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Similar responses in morphology, growth, biomass allocation, and photosynthesis in invasive *Wedelia trilobata* and native congeners to CO_2 enrichment

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Abstract Both global change and biological invasions threaten biodiversity worldwide. However, their interactions and related mechanisms are still not well elucidated. To elucidate potential traits contributing to invasiveness and whether ongoing increase in CO_2 aggravates invasions, noxious invasive *Wedelia trilobata* and native *Wedelia urticifolia* and *Wedelia chinensis* were compared under ambient and doubled atmospheric CO_2 concentrations in terms of growth, biomass allocation, morphology, and physiology. The invader had consistently higher leaf mass fraction (LMF) and specific leaf area than the natives, contributing to a higher leaf area ratio, and therefore to faster growth and invasiveness. The higher LMF of the invader was due to lower root mass fraction and higher fine root percent. On the other hand, the invader allocated a higher fraction of leaf nitrogen (N) to photosynthetic apparatus, which was associated with its higher photosynthetic rate, and resource use efficiency. All these traits collectively contributed to its invasiveness. CO_2 enrichment increased growth of all studied species by increasing actual photosynthesis, although it decreased photosynthetic capacities due to decreased leaf and photosynthetic N contents. Responses of the invasive and native plants to elevated CO_2 were not significantly different, indicating that the ongoing increase in CO_2 may not aggravate biological invasions, inconsistent with the prevailing results in references. Therefore, more comparative studies of related invasive and native plants are needed to elucidate whether CO_2 enrichment facilitates invasions.

Keywords CO_2 enrichment · Growth · Invasiveness · Morphology · Nitrogen and biomass allocation

Abbreviations

AC	Ambient atmospheric CO_2 concentration
A_{growth}	Actual photosynthetic rate measured at growth CO_2 concentration
EC	Doubled atmospheric CO_2 concentration
FRP	Fine root percent
G_{s}	Stomatal conductance
LA:RM	The ratio of leaf area to root mass
LA:FRM	The ratio of leaf area to fine root mass

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LAR	Leaf area ratio
LMF	Leaf mass fraction
N_{bioenerg}, N_{carbox}, N_{LHC}, and $N_{\mathrm{photosynth}}$	Nitrogen contents in bioenergetics, carboxylation, light-harvesting components, and all components of the photosynthetic apparatus, respectively
$N_{\mathrm{bioenerg}}/N_{\mathrm{L}}$, $N_{\mathrm{carbox}}/N_{\mathrm{L}}$, $N_{\mathrm{LHC}}/N_{\mathrm{L}}$, and $N_{\mathrm{photosynth}}/N_{\mathrm{L}}$	The fractions of leaf nitrogen allocated to bioenergetics, carboxylation, light-harvesting components, and all components of the photosynthetic apparatus, respectively
N_{L}	Total leaf nitrogen content
$P_{\max}$	Light-saturated photosynthetic rate
PNUE	Photosynthetic nitrogen-use efficiency
RGR	Relative growth rate
RMF	Root mass fraction
SLA	Specific leaf area
SMF	Support mass fraction
WUE	Water-use efficiency

Introduction

Global mean CO_2 concentration has increased from 290 to 375 μmol mol^{-1} during the last 100 years and is conservatively projected to be doubled by the end of 21st century, strongly dependent on future scenarios of anthropogenic emissions (Nagel et al. 2005). The ongoing increase in atmospheric CO_2 may cause changes in species composition of ecosystems, either by altering global climate (Chapin et al. 1995) or, more directly, by favoring certain photosynthetic pathways (Arp et al. 1993) or changing competition dynamics within ecosystems (Owensby et al. 1999). Invasive species, which may exploit the new environmental conditions caused by global change such as CO_2 enrichment, may gain footholds in previously inhospitable ecosystems, changing species composition, and biological invasions have become a serious environmental and socioeconomic problem and hot topic of ecological research worldwide (Dukes and Mooney 1999). However, the interactions between biological invasions and global change (Bond and Midgley 2000; Rogers et al. 2008) and the mechanisms underlying invasiveness are still not well elucidated (Daehler 2003; Feng et al. 2009). Identifying the factors that contribute to success of invasive alien plants is important for predicting and controlling potentially invasive plants.

It has been found that some successful invasive plants have higher light-saturated photosynthetic rate ($P_{\max}$), specific leaf area (SLA), and leaf area ratio (LAR) than native plants (Nagel and Griffin 2004; Zou et al. 2007). Pattison et al. (1998) and Zheng et al. (2009) found that $P_{\max}$ is positively correlated with relative growth rate (RGR) in some invasive plants. Higher RGR may confer competitive advantages on invasive species, facilitating invasions (Zheng et al. 2009). LAR, the product of SLA and leaf mass fraction (LMF), is the most important determinant of RGR especially at low irradiance (Feng et al. 2009). High SLA may also contribute to invasiveness of alien plants by decreasing leaf construction cost and increasing nitrogen (N) allocation to photosynthesis and photosynthetic N-use efficiency (PNUE) (Feng et al. 2009). Higher LMF and lower root mass fraction (RMF) were indeed found in some successful invasive plants in comparison with native plants (Wilsey and Polley 2006). This pattern of biomass allocation may promote irradiance capture but impair water and nutrient absorptions, suggesting that biological invasions are environment-dependent (Zheng et al. 2009). It is well known that increased availabilities of resources such as irradiance, nutrients, and water often facilitate alien plant invasions (Daehler 2003; Zheng et al. 2009).

CO_2 is necessary for photosynthesis, and increased atmospheric CO_2 supply generally increases photosynthetic rate and plant growth (Long et al. 2004). However, the effects of elevated CO_2 are significantly different among plant species and functional groups (Ainsworth et al. 2007). Many studies found that growth of invasive plants is more strongly stimulated by elevated CO_2 than growth of native plants (Raizada et al. 2009; Song et al. 2009). For example, doubled atmospheric CO_2 concentration increases biomass accumulation by 56% in invasive *Rhododendron ponticum* versus 12% in understorey native plants

(Hättenschwiler and Körner 2003). Furthermore, the intrinsically broader environmental tolerance, higher growth rate, and phenotypic plasticity, characteristics of many invasive plant species (Jia et al. 2016), may enable them to respond more positively to environmental changes that result in increased resource availability (elevated levels of water supply, atmospheric CO_2 concentrations, and N deposition) than native plants adapted to low resource conditions (Nackley et al. 2017; Zhang et al. 2017). The different responses of C_3 and C_4 plants to elevated CO_2 have been suggested as a potential explanation for invasions of native C_4 grasslands by woody C_3 plants in North America (Bond and Midgley 2000). However, the mechanisms by which these C_3 plants spread at the expense of existing native C_4 plants are poorly understood, and relatively few studies have compared the differences in responses to elevated CO_2 between invasive and native plants, especially the differences between phylogenetically related invasive and native plants.

Wedelia trilobata (L.) Hitchc. [syn. *Sphagneticola trilobata* (L.) Pruski] (creeping oxeye), native to the tropics of South America (Qi et al. 2014), is a perennial evergreen creeping clonal herb. It has been listed as one of the 100 world's worst invasive alien species (IUCN 2001). This noxious weed was introduced to South China on a large scale as a common groundcover plant in the 1970s, but it rapidly spread to the field (Li and Xie 2003). Fast dispersal through vegetative propagation (clonal growth) is one of the pivotal factors for the successful invasion of *W. trilobata*. Once established in plantations, *W. trilobata* can overgrow into a dense groundcover and prevent the regeneration of other species, including some native congeners, which are typically used as important traditional Chinese medicines (Song et al. 2010). In our study, *W. trilobata* was compared with two sympatric native congeners, *W. urticifolia* DC. and *W. chinensis* L., under ambient and doubled atmospheric CO_2 concentrations. The main aims of this study were to explore (1) the traits contributing to invasiveness of the invader; (2) how the studied plants acclimate to CO_2 enrichment in terms of growth, biomass allocation, morphology, and photosynthesis; (3) whether CO_2 enrichment aggravates invasion of the invader and related mechanisms.

Materials and methods

Plant materials and treatments

Seeds of each studied species were collected from a minimum of 15 individuals distributing around Kunming (25°06′N, 102°50′E, 2200 m a.s.l.), Yunnan Province, southwest China and mixed. The seeds were germinated on a seedbed in a greenhouse in March 2013 with average air temperature of 25 °C and relative humidity of 42% during the experimental period. In May 2013, when the seedlings were approximately 10 cm tall, similar-sized individuals were singly transplanted into 5-L pottery pots filled with 4 kg homogenized forest topsoil. After 1 month growth at an open site, 40 similar-sized seedlings per species were selected and randomly divided into two groups. Each group was moved into closed-top chambers (E-sheng Tech. Co., Beijing, China) located outdoors at Ailaoshan Station for Subtropical Forest Ecosystem Studies (24°32′N, 101°01′E, 2490 m a.s.l.), Jingdong County, southwest China. Detailed information on the chambers can be found in our previous study (Meng et al. 2013). Seedlings of each species in each chamber were randomly divided into five groups, four seedlings per group. One group of each species was put together and the 12 seedlings of the three studied species were randomly arranged and watered when necessary. No fertilizer was added during the experiment.

One chamber was supplied with compressed CO_2 gas to obtain a doubled atmospheric CO_2 concentration treatment (EC), and another chamber was used as control (AC, 320 μmol mol^{-1} CO_2). CO_2 concentration in EC chamber was controlled automatically with a computer-controlled CO_2 supply system. CO_2 concentration and temperature in each chamber were recorded at a 15-s interval. Hourly mean CO_2 concentrations were 280–340 and 590–670 μmol mol^{-1} in AC and EC chambers, respectively. There was no significant difference in temperature between chambers. Three months after CO_2 treatments, measurements were taken on five individuals per species per treatment.

Photosynthesis measurements

Under saturating photosynthetic photon flux density (PPFD, 2000 μmol m^{-2} s^{-1}), photosynthesis was measured on the youngest fully expanded leaf of each sample plant using a Li-6400 Portable Photosynthesis System (Li-Cor, Lincoln, NE). Relative humidity of the air in the leaf chamber was controlled at ≈ 70% and leaf temperature at 25 °C. Actual photosynthetic rates (A_{growth}) under growth ambient atmospheric CO_2 concentrations were measured at 320 and 640 μmol mol^{-1} CO_2 in the reference chamber for plants grown under AC and EC, respectively. For determining photosynthetic responses to intercellular CO_2 concentration, gas exchanges were measured at 380, 300, 260, 220, 180, 140, 110, 80, and 50 μmol mol^{-1} CO_2 in the reference chamber. $P_{\max}$ and stomatal conductance (G_s) were the values measured at 380 μmol mol^{-1} CO_2 and saturating PPFD. Afterwards, light- and CO_2-saturated photosynthetic rate was measured after 500 s under saturating PPFD and 1500 μmol mol^{-1} CO_2. Before measurement, each sample leaf was illuminated with saturating 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.

Two 10-mm-diameter leaf disks were taken from each sample leaf, oven-dried at 60 °C for 48 h. SLA was calculated as the ratio of leaf area to mass. Leaf N content (N_L) was determined with a Vario MAX CN Element Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Leaf chlorophyll content was measured following the method of Lichtenthaler and Wellburn (1983). Water-use efficiency and PNUE were calculated as the ratios of $P_{\max}$ to G_s and N_L, respectively.

Calculations of P_n–C_i curve-related variables

The P_n–C_i curve was fitted with a linear equation ($P_n = kC_i + i$) within 50–200 μmol mol^{-1} C_i. Maximum carboxylation rate (V_{cmax}), dark respiration rate (R_d), and maximum electron transport rate ($J_{\max}$) were calculated according to Feng et al. (2009) and Zheng et al. (2009) as follows:

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

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

$$J_{\max} = [4(P\prime_{\max} + R_d)(C_i + 2\Gamma^*)] / (C_i - \Gamma^*), \quad (3)$$

where K_c and Ko were the Michaelis–Menten constants of Rubisco for carboxylation and oxidation, respectively; Γ^* was CO_2 compensation point; O - was the intercellular oxygen concentration, close to 210 mmol mol^{-1}.

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

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

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

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

where V_{cr} and J_{mc} were 20.78 μmol CO_2 g^{-1} Rubisco S^{-1} and 155.65 μmol electrons $\mu\mathrm{mol}^{-1}$ cyt f s^{-1}, respectively. C_B was 2.15 mmol g^{-1}. 6.25 (g Rubisco g^{-1} nitrogen in Rubisco) was the conversion coefficient between nitrogen content and protein content in Rubisco, and 8.06 (μmol cyt f g^{-1} nitrogen in bioenergetics) was the conversion coefficient between cyt f and nitrogen in bioenergetics. Nitrogen contents in carboxylation (N_C) and bioenergetics (N_B) were calculated as the products of N_A and P_C, P_B, respectively.

Growth measurements

Five seedlings per species per treatment were harvested after measurements of height and ramet (> 5 cm branches originating from root collar) number. All samples were separated into leaves, support organs (including stems, branches, and petioles), and fine (diameter < 1 mm) and coarse roots. Then total leaf area was determined using Li-3000C leaf area meter (Li-Cor, Lincoln, NE). Finally, all the organs were oven-dried at 60 °C for 48 h, and weighed. Support mass fraction (SMF, support organ mass/total mass), LMF (leaf mass/total mass), RMF (root mass/total mass), fine root percent (FRP, fine root mass/total root mass × 100), leaf area-to-root mass ratio (LA:RM, total leaf area/total root mass), leaf area-to-fine root mass ratio (LA:FRM, total leaf area/fine root mass), LAR (total leaf area/total mass), and

Table 1 Differences in morphology, growth, and biomass allocation among invasive *Wedelia trilobata*, and native *W. urticifolia* and *W. chinensis* grown under ambient (AC) and doubled (EC) atmospheric CO_2 concentrations

Variables	*W. trilobata*		*W. urticifolia*		*W. chinensis*		*F* values	
	AC	EC	AC	EC	AC	EC	Species	Treatment
Height (cm)	43.8 ± 2.20ab	47.8 ± 2.65a	41.6 ± 1.94ab	46.2 ± 2.62ab	39.6 ± 2.21b	43.4 ± 2.21ab	1.730	4.775*
Total biomass (g)	13.7 ± 0.53b	17.6 ± 0.76a	5.4 ± 0.46d	6.8 ± 0.38c	4.2 ± 0.28d	5.2 ± 0.18d	317.537***	29.924***
Relative growth rate (mg g^{-1} d^{-1})	34.7 ± 0.43b	37.5 ± 0.49a	24.3 ± 0.98d	26.9 ± 0.65c	21.6 ± 0.74e	23.9 ± 0.39d	236.185***	24.354***
Total leaf area (m^2)	1.25 ± 0.11b	1.49 ± 0.14a	0.23 ± 0.02c	0.27 ± 0.02c	0.19 ± 0.01c	0.22 ± 0.01c	164.704***	2.920
Leaf area ratio (cm^2 g^{-1})	91.2 ± 6.53a	84.0 ± 5.72a	44.3 ± 4.64b	40.1 ± 2.53b	44.9 ± 4.03b	41.4 ± 1.93b	65.621***	1.814
Specific leaf area (cm^2 g^{-1})	215 ± 16.3a	191 ± 7.8b	162 ± 15.2cd	149 ± 16.4d	179 ± 27.1bc	163 ± 13.2cd	19.212***	8.114**
Leaf mass fraction	39.6 ± 0.67a	38.9 ± 0.67a	27.8 ± 0.81b	28.1 ± 1.27b	26.3 ± 0.63b	25.8 ± 1.72b	93.767***	0.097
Support mass fraction	34.1 ± 0.23ab	31.6 ± 0.42b	35.1 ± 1.59ab	33.5 ± 1.63ab	36.9 ± 1.07a	35.4 ± 1.84ab	3.365	3.230
Root mass fraction	26.3 ± 0.61b	29.4 ± 0.54b	37.1 ± 1.64a	38.3 ± 1.57a	36.7 ± 1.17a	38.8 ± 0.60a	51.375***	5.540*
Fine root percent (%)	17.5 ± 1.04b	21.1 ± 0.45a	6.9 ± 0.53c	6.9 ± 0.41c	7.9 ± 0.63c	7.8 ± 0.16c	263.360***	5.624*
Leaf area root mass ratio (cm^2 g^{-1})	346 ± 22.1a	285 ± 17.9b	121 ± 14.4c	104 ± 3.9c	122 ± 8.0c	107 ± 4.9c	145.979***	7.565*
Leaf area fine root mass ratio (cm^2 g^{-1})	530 ± 54.4ab	400 ± 33.4b	649 ± 65.8a	584 ± 37.9a	572 ± 52.6a	526 ± 19.5ab	5.328*	4.450*

Interactions between species and CO_2 treatment were not significant for all variables except total biomass ($F = 5.896$**) and fine root percent ($F = 6.262$**). Mean ± SE ($n = 5$). Different letters indicate significant differences among species and treatments ($P < 0.05$)

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

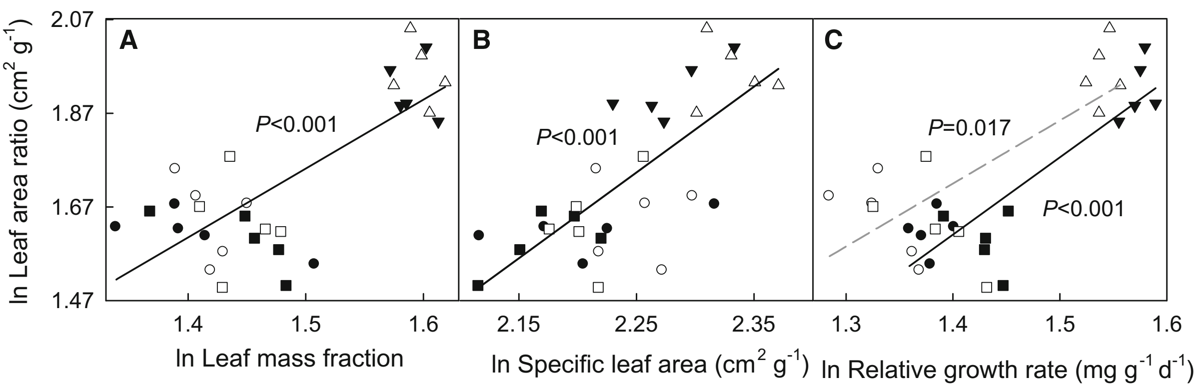

Fig. 1 Correlations between leaf area ratio (LAR) and leaf mass fraction (LMF, **a**), specific leaf area (SLA, **b**), and relative growth rate (RGR, **c**) in invasive *Wedelia trilobata* (triangles), and native *W. urticifolia* (squares) and *W. chinensis* (circles) grown under ambient (open symbols) and doubled (closed symbols) atmospheric CO_2 concentrations. Data were transformed into natural logarithms. Lines fitted for ambient (dashed line) and doubled (solid line) atmospheric CO_2 concentration treatments were given, respectively, if the difference between treatments was significant according to the result of ANCOVA. Otherwise, only one line fitted for pooled data was given

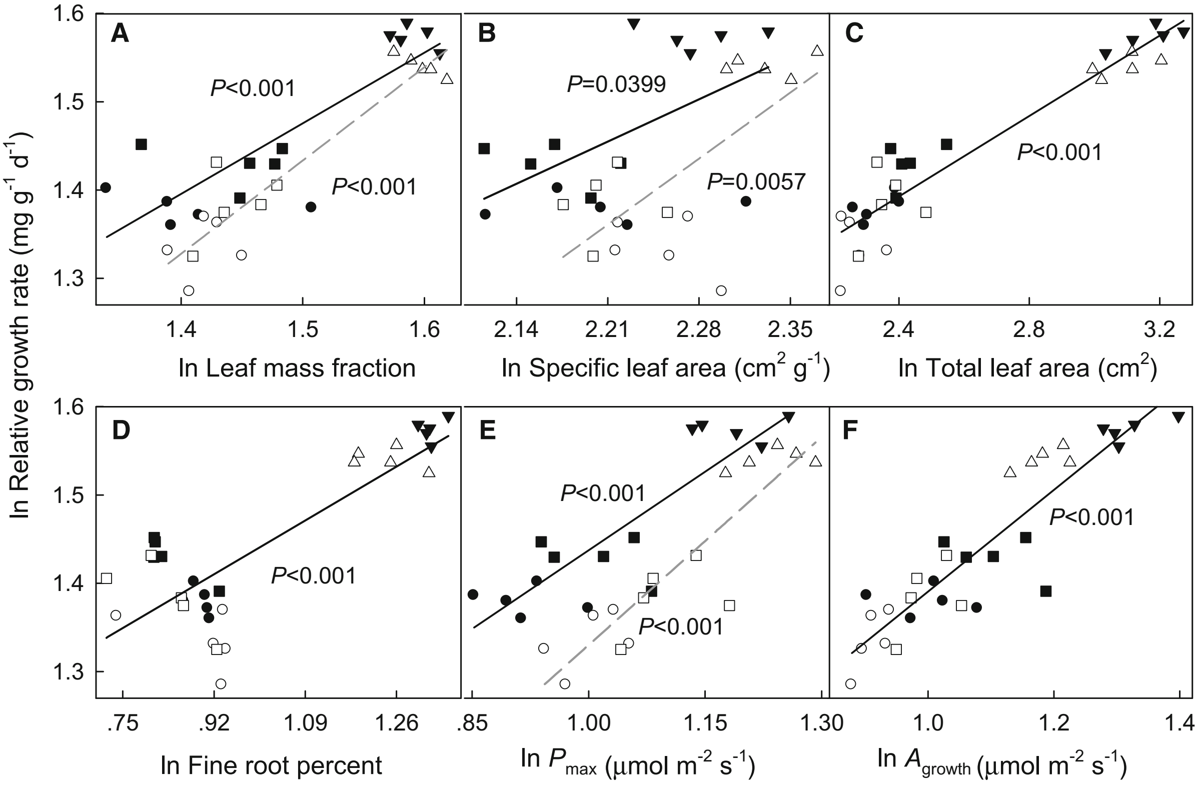

Fig. 2 Relative growth rate (RGR) as a function of leaf mass fraction (LMF, **a**), specific leaf area (SLA, **b**), total leaf area (**c**), fine root percent (FRP, **d**), light-saturated photosynthetic rate (P_{max}, **e**), and photosynthetic rate measured at growth ambient CO_2 concentration (A_{growth}, **f**) in three species and two CO_2 concentrations treatments. For species and treatment codes, as well as statistical analyses, see legend Fig. 1

Table 2 Differences in physiological traits among invasive *Wedelia trilobata* and native *W. urticifolia*, and *W. chinensis* grown under ambient (AC) and doubled (EC) atmospheric CO_2 concentrations

Variables	*W. trilobata*		*W. urticifolia*		*W. chinensis*		*F* values	
	AC	EC	AC	EC	AC	EC	AC	EC
A_{growth}	15.3 ± 0.60b	21.0 ± 1.06a	10.0 ± 0.44d	12.9 ± 0.88c	8.2 ± 0.21d	10.0 ± 0.66d	90.707***	37.910***
P_{max}	17.3 ± 0.82a	15.6 ± 0.83a	12.8 ± 0.75b	10.3 ± 0.66c	10.1 ± 0.46c	8.4 ± 0.45c	58.350***	12.428**
G_s	0.53 ± 0.02a	0.40 ± 0.01bc	0.49 ± 0.03b	0.37 ± 0.02d	0.43 ± 0.02c	0.33 ± 0.02d	50.790***	107.31***
PNUE	14.6 ± 1.32a	16.4 ± 2.09a	10.9 ± 1.27bc	11.7 ± 2.38b	8.7 ± 0.26d	9.2 ± 0.55cd	48.177***	2.405
WUE	32.5 ± 3.92b	39.0 ± 4.55a	29.6 ± 1.84bc	32.2 ± 4.06b	26.1 ± 1.99c	27.2 ± 2.02c	20.487***	8.479**
N_L	1.20 ± 0.09a	0.96 ± 0.07bc	1.18 ± 0.06a	0.92 ± 0.06c	1.16 ± 0.06ab	0.93 ± 0.06c	0.146	17.461***
$N_{photosynth}/N_L$	0.51 ± 0.035a	0.49 ± 0.017a	0.37 ± 0.018b	0.37 ± 0.018b	0.35 ± 0.014b	0.34 ± 0.013b	44.076***	0.813
N_{carbox}/N_L	0.33 ± 0.018a	0.31 ± 0.017a	0.23 ± 0.031b	0.23 ± 0.038b	0.21 ± 0.006b	0.19 ± 0.004b	44.051***	0.959
$N_{bioenerg}/N_L$	0.07 ± 0.003a	0.07 ± 0.004a	0.05 ± 0.005bc	0.05 ± 0.003b	0.04 ± 0.003c	0.04 ± 0.003bc	56.652***	3.121
N_{LHC}/N_L	0.11 ± 0.005a	0.10 ± 0.005a	0.09 ± 0.004a	0.091 ± 0.007a	0.10 ± 0.009a	0.10 ± 0.011a	1.915	1.039
$N_{photosynth}$	0.62 ± 0.12a	0.45 ± 0.05b	0.43 ± 0.04b	0.32 ± 0.02cd	0.40 ± 0.02bc	0.31 ± 0.01d	30.692***	29.601***
N_{carbox}	0.39 ± 0.05a	0.30 ± 0.06b	0.26 ± 0.04bc	0.21 ± 0.04cd	0.24 ± 0.02c	0.18 ± 0.01d	27.347***	18.97***
$N_{bioenerg}$	0.08 ± 0.01a	0.07 ± 0.01a	0.05 ± 0.01b	0.05 ± 0.01b	0.05 ± 0.01b	0.04 ± 0.01c	54.666***	11.659**
N_{LHC}	0.13 ± 0.02a	0.09 ± 0.01cd	0.11 ± 0.01bc	0.08 ± 0.01d	0.12 ± 0.02b	0.09 ± 0.01cd	4.627*	38.491***

Interactions between species and CO_2 treatment were not significant for all variables except A_{growth} ($F = 4.093$*). Statistical analyses and replications are shown as in Table 1

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

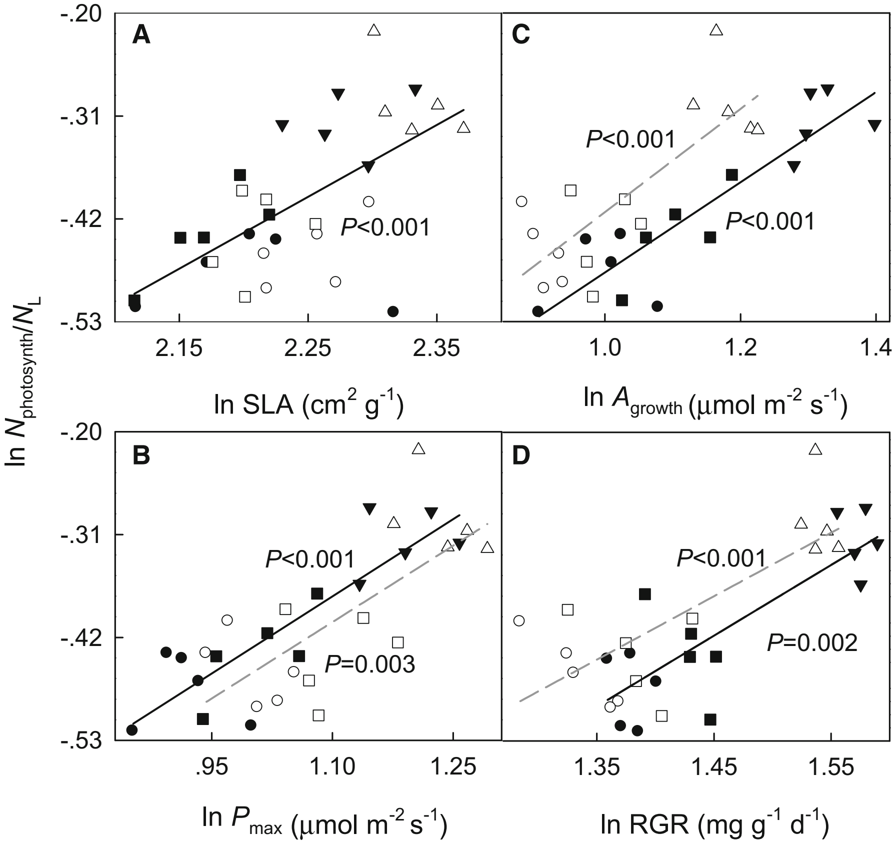

Fig. 3 Correlations between fractions of leaf nitrogen in photosynthetic apparatus ($N_{photosynth}/N_L$) and specific leaf area (SLA, **a**), light-saturated photosynthetic rate (P_{max}, **b**), photosynthetic rate measured at growth ambient CO$_2$ concentration (A_{growth}, **c**), and relative growth rate (RGR, **d**) in three species and two CO$_2$ concentrations treatments. For species and treatment codes, as well as statistical analyses, see legend Fig. 1

RGR (mass increase per unit mass per unit time) were calculated according to Poorter and Remkes (1990).

Statistical analyses

Effects of species, treatment, and their interactions on variables measured in this study were tested using two-way ANOVA. Differences among species grown at both CO$_2$ treatments were tested using one-way ANOVA. Difference between CO$_2$ treatments in correlation between each pair of variables was tested using a one-way ANCOVA. Treatment (AC vs. EC) was used as a fixed factor; variables were indicated by *y*- and *x*-axes in each figure as dependent variable and covariate, respectively. If the difference was significant, we then tested for significances of the correlations (Pearson correlation, two-tailed) for CO$_2$ treatments separately; otherwise, we pooled data from both treatments to test for significance of the correlation. All analyses were carried out using SPSS 13.0 (SPSS Inc., Chicago, Il). Principal component analysis (PCA) of ecophysiological traits was used to identify the most discriminatory effects of elevated temperature and drought. PCA analyses were performed using Canoco 5.0 (Microcomputer Power, USA).

Results

Morphology, growth, and biomass allocation

Invasive *W. trilobata* was significantly higher in biomass, RGR, total leaf area, LAR, SLA, LMF, FRP, and LA:RM than native *W. urticifolia* and *W. chinensis* (Table 1). The invader was also higher in height than the natives, although the difference was not statistically significant. In contrast, the invader was lower in RMF and LA:FRM (significant only for *W. urticifolia* under EC) than the natives. The invader showed 4.00 ± 0.32 and 4.60 ± 0.68 ramets under AC and EC, respectively, while the natives had no ramets (data not shown).

Although CO_2 enrichment significantly increased RGR in all studied species, there is no influences on other morphological and growth traits including height, TLA, LAR, SLA, LMF, SMF, RMF, FRP, LA:RM, and LA:FRM of *W. urticifolia* and *W. chinensis* except for the increased biomass of *W. urticifolia* (Table 1). However, in *W. trilobata*, elevated CO_2 significantly decreased SLA, LA:RM and increased biomass, RGR, TLA, FRP, and LA:RM.

The correlations between LAR and LMF, SLA, and RGR were significant (Fig. 1). RGR increased significantly with increasing LMF, SLA, FRP, and total leaf area (Fig. 2a–d). At given values of LMF, SLA, and LAR, plants grown under EC had higher RGR than plants grown under AC.

Photosynthesis

Invasive *W. trilobata* was significantly higher in A_{growth}, G_{s}, $P_{\max}$, PNUE, WUE (not significant for *W. urticifolia*), $N_{\mathrm{photosynth}}/N_{\mathrm{L}}$, $N_{\mathrm{carbox}}/N_{\mathrm{L}}$, $N_{\mathrm{bioenerg}}/N_{\mathrm{L}}$, $N_{\mathrm{photosynth}}$, N_{carbox}, and N_{bioenerg} than native *W. urticifolia* and *W. chinensis* (Table 2). N_{L} and $N_{\mathrm{LHC}}/N_{\mathrm{L}}$ were not significantly different between the invasive and native species. The invader was higher in N_{LHC} than the natives under AC but not under EC.

CO_2 enrichment significantly increased A_{growth} in *W. trilobata* and *W. urticifolia* (Table 2). In *W. trilobata*, CO_2 enrichment significantly increased WUE. In contrast, CO_2 enrichment significantly decreased G_{s}, N_{L}, $N_{\mathrm{photosynth}}$, N_{carbox} (not significant for *W. urticifolia*), and N_{LHC}. $P_{\max}$ in *W. urticifolia* and N_{bioenerg} in *W. chinensis* were significantly decreased by CO_2 enrichment. In contrast, CO_2 enrichment did not influence $P_{\max}$ and N_{bioenerg} in invasive *W. trilobata*. The effects of CO_2 enrichment on PNUE, $N_{\mathrm{photosynth}}/N_{\mathrm{L}}$, $N_{\mathrm{carbox}}/N_{\mathrm{L}}$, $N_{\mathrm{bioenerg}}N_{\mathrm{L}}$, and $N_{\mathrm{LHC}}/N_{\mathrm{L}}$ were not significant.

RGR increased with increasing $P_{\max}$ and A_{growth} (Fig. 2e, f). At a given value of $P_{\max}$, plants grown under EC had higher RGR than plants grown under AC. The correlations between $N_{\mathrm{photosynth}}/N_{\mathrm{L}}$ and SLA, $P_{\max}$, A_{growth}, and RGR were significant (Fig. 3). At a given value of $N_{\mathrm{photosynth}}/N_{\mathrm{L}}$, plants grown under EC had higher A_{growth} and RGR but lower $P_{\max}$ than plants grown under AC.

According to the PCA, native and invasive species were separated along the first axis of the PCA, which was strongly correlated with biomass accumulation, leaf area, fine root ratio, leaf area ratio, and accounted for 69.65% of the observed variance; meanwhile, CO_2 treatment showed modest differentiation (Fig. 4).

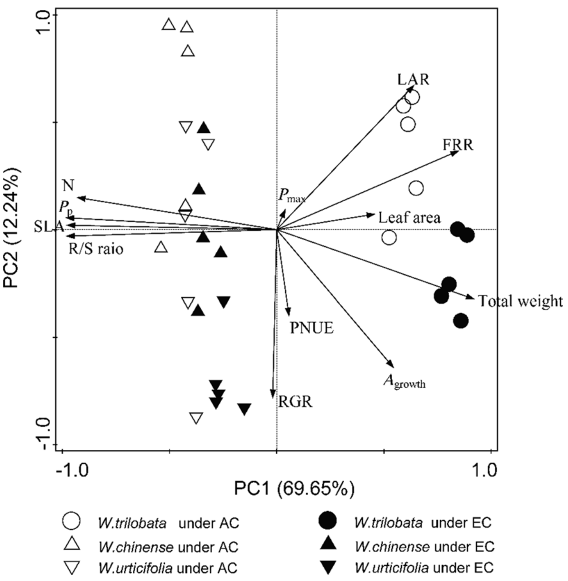

Fig. 4 Principal component analysis (PCA) based on ecophysiological traits in invasive *Wedelia trilobata* (circles), and native *W. urticifolia* (triangles) and *W. chinensis* (reverse triangles) grown under ambient (open symbols) and doubled (closed symbols) at atmospheric CO_2 concentrations

Discussion

Traits contributing to invasiveness

Higher RGR and biomass accumulation of *W. trilobata* in comparison with native congeners may contribute to invasiveness. High RGR can facilitate capture of available resources (Grotkopp and Rejmánek 2007), which is important for alien plant invasions (Davis et al. 2000). Higher RGR of the invader contributed to higher total leaf area and ramet number, and therefore to invasiveness (Table 1). Both higher LMF and SLA of *W. trilobata* contributed to higher LAR, one of the determinants of RGR (Poorter and Remkes 1990; Zheng et al. 2009), and therefore to higher RGR (Figs. 1, 4). Positive correlations between RGR and LMF, SLA, and total leaf area were indeed found (Fig. 2a–c). SLA is an important determinant of RGR (Poorter and Remkes 1990); Daehler (2003) found through reviewing published references that invasive plants have significantly higher SLA and total leaf area than co-occurring natives. Positive correlation between RGR and LMF was also found by Poorter and Remkes (1990).

The higher LMF of *W. trilobata* was due to lower RMF as SMF was not significantly different between the invader and natives (Table 1). Lower RMF of the invader contributed to higher RGR not only by increasing LMF, but also by decreasing root respiratory carbon loss (D'Antonio et al. 2001; Feng et al. 2009). The invader supported more leaves with fewer roots, as indicated by higher LA:RM (Table 1), which did not influence growth, N_L, and photosynthesis, indicating that roots of the invader were more efficient in physiological functions than those of natives. Higher FRP of the invader may explain efficient root functions; LA:FRM was even lower in the invader than in the natives (Table 1). Decreasing root diameter can increase the ratio of surface area to mass, promoting water and nutrient absorptions (Akinnifesi et al.1998; Bauhus and Messier 1999). The significantly positive correlation between RGR and FRP confirmed the role of fine roots in invasion success of the invader (Fig. 2d).

The higher SLA of *W. trilobata* contributed to higher RGR not only by increasing LAR, but also by increasing P_{max} which is positively correlated with net assimilation rate, one of the determinants of RGR (Feng et al. 2009). Generally, SLA is negatively correlated with cell wall mass (Onoda et al. 2004). Feng et al. (2009) found that 3.5–9.3% of leaf N is allocated to cell walls in *Eupatorium adenophorum* which was mediated by SLA, and the proportion of leaf N in cell walls decreases with increasing SLA, leaving more N available for allocation to photosynthesis. Higher SLA of the invader indeed contributed to higher $N_{photosynth}/N_L$, and therefore to higher RGR through higher P_{max} and A_{growth} (Figs. 2e, f, 3). The higher stomatal conductance may also contribute to higher photosynthesis in the invader, while similar N_L of the invader and natives may not (Table 2). The invader had both higher PNUE and WUE, breaking the tradeoff between them (Feng et al. 2009), which may confer competitive advantages on the invader especially under barren environments. It is a potential novel mechanism underlying alien plant invasions that invasive plants allocate higher fractions of leaf N to photosynthesis than native plants and native conspecifics (Feng et al. 2009).

Effects of CO_2 enrichment on invasiveness

Growth of *W. trilobata* and natives was significantly stimulated by EC treatment (Table 1), consistent with results of many other studies (Ainsworth and Long 2005; Hättenschwiler and Körner 2003; Raizada et al. 2009; Smith et al. 2000). LAR and SLA could not be used to explain the increased growth, which showed decrease trends under EC. The increased growth could be attributed to increased A_{growth}, which was caused by increased C_i (Table 2). The increased C_i was mainly caused by the elevated atmospheric CO_2 concentrations. CO_2 enrichment decreased N_L but did not significantly affect N allocation to photosynthesis, leading to decreased N contents in photosynthesis, and therefore to decreased photosynthetic capacity, i.e., P_{max} (Table 2). Reduced stomatal conductance may also contribute to the decreased P_{max} under EC. It has been found that P_{max} is significantly correlated with N content in photosynthesis and stomatal conductance (Feng et al. 2009). Down-regulation of photosynthetic capacity is common under prolonged elevated CO_2 concentration (Ainsworth and Long 2005; Medlyn et al. 1999), which could be explained by the decreased foliar N concentrations.

Elevated CO_2 tended to increase RMF and decrease SMF, resulting in a reallocation of biomass from support organs to roots (Table 1). The increased allocation to roots under elevated CO_2 may be driven by an increased need for belowground resources such as N to meet the increased demand associated with faster growth and additional carbon sequestration (Chapin et al. 1995), which are highly dependent on availability and cycling of N (Norby et al. 2010). However, the potential increase in N uptake may only support the increased root production and may not help improve N nutrition at the whole plant level (Johnson et al. 2004). This was confirmed by the decreased N_L (Table 2), which may be due to the dilution effect caused by faster growth. Walch-Liu et al. (2001) found that CO_2 enrichment leads to a preferential N partitioning into roots over shoots in tobacco, reducing leaf Rubisco concentration. Moreover, McGuire et al. (1995) observed a decrease of 21% in leaf and 9% in root N concentrations under CO_2 enrichment, which was confirmed by our results (Table 1).

The responses of *W. trilobata* and natives to elevated CO_2 were not significantly different, as judged by non-significant interactions between species and CO_2 treatment for RGR and many other variables (Table 1). For example, EC increased RGR in *W. trilobata*, *W. urticifolia*, and *W. chinensis* by 8, 11, and 11%, respectively. Similar results were also found by Dukes (2002) under competitive conditions but not under non-competitive conditions. The results suggest that CO_2 enrichment may not exaggerate *W. trilobata* invasion in the future with elevated CO_2. Our results are not consistent with those of many other studies, which found that CO_2 enrichment increases growth more strongly in invasive plants than in natives (Baruch and Jackson 2005; Hättenschwiler and Körner 2003; Raizada et al. 2009; Smith et al. 2000; Song et al. 2009). However, almost all these studies compared phylogenetically unrelated invasive and native species. It has been recognized that responses to CO_2 enrichment are species specific (Ainsworth and Long 2005). The responses are also significantly different between invasive plants (Rogers et al. 2008) and between natives (Ainsworth and Long 2005). Phylogenetically related plants may share more common traits and more overlapping resource requirements than unrelated plants (Goldberg 1987). Comparisons between related invasive and native plants may shed more light on invasiveness of alien plants (Feng et al. 2009), and some recent comparative studies indeed control phylogeny (Grotkopp and Rejmánek 2007; Penuelas et al. 2010).

Recently, Liu et al. (2017) summarized that invasive plants showed a slightly stronger positive response to increased N deposition and precipitation than native plants, but these differences were not statistically significant ($P = 0.051$ for N deposition; $P = 0.679$ for increased precipitation) through meta-analysis with 74 alien and 117 native species. Furthermore, Liu and Van Kleunen (2017) found that alien plant species produced more biomass only when nutrients were supplied as a single pulse in the middle of growth period instead of supplied at a constant rate, whereas the reverse was true for the native species. The findings were also supported by Godoy et al. (2011), who compared 20 invasive alien and 20 widespread native congeners in Spain across nutrient gradients, and found that both groups responded to environmental variation with similar levels of plasticity.

As for CO_2 enrichment, Hager et al. (2016) found that differences in trait means between invasive and non-invasive species tended to be similar across CO_2 levels, which was well in agreement with our results, as CO_2 enrichment showed modest differentiation (Fig. 4). The lack of response to CO_2 may be due to indirect effects of CO_2 on N, for elevated CO_2 can commonly reduce N availability, and thus indirectly limit CO_2 effects on invasion (Luo et al. 2004). For example, elevated CO_2 reduced resin-available soil N by 47%, and tissue N concentration of the invader *Bromus tectorum* by 30% (Blumenthal et al. 2016). In our study, significant decreases in foliar N concentrations were observed in both native and invasive species (Table 2). Thus, these studies collectively provide evidence, albeit circumstantial, that CO_2-induced reductions in N can limit CO_2 effects on invasion, and probably not cause large changes in competitive hierarchy. A more complete model of invasive species responses to CO_2 enrichment will require knowledge of how ecophysiological responses are likely to be mediated by factors such as light, nutrients, competition, and herbivory.

In conclusion, a suite of traits such as consistently higher LMF, SLA, LAR, total leaf area, FRP, $N_{\mathrm{carbox}}/N_{\mathrm{L}}$, $N_{\mathrm{bioenerg}}/N_{\mathrm{L}}$, $N_{\mathrm{photosynth}}/N_{\mathrm{L}}$, N_{carbox}, N_{bioenerg}, $N_{\mathrm{photosynth}}$, P_{max}, A_{growth}, and PNUE, and lower RMF contributed to higher RGR and biomass accumulation in *W. trilobata* in comparison with native *W. urticifolia* and *W. chinensis*, and therefore to invasiveness. CO_2 enrichment increased growth of all studied plants by increasing actual photosynthesis, which was due to increased CO_2 supply rather than increased photosynthetic capacity. The stimulation effect of elevated CO_2 was similar for the invader and natives, indicating that the ongoing increase in CO_2 may not enhance invasion of the invader. Our results were not consistent with the prevailing results that CO_2 enrichment stimulates growth of invasive plants more strongly than growth of natives. The difference may be associated with the fact that most studies in references compared phylogenetically unrelated invasive and native plants. Therefore, more comparative studies of related invasive and native plants are needed to elucidate whether CO_2 enrichment aggravates invasion success of alien plants. On the other hand, many other factors including light, nutrients, competition, and herbivory should be taken into consideration for a more complete understanding on the comparative responses of invasive and native species to CO_2 enrichment.

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