperature at 25°C. Stomatal conductance (G_s), C_i and P_n were recorded when the sample leaf was balanced for 200 s under each CO₂ step. Photosynthesis was measured at 380 $\mu\text{mol mol}^{-1} \text{CO}_2$, and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD was P_{max} . Afterwards, light- and CO₂-saturated photosynthetic rate (P_{max}') was detected after 500 s under 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and 1,500 $\mu\text{mol mol}^{-1} \text{CO}_2$. Prior to measurement, samples were illuminated with saturated PPFD provided by the LED light source of the equipment for 10–30 min to achieve full photosynthetic induction. No photoinhibition occurred during the measurements.

The aboveground parts of each sample plant were harvested after photosynthesis measurement. Leaf area was determined with SHY-150 leaf area meter (Harbin Optical Instrument Factory, Harbin, China). Plant parts were oven-dried for 48 h at 60°C. Leaf mass fraction (LMF) and leaf area ratio (LAR) were calculated as the ratios of leaf mass and area to total aboveground mass, respectively.

The P_n - C_i curve was fitted with a linear equation ($P_n = k C_i + i$) within 50–200 $\mu\text{mol mol}^{-1} C_i$. Maximum carboxylation rate (V_{cmax}) and dark respiration rate (R_d) were calculated according to Farquhar and Sharkey (1982) as follows:

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

$$R_d = V_{\text{cmax}}(C_i - \Gamma^*) / [C_i + K_c(1 + O/K_o)] - (k C_i + i) \quad (2)$$

where K_c and K_o were the Michaelis-Menten constants of Rubisco for carboxylation and oxidation, respectively; Γ^* was CO₂ compensation point; O was

the intercellular oxygen concentration, close to 210 mmol mol^{-1} . The values of K_c , K_o and Γ^* were temperature dependent (Bernacchi et al. 2001). Adequate linear correlation between P_n - C_i was not obtained for *A. spinosus* and *A. lividus* (C_4 plants) in this study. Thus, P_n - C_i curve-related variables were not calculated for them.

Maximum electron transport rate (J_{max}) was calculated according to Loustau et al. (1999) as:

$$J_{\text{max}} = [4(P_{\text{max}}' + R_d)(C_i + 2\Gamma^*)] / (C_i - \Gamma^*) \quad (3)$$

Leaf discs with a definite area were taken from each sample leaf, oven-dried at 60°C for 48 h. The ratio of leaf area to mass is SLA. Leaf N and carbon contents were determined with Kjeldahl (BUCHI Auto Kjeldahl Unit K-370, BUCHI Labortechnik AG, Schweiz) and H₂SO₄/K₂Cr₂O₇ oxidization-FeSO₄ titration methods, respectively. The measurements were taken at Biogeochemistry Laboratory of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. Leaf construction cost (CC) was calculated according to McDowell (2002). Leaf chlorophyll (Chl) was extracted with 80% acetone and was determined with spectrophotometric method (Lichtenthaler and Wellburn 1983). The same leaf of each sample plant was used if possible for measurements of photosynthesis, SLA, Chl, the contents of carbon and N (N_A). In this way differences among leaves of the same plant could be avoided when the relationships among variables were analyzed.

The fractions of the total leaf N allocated to carboxylation (P_C , g g^{-1}), bioenergetics (P_B , g g^{-1}) and light-harvesting components (P_L , g g^{-1}) of the photosynthetic apparatus were calculated as:

$$P_C = V_{\text{cmax}} / (6.25 V_{\text{cr}} N_A) \quad (4)$$

$$P_B = J_{\text{max}} / (8.06 J_{\text{mc}} N_A) \quad (5)$$

$$P_L = C_C / (N_M C_B) \quad (6)$$

where C_C was leaf chlorophyll concentration, N_M was mass-based leaf N content. V_{cr} , J_{mc} and C_B were constants (Niinemets and Tenhunen 1997).

The fractions of leaf N allocated to both carboxylation and bioenergetics (P_{C+B} , g g^{-1}) and to all components of the photosynthetic apparatus (P_T , g g^{-1}) were calculated as the sum of P_C and P_B and the sum of P_C , P_B and P_L , respectively. Nitrogen contents in carboxylation (N_C), bioenergetics (N_B), carboxylation and bioenergetics (N_{C+B}),

light-harvesting components (N_L) and all components of the photosynthetic apparatus (N_P) were calculated as the products of N_A and P_C , P_B , P_{C+B} , P_L and P_T , respectively. The fractions of the photosynthetic N partitioned to carboxylation, bioenergetics and light-harvesting components were indicated by N_C/N_P , N_B/N_P and N_L/N_P , respectively. Photosynthetic use

efficiency of the photosynthetic N was indicated by P_{max}/N_P .

Statistical analyses

The differences between each invasive species and its native congener in the variables presented in Table 1

Table 1 The differences between the invasive and native congeners of the three species pairs in ecophysiological traits (mean \pm SE) according to the independent-samples *t* test

Variables	<i>Peperomia</i>		<i>Piper</i>		<i>Amaranthus</i>	
	<i>P. pellucida</i> (<i>n</i> = 11)	<i>P. tetraphylla</i> (<i>n</i> = 6)	<i>P. hispidinervium</i> (<i>n</i> = 11)	<i>P. sarmentosum</i> (<i>n</i> = 11)	<i>A. spinosus</i> (<i>n</i> = 10)	<i>A. lividus</i> (<i>n</i> = 11)
LMF	0.442 \pm 0.033	0.510 \pm 0.034	0.464 \pm 0.044	0.500 \pm 0.025	0.413 \pm 0.035	0.360 \pm 0.016
LAR	324.9 \pm 24.2***	156.4 \pm 10.3	215.7 \pm 20.6*	160.3 \pm 8.1	127.5 \pm 10.8	125.1 \pm 5.6
CC	8.93 \pm 0.44***	33.47 \pm 3.37	15.53 \pm 0.99***	26.16 \pm 2.26	21.85 \pm 1.64	18.44 \pm 0.87
SLA	735.2 \pm 25.6***	306.9 \pm 21.2	465.1 \pm 20.2***	320.4 \pm 20.9	308.6 \pm 13.4*	347.4 \pm 11.9
P_{max}	7.20 \pm 0.88**	2.09 \pm 0.23	7.55 \pm 0.94	6.068 \pm 0.76	36.13 \pm 2.07*	29.49 \pm 2.34
G_s	0.112 \pm 0.02**	0.036 \pm 0.006	0.258 \pm 0.021*	0.166 \pm 0.029	0.538 \pm 0.039	0.479 \pm 0.048
C_i	245.6 \pm 11.2	279.2 \pm 10.7	301.6 \pm 7.2*	280.3 \pm 5.8	192.9 \pm 8.5	201.6 \pm 5.8
N_M	29.78 \pm 0.11	26.31 \pm 0.14	33.03 \pm 0.09	32.88 \pm 0.08	45.14 \pm 0.20	49.50 \pm 0.12
N_A	0.407 \pm 0.015***	0.862 \pm 0.020	0.724 \pm 0.038***	1.064 \pm 0.067	1.472 \pm 0.060	1.447 \pm 0.072
PNUE	17.52 \pm 1.95***	2.41 \pm 0.23	10.20 \pm 1.01**	5.68 \pm 0.54	24.50 \pm 0.91*	20.36 \pm 1.34
P_C	0.623 \pm 0.067***	0.125 \pm 0.024	0.426 \pm 0.056**	0.233 \pm 0.014	No data	No data
P_B	0.107 \pm 0.009***	0.020 \pm 0.002	0.054 \pm 0.004*	0.042 \pm 0.002	No data	No data
P_{C+B}	0.690 \pm 0.077***	0.146 \pm 0.026	0.480 \pm 0.059**	0.275 \pm 0.016	No data	No data
P_L	0.196 \pm 0.014	0.243 \pm 0.015	0.228 \pm 0.015	0.244 \pm 0.018	No data	No data
P_T	0.872 \pm 0.079***	0.388 \pm 0.032	0.708 \pm 0.050**	0.519 \pm 0.021	No data	No data
N_C	0.261 \pm 0.034**	0.110 \pm 0.023	0.320 \pm 0.051	0.248 \pm 0.221	No data	No data
N_B	0.044 \pm 0.004***	0.018 \pm 0.002	0.039 \pm 0.004	0.044 \pm 0.003	No data	No data
N_{C+B}	0.302 \pm 0.039**	0.127 \pm 0.025	0.359 \pm 0.055	0.292 \pm 0.025	No data	No data
N_L	0.078 \pm 0.005***	0.209 \pm 0.013	0.161 \pm 0.006***	0.249 \pm 0.010	No data	No data
N_P	0.360 \pm 0.040	0.336 \pm 0.031	0.520 \pm 0.053	0.541 \pm 0.022	No data	No data
N_C/N_P	0.660 \pm 0.024***	0.312 \pm 0.036	0.579 \pm 0.038*	0.451 \pm 0.024	No data	No data
N_B/N_P	0.110 \pm 0.004***	0.052 \pm 0.002	0.076 \pm 0.004	0.081 \pm 0.004	No data	No data
N_L/N_P	0.230 \pm 0.025***	0.636 \pm 0.038	0.346 \pm 0.038*	0.468 \pm 0.027	No data	No data
P_{max}/N_P	16.62 \pm 1.10***	6.19 \pm 0.19	14.22 \pm 0.73*	10.98 \pm 1.03	No data	No data

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

LMF, leaf mass fraction in $g\ g^{-1}$; LAR, leaf area ratio in $cm^2\ g^{-1}$; CC, leaf construction cost in $g\ glucose\ m^{-2}$; SLA, specific leaf area in $cm^2\ g^{-1}$; P_{max} , light-saturated photosynthetic rate in $\mu mol\ m^{-2}\ s^{-1}$; G_s , stomatal conductance in $mol\ m^{-2}\ s^{-1}$; C_i , intercellular CO_2 concentration in $\mu mol\ mol^{-1}$; N_M , mass-based leaf nitrogen content in $mg\ g^{-1}$; N_A , area-based leaf nitrogen content in $g\ m^{-2}$; PNUE, photosynthetic nitrogen use efficiency in $\mu mol\ g^{-1}\ s^{-1}$; P_C , the fraction of leaf nitrogen allocated to carboxylation in $g\ g^{-1}$; P_B , the fraction of leaf nitrogen allocated to bioenergetics in $g\ g^{-1}$; P_{C+B} , $P_C + P_B$; P_L , the fraction of leaf nitrogen allocated to light-harvesting components in $g\ g^{-1}$; P_T , the fraction of leaf nitrogen allocated to total photosynthetic apparatus in $g\ g^{-1}$; N_C , nitrogen content in carboxylation in $g\ m^{-2}$; N_B , nitrogen content in bioenergetics in $g\ m^{-2}$; N_{C+B} , $N_C + N_B$; N_L , nitrogen content in light-harvesting components in $g\ m^{-2}$; N_P , nitrogen content in total photosynthetic apparatus in $g\ m^{-2}$; N_C/N_P , N_B/N_P and N_L/N_P , the fractions of the photosynthetic nitrogen partitioned to carboxylation, bioenergetics and light-harvesting components in $g\ g^{-1}$, respectively; P_{max}/N_P , photosynthetic use efficiency of the photosynthetic nitrogen in $\mu mol\ g\ s^{-1}$

were analyzed with independent-samples t test. A one-way ANCOVA was used to determine the difference between each invader and its native congener in the correlation between each pair of the variables presented in Figs. 1–6. If the above difference was not significant, the invasive and native species of the same genus were pooled together, and the difference between genera was determined further. If the difference between congeners was significant, the difference between the invasive or native species of different genera was determined further. If the difference between the invasive or native species of different genera was not significant, the invasive or native species of different genera were pooled together, and the difference between the pooled invasive and the pooled native species was determined further. All the analyses were carried out using SPSS 13.0 (SPSS Inc., Chicago, IL).

Results

The three studied invasive species had significantly higher PNUE than their native congeners, respectively (Table 1). Compared to their native congeners, *Peperomia pellucida* and *Piper hispidinervium* exhibited significantly higher G_s , P_C , P_B , P_{C+B} , P_T , N_C/N_P , P_{max}/N_P , SLA and LAR, but lower N_A , N_L , N_L/N_P and CC. However, N_M , P_L , N_P and LMF were not significantly different between the two invasive species and their native congeners. In comparison with its native congener, *Peperomia pellucida* showed significantly higher values for N_C , N_B , N_{C+B}

and N_B/N_P . Only *Piper hispidinervium* was significantly higher in C_i than its native congener. P_{max} was significantly higher in *Peperomia pellucida* and *A. spinosus* than their native congeners, whereas it was not significantly different between the invasive and native species of *Piper*. The invasive and native species of *Amaranthus* were not significantly different in G_s , C_i , N_M , N_A , CC, LMF and LAR, while *A. spinosus* had lower SLA than *A. lividus*.

With increase of N_A , P_{max} increased significantly for the pooled invasive and the pooled native species (Fig. 1a), although only half of the studied species showed significantly positive correlation between P_{max} and N_A ($F = 9.348$, $P = 0.014$ for *Piper hispidinervium*; $F = 11.545$, $P = 0.009$ for *A. spinosus*; $F = 6.474$, $P = 0.031$ for *A. lividus*). At the same value of N_A , the invasive species showed higher P_{max} than the native species as revealed by ANCOVA ($F = 43.755$, $P < 0.000$), and it is consistent with the results obtained after employing separate ANCOVA for each genus ($F = 8.454$, $P = 0.011$ for *Peperomia*; $F = 11.296$, $P = 0.003$ for *Piper*; $F = 6.724$, $P = 0.018$ for *Amaranthus*). With the increase of G_s , P_{max} showed a similar increasing pattern for all the studied species (Fig. 1b). None of the studied species showed significant correlation between P_{max} and C_i (data not shown). For the pooled data, however, P_{max} decreased significantly with increase of C_i (Fig. 1c).

Only two of the studied species showed significantly positive correlation between PNUE and N_M ($F = 6.678$, $P = 0.029$ for *Peperomia pellucida*; $F = 5.680$, $P = 0.041$ for *Piper sarmentosum*), and none of the species showed significant correlation between PNUE

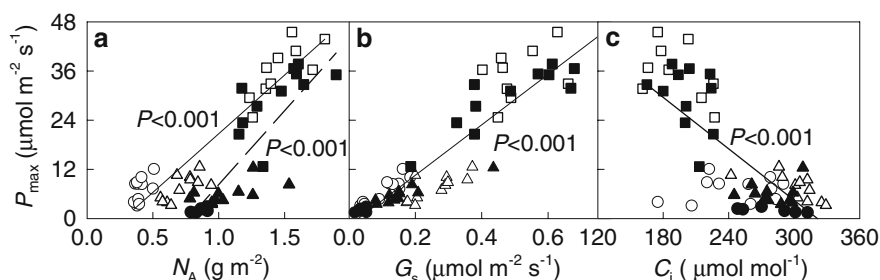


Fig. 1 Light-saturated photosynthetic rate (P_{max}) as a function of (a) leaf nitrogen content (N_A), (b) stomatal conductance (G_s) and (c) intercellular CO_2 concentration (C_i) in invasive *Peperomia pellucida* (open circles), *Piper hispidinervium* (open triangles), *Amaranthus spinosus* (open squares), and native *Peperomia tetraphylla* (filled circles), *Piper*

sarmentosum (filled triangles), *A. lividus* (filled squares). Lines fitted for the invaders (solid line) and for the natives (dash line) were given, respectively, if the difference between them was significant according to the result of ANCOVA. Otherwise, only one line fitted for all six studied species was given

and N_A (data not shown). However, PNUE increased significantly with an increase of N_M or N_A for the pooled invasive and the pooled native species (Fig. 2a, b), and the invasive species were higher in PNUE than the native species as indicated by ANCOVAs ($F = 31.927, P < 0.000$ for PNUE- N_M ; $F = 31.912, P < 0.000$ for PNUE- N_A). With an increase of P_{max} , PNUE increased significantly for the pooled invasive and the pooled native species (Fig. 2c). At the same value of P_{max} , the invasive species were significantly higher in PNUE than the

native species as revealed by ANCOVA ($F = 27.528, P < 0.000$), and the same is consistent with the results of separate ANCOVAs for *Peperomia* ($F = 10.937, P = 0.005$), *Piper* ($F = 34.191, P < 0.000$), but not for *Amaranthus* ($F = 1.510, P = 0.235$).

With increase in P_C, P_B and P_{C+B}, P_{max} increased significantly (Fig. 3a–c) and was significantly higher in *Piper* than in *Peperomia* (ANCOVAs, $F = 28.846, P < 0.000$ for P_{max} - P_C ; $F = 22.386, P < 0.000$ for P_{max} - P_B ; $F = 27.379, P < 0.000$ for P_{max} - P_{C+B}).

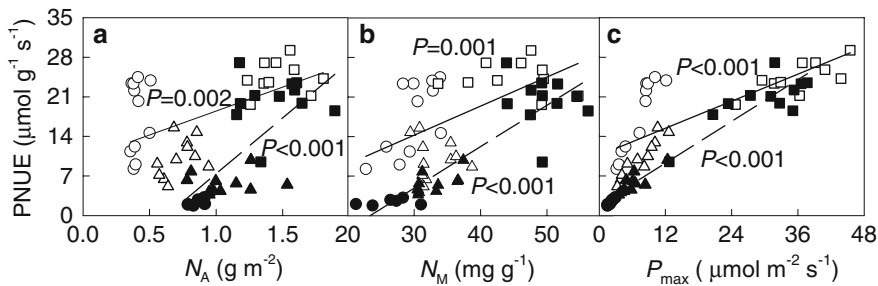


Fig. 2 Photosynthetic nitrogen use efficiency (PNUE) as a function of (a) area-based leaf nitrogen content (N_A), (b) mass-based leaf nitrogen content (N_M) and (c) light-saturated

photosynthetic rate (P_{max}) in the three species pairs of the invasive and native congeners. Species symbols and fitted lines as in Fig. 1

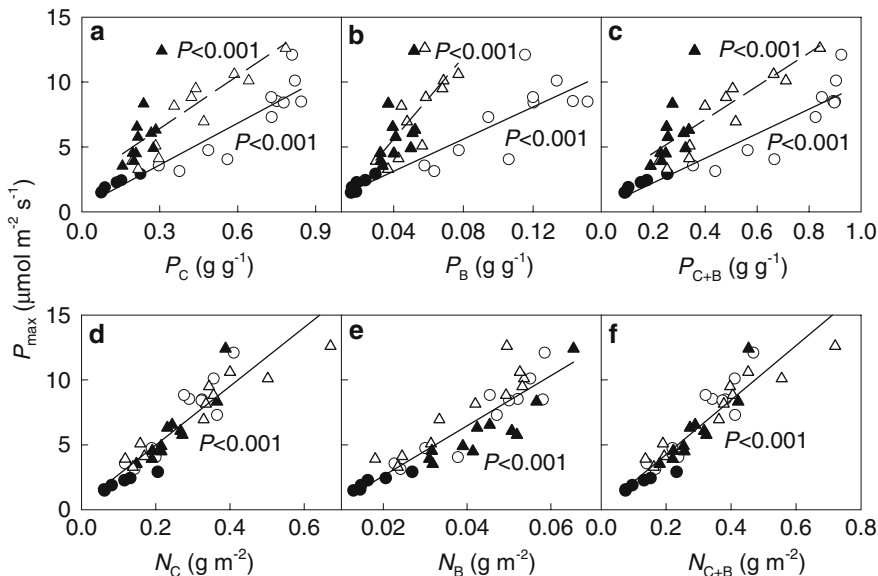


Fig. 3 Light-saturated photosynthetic rate (P_{max}) as a function of the fractions of leaf nitrogen allocated (a) to carboxylation (P_C), (b) to bioenergetics (P_B), (c) to both P_C and P_B (P_{C+B}), nitrogen contents (d) in carboxylation (N_C), (e) in bioenergetics (N_B) and (f) in both N_C and N_B (N_{C+B}) in the two species pairs of invasive and native congeners. Species symbols as in Fig. 1.

Lines fitted for *Peperomia* (solid line) and for *Piper* (dashed line) were given, respectively, if the difference between the two genera was significant according to the result of ANCOVA. Otherwise, only one line fitted for all four species was given. Photosynthesis— CO_2 curve-related variables were not available for *Amaranthus* (C_4 plants) in this study

The higher P_{\max} of *Piper* was associated with its higher N_A , which helped to increase N_C , N_B and N_{C+B} . With increase in N_C , N_B and N_{C+B} , P_{\max} showed a similar increasing pattern for the four species (Fig. 3d–f).

With increase in P_C , P_B , P_{C+B} , N_C , N_B and N_{C+B} , PNUE increased significantly for all species (Fig. 4). At the same value of N_C , N_B or N_{C+B} , PNUE was higher in *Peperomia pellucida* and *Piper hispidinervium* than in their native congeners, which was related to their lower N_A . With an increase in SLA, P_C , P_B and PNUE increased significantly in the four invasive and native species of *Peperomia* and *Piper* (Fig. 5). With increase in SLA, CC decreased exponentially (Fig. 6). The invasive and native species of each genus did not differ significantly in the relationship, while the three genera differed significantly.

Discussion

Nitrogen allocation and partitioning

The higher P_T , P_C and P_B of invasive *Peperomia pellucida* and *Piper hispidinervium* were related to

their higher SLA compared to their respective native congeners (Fig. 5a, b). Leaf mass per area (1/SLA) is positively correlated with cell wall mass and the fraction of leaf N in cell walls, but negatively correlated with the fraction of leaf N in Rubisco (Onoda et al. 2004; Takashima et al. 2004). Cell walls contain different kinds of proteins, including hydroxyproline-rich glycoproteins, proline-rich proteins, glycine-rich proteins and arabinogalactan proteins. Wall mass generally constitutes 50% of total cell mass, and about 5–10% of the primary cell wall mass is protein (Loomis 1997). Thus, the higher SLA of the two invasive species contributed to their higher N allocation to photosynthesis by reducing N allocation to cell walls at the expense of reducing leaf toughness and therefore physical defence (Onoda et al. 2004).

The two invasive species may have already possessed the above N allocation pattern before introduction. This view is partially supported by the similar SLA of the introduced and native populations of other invasive species (DeWalt et al. 2004; Güsewell et al. 2006). Alternatively, the two invaders might acquire the trait by evolution after introduction. In fact, the evolution of increased competitive ability

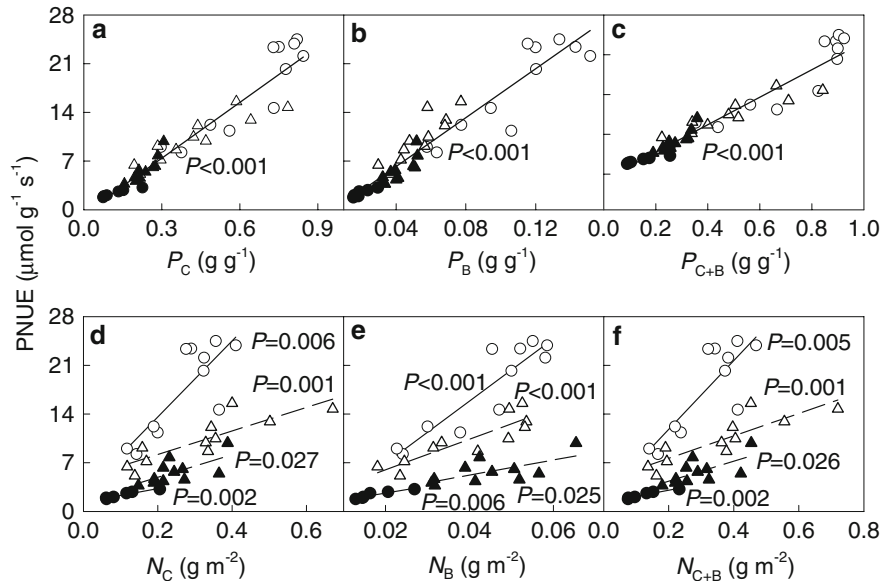


Fig. 4 Photosynthetic nitrogen use efficiency (PNUE) as a function of the fractions of leaf nitrogen allocated (a) to carboxylation (P_C), (b) to bioenergetics (P_B), (c) to both P_C and P_B (P_{C+B}), nitrogen contents (d) in carboxylation (N_C), (e) in bioenergetics (N_B) and (f) in both N_C and N_B (N_{C+B}) in the two species pairs of the invasive and native congeners. Species symbols as in Fig. 1. Line fitted for each species was

given, respectively (solid line, *Peperomia*; dashed line, *Piper*), if the difference between species was significant according to the result of ANCOVA. Otherwise, only one line fitted for all four species was given. Photosynthesis— CO_2 curve-related variables were not available for *Amaranthus* (C_4 plants) in this study

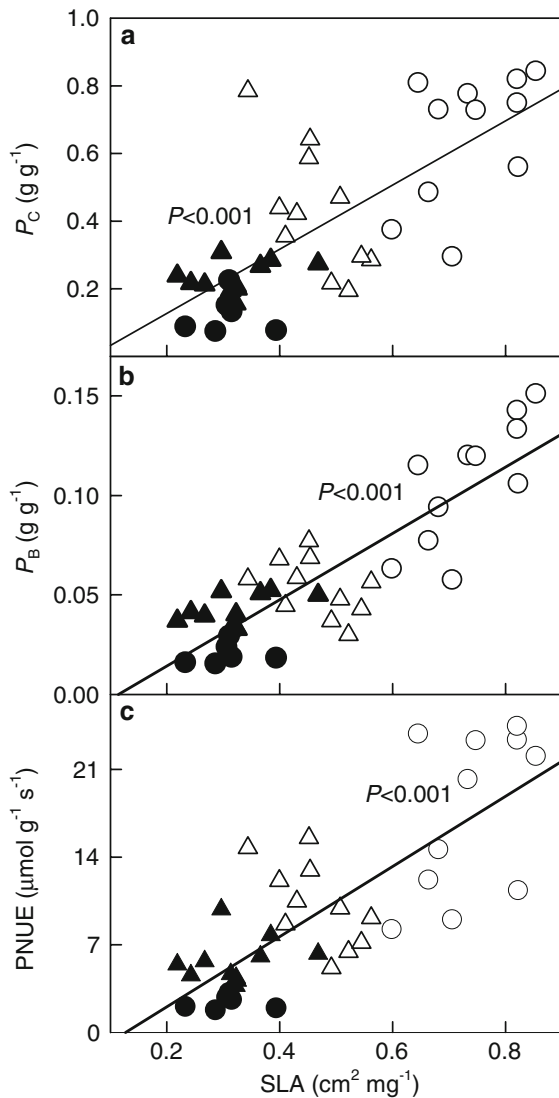


Fig. 5 The fractions of leaf nitrogen allocated (a) to carboxylation (P_C) and (b) to bioenergetics (P_B), and (c) photosynthetic nitrogen use efficiency (PNUE) as a function of specific leaf area (SLA) in the two species pairs of the invasive and native congeners. Species symbols as in Fig. 1. Only one line fitted for all four species was given as the difference between species was not significant. Photosynthesis— CO_2 curve-related variables were not available for *Amaranthus* (C_4 plants) in this study

hypothesis predicts that the resources needed by a species to defend against natural enemies (specialists) in its native range are released for its use in growth and development in the introduced range because the species is able to escape its specialist enemies in the invaded range (Blossey and Nötzold

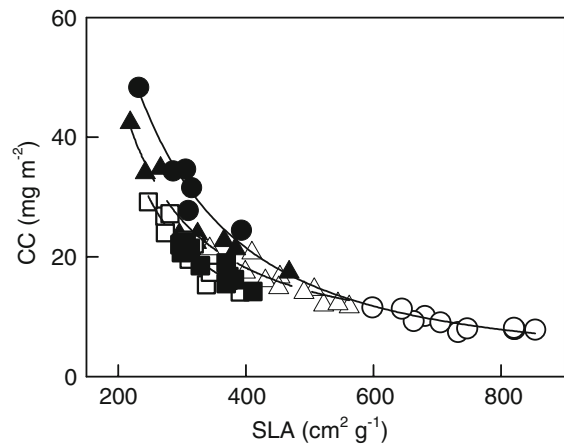


Fig. 6 Leaf construction cost (CC) as a function of specific leaf area (SLA) in the three species pairs of the invasive and native congeners. Species symbols as in Fig. 1. Line fitted for each genus was given ($P < 0.001$), respectively

1995). Particularly if the defense-related chemicals are N-based compounds, such as alkaloids or cyanogenic glycosides (Goodger et al. 2007), natural selection would favor the plant investing the liberated N into photosynthesis. Goodger et al. (2006) found that the accumulation of cyanogenic glycosides is associated with a reduction in the net assimilation rate (NAR), which is positively correlated with P_{\max} (Feng et al. 2007a), and P_{\max} is positively correlated with N allocation to photosynthesis (Fig. 3). Therefore, invasive species may increase N allocation to photosynthesis by reducing accumulation of N-based defensive chemicals due to evolutionary responses in the absence of natural enemies. The hypothesis of the evolution of N allocation is partially supported by the results of Maron et al. (2004) and Wolfe et al. (2004) who reported that invasive species indeed reduced the contents of defensive chemicals and increase susceptibility to natural enemies. To test this hypothesis further, it is necessary to compare the differences in N allocation between populations from introduced and native ranges.

Physiological consequences of N allocation and partitioning

The invasive and native species showed different photosynthetic responses to leaf N (Fig. 1a), which is consistent with the results from other invasive and native species (McDowell 2002; Niinemets et al.

2003; Feng et al. 2007c) and those from different native species (Hikosaka and Terashima 1995; Wright et al. 2001; Onoda et al. 2004; Takashima et al. 2004). At the same value of N_A , the higher P_{\max} of *Peperomia pellucida* and *Piper hispidinervium* in comparison with their native congeners was related to their higher P_C , P_B and P_{C+B} (Fig. 3a–c), which contributed to increasing N_C , N_B and N_{C+B} . The higher P_C , P_B and P_{C+B} of the two invasive species also contributed to their similar or even higher P_{\max} with lower N_A (Table 1). The results indicated that P_C , P_B and P_{C+B} are more important than N_A in explaining interspecific differences in photosynthesis. N_C , N_B and N_{C+B} influenced P_{\max} by influencing the maximum carboxylation rate and maximum electron transport rate, which were significantly correlated with P_{\max} , N_C , N_B and N_{C+B} (data not shown).

Stomatal conductance was not important in explaining the interspecific difference in P_{\max} , although it is significantly correlated with P_{\max} (Fig. 1b). With similar G_s , *A. spinosus* showed higher P_{\max} compared to *A. lividus*, while *Piper hispidinervium* showed similar P_{\max} with higher G_s compared to *Piper sarmentosum* (Table 1). Although *Peperomia pellucida* had higher G_s than its native congener, it did not have higher C_i . In addition, P_{\max} decreased significantly with increasing C_i . The results further indicated that the ability to use intercellular CO_2 (associated with N in photosynthesis) was more important than the ability to supply CO_2 (associated with G_s) in explaining the difference in P_{\max} between each invasive species and its native congener, thus again confirming the importance of N allocation.

At the same value of N_M or N_A , the higher PNUE of the invasive species was consistent with their higher P_{\max} , which was positively correlated with PNUE, P_C , P_B , P_{C+B} , N_C , N_B and N_{C+B} (Figs. 1 a, 2, 3). The similar increasing pattern of PNUE with P_C , P_B and P_{C+B} indicated that the higher PNUE of *Peperomia pellucida* and *Piper hispidinervium* in comparison with their respective native congeners could have resulted from their higher P_C , P_B and P_{C+B} (Fig. 4a–c). The positive correlation between PNUE and SLA (Fig. 5c) also showed the role of N allocation in determining PNUE. In comparison to native congeners, the lower N_A of *Peperomia pellucida* and *Piper hispidinervium* could not explain their higher PNUE because PNUE still increased with increasing N_A (Fig. 2a). Only at a high level of N_A ,

when PNUE is negatively correlated with N_A , decreasing N_A may contribute to increasing PNUE (Hikosaka and Terashima 1995). The similar N_A between *A. spinosus* and its native congener could also not explain the higher PNUE of *A. spinosus*. Niinemets et al. (2003) found that invasive species had both higher PNUE and P_{\max} than native species, which was the case for *A. spinosus* and *Peperomia pellucida*, but not for *Piper hispidinervium*. The higher P_{\max} of *Peperomia pellucida* was related to its higher P_C , P_B and P_{C+B} (Fig. 3). The higher P_C , P_B and P_{C+B} of *Piper hispidinervium* increased its N_C , N_B and N_{C+B} to similar levels as *Piper sarmentosum* in spite of its lower N_A , leading to higher PNUE (Table 1, Fig. 4). The results further indicated that the differences in N allocation and partitioning rather than in P_{\max} or N_A explained the interspecific difference in PNUE between each congeneric pair.

Ecophysiological traits and invasiveness

Five resource capture- and use-related traits (P_{\max} , PNUE, LAR, SLA and CC) were evaluated in this study, and P_{\max} is positively correlated with NAR (Feng et al. 2007a), which in turn is positively associated with growth rate (Goodger et al. 2006). Thus, P_{\max} may positively be correlated with growth rate, and evidence for the same was reported by Pattison et al. (1998). Leaf area ratio is another determinant of growth rate (ShIPLEY 2006; Feng et al. 2007a), and species with high PNUE and SLA or low CC usually have high growth rates (Lambers and Poorter 1992; Reich et al. 1997; Schieving and Poorter 1999). A higher growth rate is very important for the success of invasive plants (Grotkopp and Rejmánek 2007). Compared to their native congeners, *Peperomia pellucida* showed advantages in all five variables, *Piper hispidinervium* in PNUE, LAR, SLA and CC, and *A. spinosus* only in P_{\max} and PNUE, indicating that different invasive species may have different syndrome of traits that contributed to its invasiveness. However, further studies are necessary to explore whether the leaf-level ecophysiological advantage of the invasive species can scale up to the whole-plant level.

In conclusion, invasive *Peperomia pellucida* and *Piper hispidinervium* allocated a higher fraction of leaf N to photosynthesis and partitioned a higher fraction of the photosynthetic N to carboxylation

than their respective native congeners. These eco-physiological traits of the invaders were associated with their higher SLA. Nitrogen allocation and partitioning were important in explaining the differences between each invader and its native congener in P_{\max} and PNUE. Nitrogen allocation-related variables were not available for *Amaranthus* in this study. However, *A. spinosus* had higher P_{\max} and PNUE with similar N_A compared to its native congener (Table 1), indicating that it allocated a higher fraction of leaf N to photosynthesis. Compared to their native congeners, *Peperomia pellucida* showed advantages in P_{\max} , PNUE, LAR, SLA and CC; *Piper hispidinervium* in PNUE, LAR, SLA and CC; and *A. spinosus* only in P_{\max} and PNUE, indicating that different invasive species may have a different set of traits associated with its invasiveness. However, this study compared only three pairs of invasive and native congeners at partially shady sites. Further comparative studies on more invasive and native congeners at a wide range of irradiance are necessary for understanding the generality of the patterns found in this study and fully determining the competitive advantages provided by these eco-physiological features.

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References

- Bernacchi CJ, Singaas EL, Pimentel C, Portis AR, Long SP (2001) Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant Cell Environ* 24:253–259
- Blossey B, Nötzold R (1995) Evolution of increased competitive ability in invasive non-indigenous plants: a hypothesis. *J Ecol* 83:887–889
- Burns JH (2004) A comparison of invasive and non-invasive dayflowers (Commelinaceae) across experimental nutrient and water gradients. *Divers Distrib* 10:387–397
- Burns JH (2006) Relatedness and environment affect traits associated with invasive and noninvasive introduced Commelinaceae. *Ecol Appl* 16:1367–1376
- Daehler CC (2003) Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annu Rev Ecol Evol Syst* 34: 183–211
- D’Antonio CM, Kark S (2002) Impacts and extent of biotic invasions in terrestrial ecosystems. *Trends Ecol Evol* 17:202–204
- Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities, a general theory of invasibility. *J Ecol* 88:528–534
- DeWalt SJ, Denslow JS, Hamrick JL (2004) Biomass allocation, growth, and photosynthesis of genotypes from native and introduced ranges of the tropical shrub *Clidemia hirta*. *Oecologia* 138:521–531
- Ewe SML, Sternberg LSL (2003) Seasonal exchange characteristics of *Schinus terebinthifolius* in a native and disturbed upland community in Everglade National Park, Florida. *For Ecol Manage* 179:27–36
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol* 11:191–210
- Feng Y-L, Wang J-F, Sang W-G (2007a) Biomass allocation, morphology and photosynthesis of invasive and noninvasive exotic species grown at four irradiance levels. *Acta Oecol* 31:40–47
- Feng Y-L, Wang J-F, Sang W-G (2007b) Irradiance acclimation, capture ability and efficiency in invasive and noninvasive alien plant species. *Photosynthetica* 45:245–253
- Feng Y-L, Auge H, Ebeling SK (2007c) Invasive *Buddleja davidii* allocates more nitrogen to its photosynthetic machinery than five native woody species. *Oecologia* 153:501–510
- Goodger JQD, Gleadow RM, Woodrow IE (2006) Growth cost and ontogenetic expression patterns of defence in cyanogenic *Eucalyptus* spp. *Trees – Struct Funct* 20:757–765
- Goodger JQD, Choo TYS, Woodrow IE (2007) Ontogenetic and temporal trajectories of chemical defence in a cyanogenic eucalypt. *Oecologia* 153:799–808
- Goodwin BJ, McAllister AJ, Fahrig L (1999) Predicting invasiveness of plant species based on biological information. *Conserv Biol* 13:422–426
- Grotkopp E, Rejmánek M (2007) High seedling relative growth rate and specific leaf area are traits of invasive species: phylogenetically independent contrasts of woody angiosperms. *Am J Bot* 94:526–532
- Grotkopp E, Rejmánek M, Rost TL (2002) Toward a causal explanation of plant invasiveness: seedling growth and life-history strategies of 29 pine (*Pinus*) species. *Am Nat* 159:396–419
- Güsewell S, Jakobs G, Weber E (2006) Native and introduced populations of *Solidago gigantea* differ in shoot production but not in leaf traits or litter decomposition. *Funct Ecol* 20:575–584
- Hamilton MA, Murray BR, Cadotte MW, Hose GC, Baker AC, Harris CJ, Licari D (2005) Life-history correlates of plant invasiveness at regional and continental scales. *Ecol Lett* 8:1066–1074
- Hikosaka K, Terashima I (1995) A model of the acclimation of photosynthesis in the leaves of C_3 plants to sun and shade with respect to nitrogen use. *Plant Cell Environ* 18: 605–618
- Lambers H, Poorter H (1992) Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Adv Ecol Res* 23:188–261
- Li Z-Y, Xie Y (2002) Invasive alien species in China. China forestry publishing house, Beijing

- Lichtenthaler HK, Wellburn AR (1983) Determination of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochem Soc Trans* 603:591–592
- Loomis RS (1997) Commentary on the utility of nitrogen in leaves. *Proc Natl Acad Sci USA* 94:13378–13379
- Loustau D, Beahm M, Gaudillère JP, Dreyer E (1999) Photosynthetic responses to phosphorous nutrition in two-year-old maritime pine seedlings. *Tree Physiol* 19:707–715
- Maron J, Vilà M, Arnason J (2004) Loss of enemy resistance among introduced populations of St. John's wort (*Hypericum perforatum*). *Ecology* 85:3243–3253
- McDowell SCL (2002) Photosynthetic characteristics of invasive and noninvasive species of *Rubus* (Rosaceae). *Am J Bot* 89:1431–1438
- Mitchell CE, Power AG (2003) Release of invasive plants from fungal and viral pathogens. *Nature* 421:625–627
- Niinemets Ü, Tenhunen JD (1997) A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant Cell Environ* 20:845–866
- Niinemets Ü, Valladares F, Ceulemans R (2003) Leaf-level phenotypic variability and plasticity of invasive *Rhododendron ponticum* and non-invasive *Ilex aquifolium* co-occurring at two contrasting European sites. *Plant Cell Environ* 26:941–956
- Onoda Y, Hikosaka K, Hirose T (2004) Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency. *Funct Ecol* 18:419–425
- Pattison RR, Goldstein G, Ares A (1998) Growth, biomass allocation and photosynthesis of invasive and native Hawaiian rain-forest species. *Oecologia* 117:449–459
- Poorter L (1999) Growth responses of 15 rain-forest species to a light gradient: the relative importance of morphological and physiological traits. *Funct Ecol* 13:396–410
- Poorter H, Evans JR (1998) Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. *Oecologia* 116:26–37
- Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. *Proc Natl Acad Sci USA* 94:13730–13734
- Schieving F, Poorter H (1999) Carbon gain in a multispecies canopy: the role of specific leaf area and photosynthetic nitrogen-use efficiency in the tragedy of the commons. *New Phytol* 143:201–211
- Shipley B (2006) Net assimilation rate, specific leaf area and leaf mass ratio: which is most closely correlated with relative growth rate? A meta-analysis. *Funct Ecol* 20:565–574
- Takashima T, Hikosaka K, Hirose T (2004) Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. *Plant Cell Environ* 27:1047–1054
- Wolfe LM, Elzinga JA, Biere A (2004) Increased susceptibility to enemies following introduction in the invasive plant *Silene latifolia*. *Ecol Lett* 7:813–820
- Wright IJ, Reich PB, Westoby M (2001) Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Funct Ecol* 15:423–434
- Xu C-Y, Griffin KL, Schuster WSF (2007) Leaf phenology and seasonal variation of photosynthesis of invasive *Berberis thunbergii* (Japanese barberry) and two co-occurring native understory shrubs in a northeastern United States deciduous forest. *Oecologia* 154:11–12