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Effects of elevated CO_2 on photosynthetic traits of native and invasive C_3 and C_4 grasses

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Abstract

Background: Rising CO_2 is expected to result in changes in plant traits that will increase plant productivity for some functional groups. Differential plant responses to elevated CO_2 are likely to drive changes in competitive outcomes, with consequences for community structure and plant diversity. Many of the traits that are enhanced under elevated CO_2 also confer competitive success to invasive species, and it is widely believed that invasive species will be more successful in high CO_2. However, this is likely to depend on plant functional group, and evidence suggests that C_3 plants tend to respond more strongly to CO_2.

Results: We tested the hypothesis that invasive species would be more productive than noninvasive species under elevated CO_2 and that stronger responses would be seen in C_3 than C_4 plants. We examined responses of 15 grass species (eight C_3, seven C_4), classified as noninvasive or invasive, to three levels of CO_2 (390, 700 and 1000 ppm) in a closed chamber experiment. Elevated CO_2 decreased conductance and %N and increased shoot biomass and C/N ratio across all species. Differences between invasive and noninvasive species depended on photosynthetic mechanism, with more differences for traits of C_3 than C_4 plants. Differences in trait means between invasive and noninvasive species tended to be similar across CO_2 levels for many of the measured responses. However, noninvasive C_3 grasses were more responsive than invasive C_3 grasses in increasing tiller number and root biomass with elevated CO_2, whereas noninvasive C_4 grasses were more responsive than invasive C_4 grasses in increasing shoot and root biomass with elevated CO_2. For C_3 grasses, these differences could be disadvantageous for noninvasive species under light competition, whereas for C_4 grasses, noninvasive species may become better competitors with invasive species under increasing CO_2.

Conclusions: The ecophysiological mechanisms underlying invasion success of C_3 and C_4 grasses may differ. However, given that the direction of trait differences between invasive and noninvasive grasses remained consistent under ambient and elevated CO_2, our results provide evidence that increases in CO_2 are unlikely to change dramatically the competitive hierarchy of grasses in these functional groups.

Keywords: C_3 photosynthesis, C_4 photosynthesis, Climate change, Ecophysiology, Elevated CO_2, Grasslands, Invasive species, Plant competition

Background

Rising atmospheric CO_2 is known to alter an array of plant traits, often resulting in enhanced plant growth. Elevated CO_2 has been shown to enhance photosynthetic output, above- and below ground biomass production, and the concentration of photosynthate, resulting in higher C/N ratios [1, 2]. Water use efficiency, as a result of stomatal closure in high CO_2, has also been shown to increase [3–5], contributing to increases in plant biomass through improved drought tolerance. Such changes can enhance primary productivity in a variety of grassland ecosystems, including shortgrass steppe [6], arid

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grasslands [7], calcareous grasslands [8], and tallgrass prairies [9].

Increased CO_2 concentration can alter plant competition in cases where species respond differentially to changes [10]. Competitive outcomes are likely to be altered in favour of species responding positively to elevated CO_2, with consequences for plant community composition and diversity. For example, global change factors, including elevated CO_2, will likely alter the effects of invasive plants on native and managed ecosystems [11]. Invasive species may be more productive under elevated CO_2 for several reasons. The success of invasive species is often greatest in novel, resource-rich environments, and ecosystem invasibility is also related to resource availability [11]. Also, many of the traits that are enhanced in high CO_2 are also those that confer a competitive advantage to successful invaders [12]. Several important invasive species have been shown to respond positively to rising CO_2. For example, the biomass of *Pueraria lobata* (kudzu) increased by 51 % in response to elevated CO_2 [13]. Canada thistle (*Cirsium arvense*), widely considered to be one of the most invasive species in the continental United States, showed a 180 % increase in biomass under elevated CO_2 [14]. In an even more extreme example, *Centaurea solstitialis* (yellow starthistle), one of California's worst weeds, grew 600 % larger in elevated CO_2 relative to ambient, while native plants responded much less strongly or not at all [15]. Within-species studies suggest that traits associated with invasion success, rather than just phylogenetic differences, may account for the response of invasive species to CO_2. For example, Mozdzer and Megonigal [16] examined the responses of two different populations of the same grass species to elevated CO_2 (North American-native and Eurasian-introduced genotypes of *Phragmites australis*) and found that the introduced genotype had stronger responses to CO_2 for all ecophysiological traits measured.

Plant responses to elevated CO_2 are highly dependent on plant functional group (e.g., photosynthetic mechanism, nitrogen fixation, reproductive system, growth form; [2]). Robinson et al.'s [2] meta-analysis of 152 plant species found the largest and most consistent differences between C_3 and C_4 plant groups. Plants with a C_4 photosynthetic mechanism are adapted for low CO_2 environments and contain a biochemical pump that concentrates CO_2 at the site of carboxylation, thus reducing carbon loss through photorespiration. At current levels of CO_2, the carboxylation function of Rubisco in C_4 plants is thought to be near saturation. C_3 plants do not possess this CO_2 concentrating ability, and carbon gains are expected under elevated CO_2 as the concentration gradient of CO_2 from the air to the site of carboxylation increases. Of 365 C_3 plant responses and 37 C_4 plant responses to elevated CO_2 measured, on average, plant biomass was significantly increased in C_3 species but was unchanged in C_4 species [2]. Additionally, the variance associated with C_4 responses was substantially higher than for C_3 plants [2], and this variability is reflected in the literature. For example, Ziska and Bunce [17] found that four of ten C_4 species had higher biomass under elevated CO_2, while eight of ten species had increased rates of photosynthesis, suggesting that not all C_4 species are unresponsive. Additionally, a meta-analysis of C_3 and C_4 responses restricted to the Poaceae found that while C_3 plant biomass increased by 44 % in response to elevated CO_2, C_4 biomass increased by 33 %, suggesting that responses are not readily predicted by photosynthetic mechanism alone [18].

Differences in the average growth responses of individual C_3 and C_4 plants have generally resulted in the predicted competitive outcomes when grown in mixtures. A meta-analysis of competition outcomes for different plant functional groups grown in elevated CO_2 found that when grown in competition, C_3 plants tended to outperform C_4 plants [10]. However, this occurred only in high-nutrient conditions; there were no differences between these groups for low nutrient conditions, and nitrogen-fixing plant species tended to dominate over other plant groups [10]. Thus, functional groups such as C_3 and N-fixing plants that have the ability to exploit enhanced resource availability under elevated CO_2 are likely to be more competitive. Invasive species that fall into these categories are likely to become more aggressive invaders, potentially with increased success of C_3 trees, shrubs, forbs, and grasses invading C_4 grasslands. On the other hand, native and crop C_3 plants may have a competitive advantage over potential invaders (e.g. invasion of C_4 weeds in C_3 crop fields; [19]). However, there is still much to be learned about C_4 plant responses to elevated CO_2, and exceptions to these general responses have been noted. For example, Owensby et al. [9] found that CO_2 increased the production of C_4 grasses but not C_3 grasses in a three-year study of grassland ecosystems using open-top chambers.

Here, we test the hypothesis that plant invasive potential under elevated CO_2 is dependent on photosynthetic mechanism using multiple species in a closed-chamber experiment. We examine responses to elevated CO_2 in 15 grass species (eight C_3 and seven C_4) classified as either "noninvasive" or "invasive" (Table 1) and measured at two separate time points to account for possible CO_2 acclimation phenomena. Specifically, we examine whether photosynthetic and morphological traits associated with productivity, competitive ability, and invasiveness are differentially altered in these groups under elevated CO_2.

Table 1 List of plant species and their photosynthetic, invasiveness, and phylogenetic characteristics

Species name	Common name	Invasiveness	Subfamily (tribe)[a]	Seed source[b]
C_3 photosynthesis				
Brachypodium sylvaticum	Slender false brome	Invasive	Pooideae	Collected: Grey County
Bromus inermis	Smooth brome	Invasive	Pooideae	Collected: Wellington County
Dactylis glomerata	Orchard grass	Invasive	Pooideae	Collected: Wellington County
Elymus repens	Quackgrass	Invasive	Pooideae	Collected: Wellington County
Phalaris arundinacea	Reed canary grass	Invasive	Pooideae	Collected: Wellington County
Schedonorus arundinaceus cv. KY-31 E-	Tall fescue	Invasive	Pooideae	T. Phillips, University of Kentucky
Elymus virginicus	Virginia wild rye	Noninvasive	Pooideae	Wildflower Farms, Ontario
Lolium perenne cv. Nui (A8385)	Perennial ryegrass	Noninvasive	Pooideae	D. Hume, AgResearch, New Zealand
C_4 photosynthesis				
Miscanthus sinensis	Miscanthus	Invasive	Panicoideae (Paniceae)[c]	Jelitto Perennial Seed, Schwarmstedt, Germany
Miscanthus giganteus	Miscanthus	Invasive	Panicoideae (Paniceae)[c]	Mendel Biotechnology, Hayward, California
Panicum miliaceum	Proso millet	Invasive	Panicoideae (Paniceae)[d]	Collected: Wellington County
Andropogon gerardii	Big bluestem	Noninvasive	Panicoideae (Andropogoneae)[c]	Wildflower Farms, Ontario
Bouteloua curtipendula	Sideoats gramma	Noninvasive	Chloridoideae[d]	Wildflower Farms, Ontario
Panicum virgatum cv. Cave-in-Rock	Switchgrass	Noninvasive	Panicoideae (Paniceae)[d]	Ernst Conservation Seeds, Meadville, Pennsylvania
Schizachyrium scoparium	Little bluestem	Noninvasive	Panicoideae (Andropogoneae)[c]	Wildflower Farms, Ontario

[a] Based on GPWG II [46]

[b] Collected from field populations in southern Ontario, unless otherwise indicated

[c] NADP-me C_4 photosynthetic subtype [20]

[d] NAD-me C_4 photosynthetic subtype [20]

Results

Photosynthetic characteristics

There was a significant effect of time on photosynthetic response (Table 2) whereby photosynthesis was higher at 7 than 14 weeks of growth. However, this was dependent on plant species (time × species interaction; Table 2, Additional file 1a). Five species showed large decreases in photosynthetic rate at 14 weeks (C_3: *Elymus virginicus*, C_4: *Bouteloua curtipendula*, *Miscanthus giganteus*, *Miscanthus sinensis*, and *Panicum virgatum*), whereas only the C_3 *Schedonorus arundinaceus* showed a small increase, although none of the within-species changes were significant in a post hoc Tukey's test. Pre-planned contrasts found no differences in photosynthetic rates between C_3 and C_4 plants or invasive and noninvasive plants at any CO_2 levels at 14 weeks of growth (Table 3). Although not statistically significant, photosynthetic rate was 18.4 % higher in C_3 than C_4 plants at ambient CO_2 (390 ppm), but differed by 0–1.7 % at elevated CO_2 (not shown).

There was a significant effect of CO_2 on plant conductance, with lower conductance at higher CO_2 concentrations (Fig. 1a; Table 2). Significant species, time, and species x time effects (Table 2) indicated that conductance was generally lower at 14 than 7 weeks, with the exception of *Schizachyrium scoparium*, which showed the opposite pattern. Contrasts at 14 weeks showed that conductance was higher in C_3 than C_4 plants, and this relationship held across all CO_2 concentrations (Fig. 1a; Table 3). Invasive and noninvasive species had no detectable differences in conductance, with the exception of lower conductance in invasive than noninvasive C_3 species at 700 ppm (Fig. 1a; Table 3).

Stomatal density differed among species, and these differences were dependent on time for the upper leaf surface and on time and CO_2 concentration for the lower leaf surface (species × time and CO_2 × species × time interactions, respectively; Table 2). For the upper surface, there was little change in stomatal density between 7 and 14 weeks except for *Andropogon gerardii,* which showed a large decrease. For the lower surface, stomatal density was generally greater at 14 than 7 weeks, but this pattern differed inconsistently for some species at some CO_2 concentrations. Contrasts at 14 weeks showed that upper leaf stomatal density was lower overall in C_3 than C_4 plants, but this was inconsistent across CO_2 levels, being higher in C_3 than C_4 plants at 700 ppm (Table 3).

Table 2 Summary of ANOVA results

Source	Photo.	Cond.	SD (top)	SD (bot.)	SLA	Tillers	% N	% C	C:N	Shoot	Root
CO_2		***					*		†	†	
	$F_{2,4} = 0.5$	*$F_{2,4} = 234.9$*	$F_{2,4} = 0.3$	$F_{2,4} = 0.3$	$F_{2,4} = 0.6$	$F_{2,4} = 0.5$	*$F_{2,4} = 9.1$*	$F_{2,4} = 0.3$	$F_{2,4} = 6.3$	$F_{2,4} = 5.6$	$F_{2,4} = 4.3$
SP	†	***	***	***	***	***	***	***	***	***	***
	$F_{12,24} = 1.9$	*$F_{12,24} = 16.5$*	*$F_{12,24} = 41.3$*	*$F_{12,24} = 98.0$*	*$F_{12,24} = 10.8$*	*$F_{12,24} = 44.7$*	*$F_{14,28} = 14.4$*	*$F_{14,28} = 9.5$*	*$F_{14,28} = 13.1$*	*$F_{14,26} = 11.4$*	*$F_{14,26} = 21.2$*
$CO_2 \times SP$									†		
	$F_{24,48} = 1.1$	$F_{24,48} = 1.0$	$F_{24,48} = 0.8$	$F_{24,48} = 1.2$	$F_{24,48} = 1.3$	$F_{24,43} = 1.3$	$F_{28,56} = 1.5$	$F_{28,56} = 1.0$	$F_{28,56} = 1.6$	$F_{28,52} = 1.2$	$F_{28,52} = 1.0$
T	**	*		†	*	**					
	$F_{1,2} = 109.1$	*$F_{1,2} = 37.2$*	$F_{1,2} = 1.5$	$F_{1,2} = 10.8$	*$F_{1,2} = 38.3$*	*$F_{1,2} = 197.7$*					
$CO_2 \times T$	$F_{2,4} = 1.8$	$F_{2,4} = 0.2$	$F_{2,4} = 2.4$	$F_{2,4} = 0.1$	$F_{2,4} = 3.9$	$F_{2,4} = 1.1$					
SP x T	†	*	*		***	***					
	$F_{12,24} = 1.9$	*$F_{12,24} = 2.3$*	*$F_{12,24} = 2.8$*	$F_{12,24} = 1.7$	*$F_{12,24} = 6.2$*	*$F_{12,24} = 15.2$*					
$CO_2 \times SP \times T$			†	*	*	†					
	$F_{24,48} = 1.1$	$F_{24,48} = 1.3$	$F_{24,48} = 1.7$	*$F_{24,48} = 176$*	*$F_{24,48} = 1.8$*	$F_{24,41} = 1.6$					

Values in italics represent significant effects

† P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001

Blank fields not analysed, *SP* species, *T* time, *Photo*. photosynthetic rate, *Cond*. conductance, *SLA* specific leaf area, *SD* stomatal density, *bot*. bottom

Table 3 Results of pre-planned contrasts for photosynthetic and growth measures of C_3 vs. C_4 and invasive vs. noninvasive species groups at 14 weeks of growth (see Table 1)

	C_3 vs. C_4 photosynthesis			
	All species	Within CO_2		
		390 ppm	700 ppm	1000 ppm
Photo.	$F_{1,44} = 0.5$	$F_{1,138} = 1.6$	$F_{1,138} = 0.0$	$F_{1,138} = 0.0$
Cond.	***	***	**	**
SD (top)	$F_{1,47} = 31.9$ ***	$F_{1,140} = 15.6$ **	$F_{1,140} = 7.8$ ***	$F_{1,140} = 10.5$ ***
SD (bot.)	$F_{1,45} = 12.3$ ***	$F_{1,112} = 10.0$ ***	$F_{1,112} = 16.4$ ***	$F_{1,112} = 15.0$ ***
SLA	$F_{1,48} = 494.2$ *	$F_{1,129} = 234.0$	$F_{1,129} = 240.1$	$F_{1,129} = 244.3$
Tillers	$F_{1,46} = 5.4$ ***	$F_{1,140} = 2.4$ ***	$F_{1,140} = 0.6$ ***	$F_{1,140} = 2.4$ ***
%N	$F_{1,37} = 319.5$ ***	$F_{1,109} = 165.5$ ***	$F_{1,107} = 90.0$ ***	$F_{1,107} = 121.1$ ***
%C	$F_{1,28} = 131.2$ ***	$F_{1,84} = 58.7$ *	$F_{1,84} = 57.2$ **	$F_{1,84} = 28.0$
C:N	$F_{1,28} = 16.13$ ***	$F_{1,84} = 6.7$ ***	$F_{1,84} = 11.0$ ***	$F_{1,84} = 2.1$ ***
Shoot	$F_{1,28} = 121.0$ ***	$F_{1,83} = 56.6$ ***	$F_{1,83} = 58.8$ ***	$F_{1,83} = 27.2$ ***
Root	$F_{1,26} = 67.5$ ***	$F_{1,75} = 45.9$ ***	$F_{1,76} = 24.2$ ***	$F_{1,76} = 27.7$ ***
	$F_{1,26} = 171.2$	$F_{1,82} = 73.4$	$F_{1,82} = 39.3$	$F_{1,82} = 75.0$

	Invasive vs. Noninvasive											
	All species	C_3 species	C_4 species	Within CO_2			C_3 species within CO_2			C_4 species within CO_2		
				390 ppm	700 ppm	1000 ppm	390 ppm	700 ppm	1000 ppm	390 ppm	700 ppm	1000 ppm
Photo.	$F_{1,44} = 0.4$	$F_{1,44} = 0.2$	$F_{1,44} = 0.1$	$F_{1,138} = 0.4$ †	$F_{1,138} = 2.7$	$F_{1,138} = 0.0$	$F_{1,138} = 1.0$	$F_{1,138} = 3.0$ † **	$F_{1,138} = 0.0$	$F_{1,138} = 0.2$	$F_{1,138} = 0.5$	$F_{1,138} = 0.1$
Cond.	$F_{1,47} = 1.9$ ***	$F_{1,47} = 0.0$	$F_{1,47} = 0.0$ ***	$F_{1,140} = 3.5$ †	$F_{1,140} = 0.2$ *	$F_{1,140} = 0.9$ ***	$F_{1,140} = 1.9$	$F_{1,140} = 2.6$	$F_{1,140} = 0.1$	$F_{1,140} = 0.0$ *	$F_{1,140} = 0.0$ *	$F_{1,140} = 0.0$ **
SD (top)	$F_{1,45} = 12.3$	$F_{1,45} = 1.3$	$F_{1,45} = 17.5$	$F_{1,112} = 3.5$	$F_{1,112} = 6.6$	$F_{1,112} = 11.4$	$F_{1,112} = 1.2$	$F_{1,112} = 0.2$	$F_{1,112} = 1.0$	$F_{1,112} = 6.2$	$F_{1,112} = 6.7$	$F_{1,112} = 17.9$

Table 3 continued

	Invasive vs. Noninvasive											
	All species	C_3 species	C_4 species	Within CO_2			C_3 species within CO_2			C_4 species within CO_2		
				390 ppm	700 ppm	1000 ppm	390 ppm	700 ppm	1000 ppm	390 ppm	700 ppm	1000 ppm
		***	**				***	**	***	*	†	**
SD (bot.)	$F_{1,48} = 0.1$ ***	*$F_{1,48} = 24.2$* ***	*$F_{1,48} = 11.9$*	$F_{1,129} = 0.3$ *	$F_{1,129} = 0.4$	$F_{1,129} = 0.8$ **	*$F_{1,129} = 13.7$* ***	*$F_{1,129} = 8.2$*	*$F_{1,129} = 13.8$* ***	*$F_{1,129} = 5.9$*	$F_{1,129} = 3.0$	*$F_{1,129} = 9.4$*
SLA	*$F_{1,46} = 12.4$* *	*$F_{1,46} = 21.8$* ***	$F_{1,46} = 0.0$ ***	*$F_{1,140} = 6.2$*	$F_{1,140} = 0.1$ **	*$F_{1,140} = 9.9$*	*$F_{1,140} = 14.3$* *	$F_{1,140} = 0.2$ ***	*$F_{1,140} = 12.5$* ***	$F_{1,140} = 0.2$	$F_{1,140} = 0.1$ ***	$F_{1,140} = 0.5$ †
Tillers	*$F_{1,37} = 4.3$*	*$F_{1,36} = 33.0$* ***	*$F_{1,37} = 13.2$*	$F_{1,110} = 0.2$	*$F_{1,107} = 8.1$*	$F_{1,107} = 2.3$	*$F_{1,107} = 6.4$* *	*$F_{1,107} = 13.5$* *	*$F_{1,107} = 20.5$* **	$F_{1,111} = 1.8$	*$F_{1,107} = 14.3$*	$F_{1,107} = 2.8$
% N	$F_{1,28} = 0.7$ †	*$F_{1,28} = 19.5$* ***	$F_{1,28} = 0.1$	$F_{1,84} = 1.7$	$F_{1,84} = 0.8$	$F_{1,84} = 1.1$ *	*$F_{1,84} = 5.9$* *	*$F_{1,84} = 4.3$* *	*$F_{1,84} = 11.6$* **	$F_{1,84} = 0.3$	$F_{1,84} = 0.1$	$F_{1,84} = 0.7$
% C	$F_{1,28} = 4.1$	*$F_{1,28} = 20.4$* ***	$F_{1,28} = 0.4$	$F_{1,84} = 0.8$	$F_{1,84} = 0.0$	*$F_{1,84} = 5.9$*	*$F_{1,84} = 6.6$* *	*$F_{1,84} = 5.0$* *	*$F_{1,84} = 8.0$* ***	$F_{1,84} = 0.0$	$F_{1,84} = 0.1$	$F_{1,84} = 2.4$
C:N	$F_{1,28} = 0.1$ ***	*$F_{1,28} = 20.6$* †	$F_{1,28} = 0.2$ **	$F_{1,83} = 1.3$ ***	$F_{1,83} = 0.7$ **	$F_{1,83} = 1.8$ ***	*$F_{1,83} = 6.7$*	*$F_{1,83} = 5.0$*	*$F_{1,83} = 12.9$*	$F_{1,83} = 0.3$ *	$F_{1,83} = 0.1$ †	$F_{1,83} = 1.1$ **
Shoot	*$F_{1,26} = 39.7$* ***	$F_{1,26} = 3.0$ **	*$F_{1,27} = 12.9$* *	*$F_{1,75} = 24.0$* ***	*$F_{1,76} = 10.7$* *	*$F_{1,76} = 23.4$* ***	$F_{1,75} = 2.0$ **	$F_{1,76} = 0.4$	$F_{1,75} = 2.2$ †	*$F_{1,75} = 6.5$*	$F_{1,75} = 3.2$	*$F_{1,77} = 9.1$* *
Root	*$F_{1,26} = 62.4$*	*$F_{1,26} = 9.5$*	*$F_{1,27} = 5.4$*	*$F_{1,82} = 33.3$*	*$F_{1,82} = 6.8$*	*$F_{1,82} = 33.9$*	*$F_{1,82} = 8.4$*	$F_{1,82} = 0.5$	$F_{1,82} = 3.9$	$F_{1,83} = 2.5$	$F_{1,82} = 0.0$	*$F_{1,83} = 5.6$*

Values in italics represent significant effects

† P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001

Photo. photosynthetic rate, *Cond.* conductance, *SD* stomatal density; *bot.* bottom, *SLA* specific leaf area

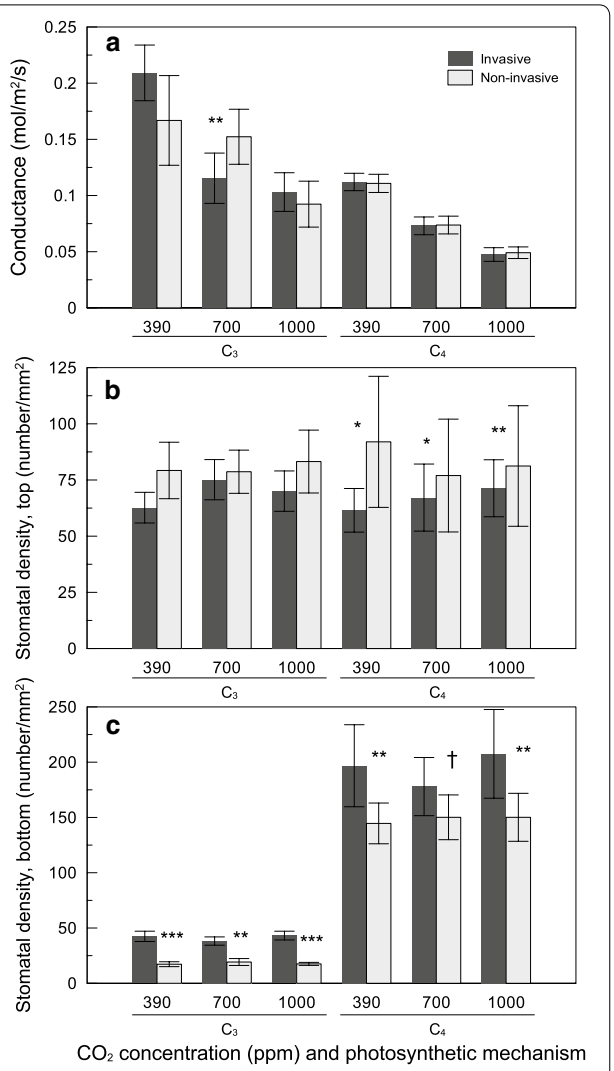

Fig. 1 The effect of CO_2 concentration (ppm), photosynthetic mechanism, and invasive status on **a** conductance, **b** adaxial stomatal density, and **c** abaxial stomatal density (mean ± SE). *Asterisks* represent the degree of significance between invasive status: †P < 0.10, *P < 0.05, **P < 0.01, ***P < 0.001

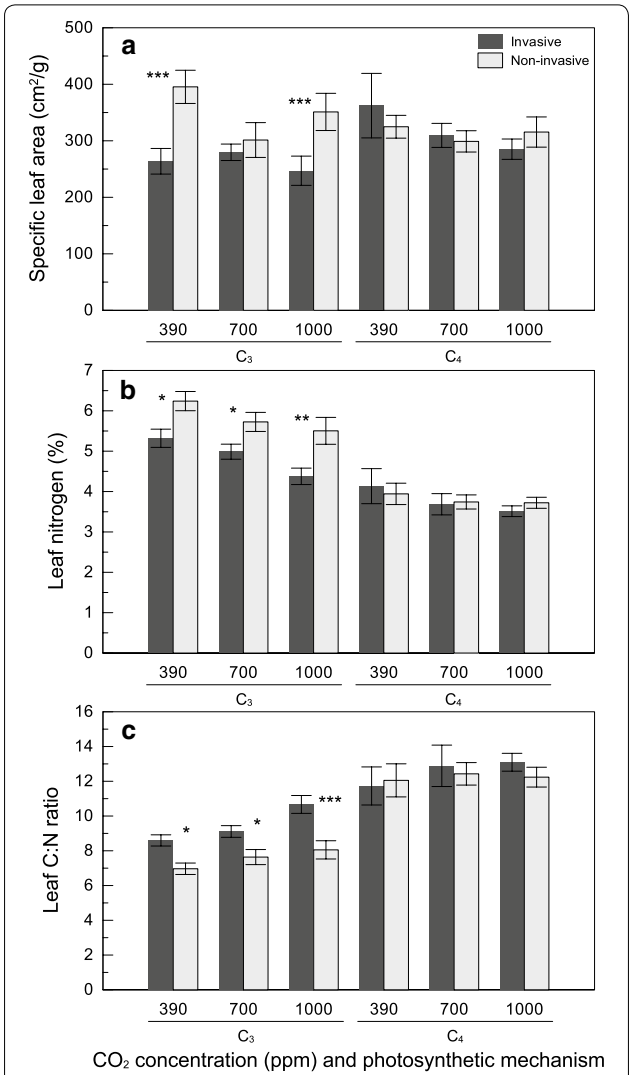

Fig. 2 The effect of CO_2 concentration (ppm), photosynthetic mechanism, and invasive status on **a** specific leaf area, **b** leaf N, and **c** leaf C:N ratio (mean ± SE). *Asterisks* represent the degree of significance between invasive status: †P < 0.10, *P < 0.05, **P < 0.01, ***P < 0.001

Upper stomatal density was lower overall in invasive than noninvasive species, and this pattern was driven by differences between invasive and noninvasive C_4 species, with no differences between invasive and noninvasive C_3 species (Fig. 1b; Table 3). However, absolute differences in upper leaf stomatal density were small. Lower leaf stomatal density was consistently lower in C_3 than C_4 plants across all CO_2 levels, and was consistently higher in invasive than noninvasive C_3 and C_4 species across CO_2 levels (Fig. 1c; Table 3).

Specific leaf area (SLA, unit leaf area per unit leaf weight) differed among species and with time, and those differences depended on CO_2 concentration (Table 2). SLA decreased between 7 and 14 weeks for six of the species, and increased or showed no change over time for the remainder, with no clear trends among CO_2 concentrations. Contrasts at 14 weeks showed lower overall SLA in C_3 than C_4 plants, but this pattern was not detected when CO_2 levels were examined individually (Table 3). SLA was also lower in invasive than noninvasive C_3 species, except at 700 ppm (Fig. 2a; Table 3).

Nitrogen and carbon

Nitrogen concentration (%N) decreased significantly under elevated CO_2 (Fig. 2b; Table 2). There was also an effect of species on %N, with highest concentrations in the C_3 species *Lolium perenne, Elymus virginicus*, and

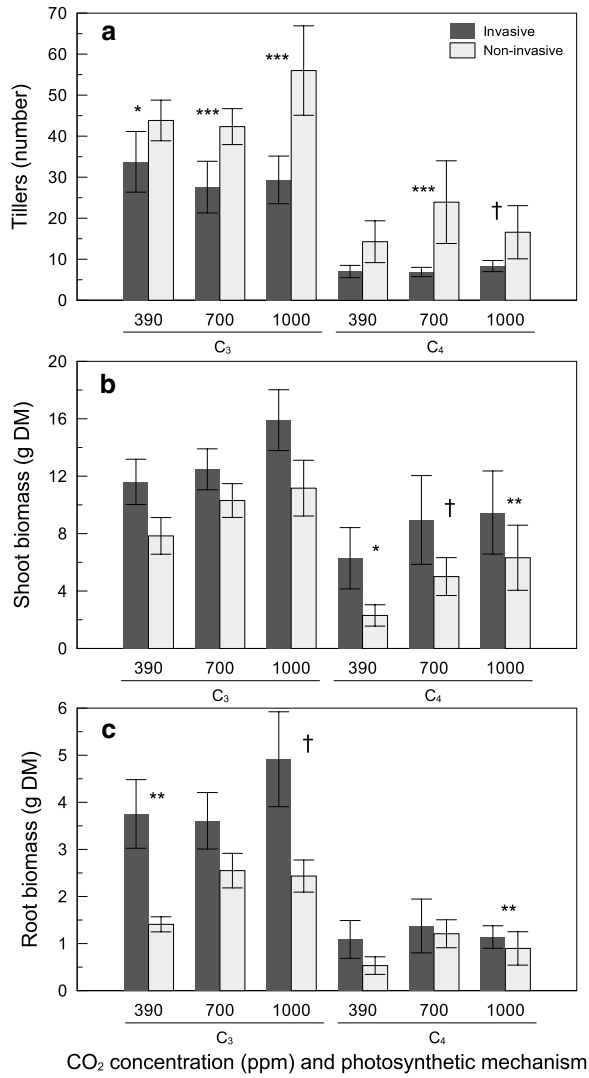

Fig. 3 The effect of CO_2 concentration (ppm), photosynthetic mechanism, and invasive status on **a** tillers, **b** shoot biomass, and **c** root biomass (mean ± SE). *DM* dry mass. *Asterisks* represent the degree of significance between invasive status: †P < 0.10, *P < 0.05, **P < 0.01, ***P < 0.001

Phalaris arundinacea, and lowest concentrations in the C_4 species *Miscanthus sinensis, Miscanthus giganteus,* and *Bouteloua curtipendula* (Additional file 1b). Contrasts showed that %N was significantly higher for C_3 than C_4 plants at all CO_2 concentrations (Table 3). %N was lower in invasive than noninvasive C_3 species across CO_2 levels but did not differ for C_4 species (Fig. 2b; Table 3).

Carbon concentration (%C) differed among plant species (Table 2) and was lower in *Schedonorus arundinaceus* and *Lolium perenne* than in all other species. Contrasts revealed that %C was slightly lower in C_3 than C_4 plants except at the highest CO_2 level. %C was higher in invasive than noninvasive C_3 species across CO_2 levels but did not differ for C_4 species (Table 3).

There was an effect of species on the C/N ratio (Table 2), with highest C/N in the C_4 species *Miscanthus sinensis, Miscanthus giganteus, Bouteloua curtipendula,* and *Andropogon gerardii,* and lowest C/N in the C_3 species *Bromus inermis, Phalaris arundinacea, Elymus virginicus*, and *Lolium perenne.* Both CO_2 and CO_2 x species were weakly significant (Table 2), with C/N tending to increase under elevated CO_2, but more for some species than others. Contrasts showed that differences in C/N followed a similar pattern to %C. That is, C/N was lower in C_3 than C_4 plants across CO_2 levels, and was higher in invasive than noninvasive C_3 species across CO_2 levels but did not differ for invasive and noninvasive C_4 species (Fig. 2c; Table 3).

Plant growth and dry mass

Tiller production was affected by species, time, and their interaction, but not CO_2 (Table 2). Tiller number increased between 7 and 14 weeks for all species except *Andropogon gerardii,* which did not change. Contrasts at 14 weeks showed that tiller number was higher in C_3 than C_4 plants across CO_2 levels (Table 3). Invasive C_3 and C_4 species had fewer tillers than their respective invasive species across all CO_2 levels except for C_4 plants at ambient CO_2 (Fig. 3a; Table 3).

There was a significant effect of species on both shoot and root biomass (Table 2). Shoot biomass was significantly greater in *Elymus repens, Dactylis glomerata, Lolium perenne, Phalaris arundinacea, Schedonorus arundinaceus,* and *Panicum miliaceum* than in *Miscanthus sinensis, Bouteloua curtipendula,* and *Andropogon gerardii.* Root biomass was significantly greater in *Elymus repens, Dactylis glomerata, Lolium perenne, Phalaris arundinacea, Schedonorus arundinaceus, Bromus inermis, Elymus virginicus,* and *Brachypodium sylvaticum* than in *Miscanthus sinensis, Bouteloua curtipendula,* and *Panicum virgatum.* There was a weak effect of CO_2 on shoot biomass whereby mass tended to increase under elevated CO_2 (Table 2). Contrasts detected greater shoot and root mass in invasive than noninvasive species when pooled as well as separated by photosynthetic mechanism (Table 3). However, patterns were weaker when examined across CO_2 levels. For C_4 plants, invasive species had greater shoot mass across CO_2 levels (Fig. 3b) and greater root mass at 1000 ppm (Fig. 3c). For C_3 plants, invasive species had greater root mass at ambient and 1000 ppm, but no differences were detected in shoot mass across CO_2 levels (Fig. 3b, c; Table 3).

Discussion

C_3 vs. C_4 responses to CO_2

Elevated CO_2 resulted in the typically expected changes [2, 18] for some photosynthetic and growth responses at 14 weeks in the grasses studied but not for others. As expected, conductance was greater for C_3 than C_4 grasses at all CO_2 levels and decreased with increasing CO_2. Similarly, %N was higher in C_3 than C_4 grasses at all CO_2 levels and decreased with increasing CO_2, whereas the opposite pattern held for C:N, most strongly due to the contribution of %N (however, Taylor et al. [20] raise the possibility that the commonly observed C_3–C_4 differences in grass leaf N could be a partial effect of phylogeny, which was not examined here). In contrast, photosynthetic rates are expected to be lower in C_3 than C_4 grasses at ambient CO_2 and to increase more for C_3 than C_4 grasses with elevated CO_2 (but see [18]). However, we detected no differences in photosynthetic rates between C_3 and C_4 grasses at 14 weeks. Although this result might have been caused by greenhouse conditions that were more optimal for C_3 than C_4 growth (but see [18]), such an effect should emphasize a greater increase in C_3 than C_4 photosynthetic rates with increases in CO_2, which was not the case. Overall, photosynthetic rates decreased with time, and additional contrasts at 7 weeks detected the expected lower photosynthetic rates in C_3 than C_4 grasses at ambient CO_2, and a loss of that difference with elevated CO_2 (Additional file 2). Decreasing photosynthetic rates over time could be attributed to increasing light limitation (although natural-light day-length had increased) and/or a CO_2 acclimation response, for example, due to root restriction [21], with corresponding downregulation of photosynthetic enzymes [22, 23]. Indeed, photosynthetic rate decreased with time for more C_4 than C_3 grasses (4 of 7 vs. 1 of 8, respectively), but there was no change with time for the remaining C_4 and 6 of the 7 remaining C_3 grasses, so evidence for either mechanism of decline is equivocal.

Typical expected photosynthetic differences should also translate to biomass responses, with greater increases in productivity for C_3 than C_4 plants with elevated CO_2 [10, 22]. We detected marginally significant increases in shoot biomass with increases in CO_2, but the lack of CO_2 x species interaction suggests that the increases were similar for C_3 and C_4 grasses. The lack of a root biomass or tiller number response to elevated CO_2 corresponds with results for photosynthetic rate. The overall higher productivity of C_3 than C_4 grasses could be a result of potentially preferential conditions for C_3 growth; i.e., C_4 usually prefer high light and warmer, drier conditions than do C_3 plants [22].

Finally, the responses of both SLA and stomatal density to elevated CO_2 have been observed to vary inconsistently among grass species, even within photosynthetic mechanism. Although SLA is generally expected to decrease with increasing CO_2 (e.g., [10, 18, 24]), studies of C_3 grasses find that different species respond differently to elevated CO_2 [24–26]. Our results were consistent with previous findings in that the effect of CO_2 varied among species. Overall, however, SLA was lower for C_3 than C_4 grasses, indicating that C_3 grasses tended to have thicker or denser leaf tissue. Although stomatal density has been proposed to decrease with elevated CO_2 because of energetic costs [27] or redistribution of stomata due to increases in vascular tissue [28], stomatal density has been found to differ by species in response to elevated CO_2, even within photosynthetic mechanism [28] and genus (e.g., *Panicum* [29]). Species-specific differences would explain our nonsignificant CO_2 effect but significant CO_2 x species x time interaction. Although we were unable to detect CO_2-based differences within species (within the species x CO_2 x time interaction), trends indicate different responses to elevated CO_2 for within-genus pairs (i.e., *Elymus, Miscanthus,* and *Panicum*). The lack of a strong CO_2 main effect on stomatal density suggests that differences in conductance among CO_2 levels are a result of physiological control of stomatal aperture behaviour, rather than plasticity in stomatal density [28].

Invasive vs. noninvasive responses to CO_2

Although we detected differences between invasive and noninvasive grasses for some photosynthetic and growth responses across CO_2 levels, the differences frequently depended on the photosynthetic mechanism. Invasive C_3 grasses had lower SLA and leaf N content, and higher leaf C and C:N ratio than did noninvasive C_3 grasses, whereas invasive C_4 grasses had lower upper leaf stomatal density than noninvasive C_4 grasses. When the responses did not differ by photosynthetic mechanism, they were always in the same direction. That is, invasive grasses had higher stomatal density on the lower leaf surface, produced fewer tillers, and had greater shoot and root biomass than native grasses for both C_3 and C_4 grasses.

Differences between invasive and noninvasive grasses were consistent across CO_2 levels for many of the traits measured (i.e., magnitudes of the differences were <10 %). Thus, invasive and noninvasive C_3 grasses responded similarly to elevated CO_2 for lower leaf stomatal density, SLA, leaf N, and C:N. Invasive and noninvasive C_4 grasses responded similarly to elevated CO_2 for lower leaf stomatal density and number of tillers. In contrast, invasive grasses were either more or less responsive than noninvasive grasses to elevated CO_2 for some traits.

For C_3 plants, noninvasive grasses responded to elevated CO_2 with increases in tiller numbers, whereas invasive grasses did not, as well as with greater per-gram increases

in root biomass than did invasive grasses (although absolute increases were similar). Thus, under the nonlimiting nutrient and water conditions of our experiment, noninvasive C_3 grasses appear to invest more in belowground tissue and clonal expansion under elevated CO_2 than do invasive C_3 grasses, which could be disadvantageous in competition for light. However, we did not measure plant height or total leaf area, which would allow better determination of this potential trade-off.

For C_4 plants, the difference between invasive and noninvasive upper leaf stomatal density decreased with elevated CO_2, but persisted. Noninvasive grasses also had greater per-gram increases in shoot and root biomass than did invasive grasses (slightly greater absolute increases). Thus, although the invasive grasses always had greater absolute shoot and root biomass than the noninvasive grasses, noninvasive C_4 grasses may become less disadvantaged in competition with invasive C_4 grasses under elevated CO_2. This idea contrasts with previous findings of potentially increased success of invasive grasses under elevated CO_2 [30, 31].

Given that the direction of differences between invasive and noninvasive grasses did not change with elevated CO_2 for any of the measured traits, we conclude that elevated CO_2 is unlikely to alter significantly the competitive hierarchy of species within these functional groups given that many of these traits are considered indicative of invasive ability [32, 33]. Our findings echo those of previous studies that found no effects of elevated CO_2 on the relative growth rate rankings of 19 species [34] or on the competitive rankings of 14 species pairs [31] from multiple functional groups, suggesting that "winners always win" [34]. However, chamber and field experiments examining competitive outcomes under elevated CO_2 as well as in combination with various resource limitations (e.g., [35]) will be required to determine which species are winners under other conditions because individual plant responses to CO_2 may not scale predictably to the community level [10, 36].

Invasive traits of grasses

Overall differences between the invasive and noninvasive grasses were not always in the expected directions based on previous large-scale multispecies trait analyses (e.g., [37–39]. For example, we found that invasive grasses had lower SLA and leaf N than noninvasive grasses, although their photosynthetic rates were similar. However, the invasive grasses we studied had greater biomass allocation to shoot and root production than the noninvasive grasses, indicating higher nitrogen productivity [40]. The greater shoot biomass but lower tiller production of invasive grasses suggests that they were taller or had greater total leaf area than the noninvasive grasses, and they may have had an early higher growth rate advantage. In a greenhouse experiment, Reichmann et al. [41] also found that an invasive grass was able to maintain greater biomass than three native grasses, even though its initially higher SLA and relative growth rate converged with those of the natives over time. A field study that surveyed one invasive and three noninvasive C_4 grasses also found that the invasive grass had lower SLA and leaf N but higher photosynthetic activity, suggesting higher nitrogen productivity, and the invasive grass began its growing season earlier than the natives [42]. Thus, invasive grasses may be successful because of early season advantages that allow competitive resource pre-emption [41], and further research should pursue this area of inquiry. We note also that quantitative syntheses lumping functional groups, experimental environments, and different physiological traits into trait groups may be obscuring some trait relations that could be important determinants of invasive success in certain species groups.

Overall, invasive species had fewer stomata on the top leaf surface than did noninvasive species, although this relationship was driven by the C_4 grasses and was not statistically significant in the C_3 grasses. To our knowledge, stomatal density has not been examined previously as a potential trait related to invasion success. However, in an extensive quantitative review of stomatal distribution, Muir [43] concluded that the proportion of stomata on each leaf surface is highly constrained by selective pressures to maximize photosynthesis rates while minimizing fitness costs. Minimizing the number of stomata on the upper leaf surface could reduce the risk of infection by foliar pathogens [43]. Thus, it is possible that some invasive plants are escaping natural enemies via altered stomatal distribution. This idea remains to be tested.

Conclusion

Our experimental design allowed us to examine traits in a suite of species for different plant functional groups over time. Plant traits associated with increased invasion success are not always enhanced in invasive species under elevated CO_2, and the ecophysiological mechanisms underlying invasion success of C_3 and C_4 grasses may differ. Given that the direction of trait differences between invasive and noninvasive grasses remained consistent under ambient and elevated CO_2, our results provide evidence that increases in CO_2 are unlikely to change dramatically the competitive hierarchy of grasses in these functional groups. A more complete model of invasive species responses to global change will require knowledge of how ecophysiological responses are likely to be mediated by factors such as light, nutrients, and herbivory.

Methods

CO_2 growth chambers

The experiment was conducted in the E.C. Bovey Greenhouse at the University of Guelph, Ontario, in nine CO_2-controlled plexiglass closed-top chambers arranged in a 3 × 3 square. Chambers were constructed and operated according to Grodzinski et al. [44]; they were 82 (height) × 52 × 45 cm and were computer controlled to maintain CO_2, temperature (23 °C), and humidity (~40 %) levels using an Argus Greenhouse Control System (Argus, Surrey, British Columbia). We used three CO_2 concentrations that are within the range of the projected increase by the year 2100 [45]: ambient (390 ppm) and two elevated (700 and 1000 ppm). The nine chambers were blocked according to a light gradient in the greenhouse, with one chamber of each CO_2 concentration per block, for a total of 3 blocks. Lighting followed a 16:8 light/dark cycle. Supplementary artificial metal halide lights (approx. 150 μmol/m^2/s in the absence of daylight) were used when natural light fell below 600 μmol/m^2/s. Maximum external ambient light levels during the experimental period ranged from 2120 μmol/m^2/s (October) to 1371 μmol/m^2/s (December; estimated interior max. of 1000–1570 μmol/m^2/s); these were 25–65 % of external light levels in August (max. 3032 μmol/m^2/s).

Plant material

Fifteen grass species (eight C_3 and seven C_4 species; see Table 1 for details and sources) were chosen for the experiment based on invasive status and seed availability. These species grow and can co-occur in pastures, grasslands, and roadside ditches, and *Miscanthus giganteus* is currently cultivated as a bioenergy feedstock, in Ontario and elsewhere in North America. Species were classified as invasive or noninvasive based on information from several databases: the Invasive Species Compendium (http://www.cabi.org/isc/); Ontario Ministry of Agriculture and Food *Ontario Weeds* (http://www.omafra.gov.on.ca/english/crops/facts/ontweeds/weedgal.htm), and Urban Forest Associates Inc. (http://ufora.ca/index.php/resources/invasive-species/). Many of these species are well-known invaders.

Grasses were germinated from seed at their CO_2 treatment concentrations in greenhouse flats with LC-1 potting soil (Sun Gro-sunshine soil mix containing Canadian Sphagnum peat moss, coarse perlite, organic starter nutrient charge, Gypsum and dolomitic limestone). Three weeks after planting, seedlings were transferred into PVC pots (0.6 cm thick, 7.6 cm diameter PVC pipe cut to 45.7 cm height [1.73 L] and the bottom covered with mesh for drainage) containing the same potting mix. Each species was replicated once per chamber and three times per CO_2 concentration for a total of 189 pots. Plants were watered ad libitum with alternating deionized and fertilized water (1.25 g/L N-P-K, 20-8-20). On days when photosynthesis was measured, all chambers received deionized water on the morning of data collection. Plants were grown for 14 weeks; any inflorescences that grew during this time were removed, dried, and weighed. At the end of the experiment, plants were harvested and separated into shoots and roots. Although root growth was extensive, roots were not observed to fill the pot volume. Roots were thoroughly washed, and all material was dried for at least 48 h at 55 °C in a forced air oven before being weighed.

Measurement of plant traits

We measured photosynthetic rate, conductance, vegetative tiller number, and stomatal density at two time points over the course of the experiment (~7 and 14 weeks post-germination). Photosynthesis and conductance were measured using a portable infrared gas analyzer (LI-6400 Portable Photosynthesis System; LI-COR, Lincoln, Nebraska). The 2 × 3 cm LI-COR leaf clamp had an opaque LED light source (LI-6400-02B red/blue LED #670) set to 1600 μmol/m^2/s and a CO_2 injector (LI-6400-01 CO_2 Injector System) that controlled the clamp chamber concentration to that of the growth chamber in which each plant was grown. The fully expanded, upper canopy leaf was measured between 9 am and 4 pm on data collection days. Due to time and daylight constraints, measurements were staggered such that plants from different blocks were measured on different days. After clamping the leaf into the LI-COR, each plant was allowed to acclimate to the light intensity until readings stabilized. An automatic logger was then initiated to record values every 20 s for 2 min (total of six measurements per species), which were subsequently averaged. Most of the leaf blades were not wide enough to cover the entire 2 × 3 cm leaf clamp. In these cases, the leaf was marked while still in the clamp, removed from the plant, and the width at each end measured using callipers; area was calculated as the area of a trapezoid. The leaf segment was then dried for 48 h at 55 °C in a forced air oven and used to calculate specific leaf area (SLA; leaf area to dry mass ratio). This tissue was then analyzed for carbon and nitrogen content (second time point only) using an elemental analyzer (vario Max CN analyzer, Elementar Analysesysteme Gmbh, Hanau, Germany).

A small section of leaf blade directly adjacent to the clamp section was used for taking cuticle prints from both the top and bottom of the leaf blade. A thin film of clear nail polish was brushed onto the cuticle. Once dried, the polish was removed with clear tape and placed onto a microscope slide. The total number of

stomata was counted under a light microscope at 40× magnification and expressed as the number per area (in mm^2) of plant tissue on both the top and bottom prints.

Statistical analysis

Responses that were measured only at 14 weeks were analysed using a blocked split-plot design with CO_2 as the whole-plot factor and species as a sub-plot factor, where individual chambers constituted the unit of replication. Responses that were measured at 7 and 14 weeks were analysed using the same design with an additional split-plot effect of time to account for the repeated measures. All analyses were performed using mixed effects ANOVA with species, CO_2, and time as fixed factors, and block as a random factor. All block-factor interactions (except the highest order interaction) were included as error terms. Box-Cox transformation was used to homogenize the residual variance, and examination of the residuals following transformation suggested that assumptions of ANOVA were met. Two species (*Brachypodium sylvaticum* and *Phalaris arundinacea*) were excluded from analyses of photosynthesis, conductance, and stomatal density due to missing values. For each response variable at 14 weeks, we conducted several pre-planned contrasts: C_3 vs. C_4, invasive vs. noninvasive, C_3 invasive vs. C_3 noninvasive, C_4 invasive vs. C_4 noninvasive, and all interactions involving CO_2. Analyses were conducted in JMP 10.0 and 12.0 (SAS Institute, Cary, NC). In text and figures, we report untransformed means and standard errors as a measure of data dispersion. Individual plant species means and standard errors are provided in Additional file 1 in the supplemental material for all CO_2 concentrations and time points.

Additional files

Additional file 1. (a) Mean ± SE for individual species across CO_2 concentrations for responses measured at 7 and 14 weeks, (b) mean ± SE for individual species across CO_2 concentrations for responses measured at 14 weeks only.

Additional file 2. Results of contrasts for photosynthetic rate at 7 weeks.

Abbreviations

C: carbon; C_3: Calvin cycle photosynthetic pathway; C_4: Hatch-Slack cycle photosynthetic pathway; CO_2: carbon dioxide; N: nitrogen; ppm: parts per million; SLA: specific leaf area; ANOVA: analysis of variance.

Authors' contributions

HAH and GDR collected seeds, designed the experiment, collected and analysed data, and wrote the manuscript. HMK cared for the plants, collected and processed data, and helped write the methods. JAN designed the experiment, helped with statistical analysis, and performed manuscript edits. All authors read and approved the manuscript.

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Availability of supporting data

The data sets supporting the results of this article are available in the University of Guelph Agri-environmental Research Data Repository, http://www.hdl.handle.net/10864/TZBTY [47].

Competing interests

The authors declare that they have no competing interests.

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