

Ozone and density affect the response of biomass and seed yield to elevated CO₂ in rice

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Abstract

Tropospheric O₃ reduces growth and yield of many crop species, whereas CO₂ ameliorates the negative effects of O₃. Thus, in a combined elevated CO₂ and O₃ atmosphere, seed yield is at least restored to that of charcoal-filtered (CF) air at ambient CO₂. The CO₂-induced yield increase in CF air is highly variable, suggesting other potential resource limitations. To understand such variability in response, we tested that (1) competition for resources precludes some of the CO₂ enhancement on biomass and yield; and (2) O₃ reduces competition in elevated CO₂. We grew rice (*Oryza sativa* L.) at five densities in CF and O₃-fumigated (+O₃) air at ambient (A) and elevated [CO₂] (+CO₂) in 1997 and 1998. O₃ reduced biomass by 25% and seed yield by 13–20% in A, but had little effect in +CO₂. A competition model of biomass and yield response to density based on resource availability without competition showed that fewer resources were used for biomass in +O₃ than in CF (average 53% vs. 70%) in A, while in +CO₂ 85% of resources were used for biomass regardless of O₃ suggesting greater depletion of resources. The enhanced biomass response to CO₂ with O₃ is consistent with a 22% greater CO₂ enhancement ratio [mass in +CO₂ air/mass in A air; enhancement ratio (ER)] in +O₃ than in CF air. For seed yield, few resources were used (average 17% and 25% for CF in 1997 and 1998, respectively), and ER was 13% greater in +O₃. With competition the rate of change of individual plant biomass to density was not affected by +CO₂ in CF air in 1997 but was increased 19% with more nutrients in 1998, indicating resource limitations with +CO₂. The rate of change of individual plant yield to density was reduced with CO₂ in 1997 and unchanged in 1998 showing a different response to resource limitation for reproductive biomass. The resource use in +O₃-A suggested that increased density and soil fertility might compensate for pollutant damage. Although ambient [O₃] can modulate the response to elevated CO₂, resource limitation precludes the CO₂ fertilization impact and both factors need consideration for better management and forecasts of future productivity.

Keywords: competition model, CO₂ × O₃ interaction, density, nutrients, *Oryza sativa*, resource use

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Introduction

Plant growth is directly affected by two important trace gases associated with global change, atmospheric CO₂ and tropospheric O₃. Atmospheric CO₂ will likely double from its preindustrial concentration during the next century; likewise, tropospheric O₃ is rising globally (IPCC, 2001). Numerous studies examining the indi-

vidual effects of these trace gases have reported that elevated CO₂ usually increases plant growth and often increases yield for annual species, while pollutant O₃ reduces both (Ainsworth *et al.*, 2002; Jablonski *et al.*, 2002; Long *et al.*, 2004; for CO₂; Davidson & Barnes, 1998; Morgan *et al.*, 2003 for O₃). Studies investigating the combined effects of these trace gases on herbaceous species show that elevated CO₂ usually ameliorates the negative effects of pollutant O₃ on plant growth and yield (Fiscus *et al.*, 1997, 2002; McKee *et al.*, 1997; Mulholland *et al.*, 1997; Volin *et al.*, 1998;

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Cardoso-Vilhena & Barnes, 2001; Heagle *et al.*, 2002) and can result in an increased CO₂ enhancement ratio [mass in +CO₂ air/mass in A air, enhancement ratios (ER); Fiscus *et al.*, 2002, 2005]. Such studies were designed to examine plants grown individually in optimal environmental conditions. However, not all managed or natural systems subjected to increased atmospheric CO₂ and O₃ will be free of limitations by other resources. Plants will experience simultaneous enhanced CO₂ and O₃ in conditions that are likely to be resource limited. Because of their impact on food production and on native species dispersal and recruitment, understanding limitations of seed yield in projected future atmospheres is critical for proper management of agricultural and native species.

Singly, elevated CO₂ causes a range of responses depending on species and growth conditions. For vegetative plant growth, the responses ranged from large enhancements to some reductions of biomass (Ainsworth *et al.*, 2002; Poorter & Navas, 2003). Poorter & Navas (2003) speculated that the range was due to a nutrient stress effect that might limit the CO₂ enhancement while experiments in a high-O₃ environment would overestimate the CO₂ enhancement. For reproductive yield, the responses varied (Jablonski *et al.*, 2002) and often included a lack of CO₂ effect as, for example, soybean (Amthor *et al.*, 1994; Prior & Rogers, 1995), wheat (*Triticum aestivum* L.) and rice (*Oryza sativa*) at low nitrogen (Kim *et al.*, 2003). Ainsworth *et al.* (2002) suggested that seed yield of soybean increased with elevated CO₂ only in 'unstressed conditions,' the nutrient status being more important in limiting production than CO₂. Although seed yield of crop species increased on average 28%, yield of wild species decreased ca. 4% (Jablonski *et al.*, 2002). Most wild annuals lacking a reproductive response to CO₂ were grown in their natural competitive environment probably with varying resource availability. Körner (2003) suggested that soil fertility might help explain the range of reproductive responses to elevated CO₂ reported in Jablonski's (2002) survey. We also suggest, as pointed out by Poorter & Navas (2003) for vegetative growth, that inadvertent exposure to pollutant O₃ in some studies may also explain the observed variation in seed yield. Fiscus *et al.* (1997, 2002) already reported an average CO₂ effect of 3% on seed yield for annual crops in CF but a mean enhancement of 50% in O₃-fumigated air; however, shoot biomass showed a mean enhancement above 30% in CF air rising to 80% in O₃-fumigated air. All of these studies suggest that factors other than CO₂ may modulate the biomass and yield response in changing environments.

Singly, tropospheric O₃ reduces annual plant growth and yield although the response depends on species, level of chronic exposure and other environmental

conditions (Mauzerall & Wang, 2001; Morgan *et al.*, 2003). The magnitude of response to O₃ exposure is associated with the O₃ flux into the leaf (Fiscus *et al.*, 1997, 2005) and O₃ tolerance has been related to leaf conductance (Nebel & Fuhrer, 1994; Bungener *et al.*, 1999). The plant's capacity to avoid or repair oxidative damage also determines the plant sensitivity to O₃ (Fuhrer & Booker, 2003), including increased antioxidant levels (Chernikova *et al.*, 2000; Conklin & Barth, 2004) and increased maintenance costs (Amthor, 1988). The plant oxidative defense likely alters carbon partitioning between maintenance and growth, and between above- and belowground biomass (Fuhrer & Booker, 2003; Fiscus *et al.*, 2005). The reduced carbohydrate allocation to roots observed in O₃-injured plants (Andersen, 2003) suggests that nutrient acquisition may also become limiting. Using different pot sizes with O₃ fumigation, Whitfield *et al.* (1996) found that the root to shoot ratio increased the most in the smallest pots and suggested that belowground limitations altered the O₃ response.

Elevated atmospheric CO₂ increases the availability of one potentially limiting resource for photosynthesis and growth and, thus, may increase the significance of limitations by other resources. In greenhouse studies, elevated CO₂ increased leaf area index and shoot biomass for wheat grown at low but not at high density (Du Cloux *et al.*, 1987), suggesting such competition for resources. The reproductive output was decreased with increasing density for *Abutilon theophrasti* in greenhouse studies, with no additional effect of elevated CO₂ (Bazzaz *et al.*, 1992); for *Avena barbata* in open-top chambers (OTCs) at elevated CO₂ (Jackson *et al.*, 1994); and for *Bromus erecta* at high density in a grassland Free-Air Carbon dioxide Enrichment (FACE) experiment (van Kleulen *et al.*, 2006). These studies suggest that increased competition for resources has a direct effect on reproductive yield at elevated CO₂ and that increased competition at high-density limits the potential enhancement effect of CO₂. In FACE experiments using current standard cultural practices, that is optimal density and fertilization, the CO₂ enhancement effect on yield of soybean (Morgan *et al.*, 2005) or rice (Kim *et al.*, 2003) was lower than suggested by previous studies. These lower yields may reflect enhanced competition for resources in the future. Although studies have reported a reduced growth response to elevated CO₂ in herbaceous species when grown at high density in a monoculture (Du Cloux *et al.*, 1987; Bazzaz *et al.*, 1992), most elevated CO₂ studies on plant competition have dealt with interspecific competition among multiple species (e.g. Berntson *et al.*, 1998; Navas *et al.*, 1999; Edwards *et al.*, 2001; Thürig *et al.*, 2003). To our knowledge, none of them examined the potential effect of

pollutant O₃ as a mediator of these competitive interactions on annual species.

The objective of this study was to determine whether competition for resources other than CO₂ affects the biomass and seed yield response of annual plants grown in elevated CO₂ at different [O₃]. The hypotheses tested were that (1) plant competition for resources limits the growth and seed yield response to elevated CO₂; and (2) pollutant O₃ reduces or eliminates that competition for resources because of reduced growth.

Materials and methods

Plant material and cultural practices

Rice (*O. sativa*, hybrid Lemont/Qi Gui Zao) is an important crop globally and provides a good model for annual species responses to trace gases. Its upright growth habit permitted high density and yield that was advantageous in the limited space of the OTC (7 m²) used at the USDA/ARS Plant Science Research Unit field site, 5 km south of Raleigh, NC, USA. In April 1997, two seeds each were planted in peat pellets, 35-mm diameter [Jiffy-9[®], Jiffy Products Ltd, Hummert International, Earth City, MO, USA (The use of trade names in this publication does not imply endorsement by the US Department of Agriculture, the North Carolina Agricultural Research Service, North Carolina State University or Duke University or criticism of similar ones not mentioned)], that were watered daily and kept on wet sand in flats (Table 1). Seeds were germinated in the greenhouse under supplemental lighting provided by 400 W multivapor lamps (Sylva-

nia, GTE-Sylvania, Charlotte, NC, USA). Before transplanting, the pellets were transferred to open-top field chambers and placed under neutral shade cloth for acclimation. Seedlings were transplanted and the CO₂ enrichment began on May 27. Because of cool overcast weather in 1997, initial growth and seedling establishment were slow so the O₃ exposure was started 3 weeks later. In 1998, three seeds were planted in each peat pellet because of low rates of germination in 1997. Also, the seeds were germinated in the open-top field chambers at their respective CO₂ treatments because of the significant CO₂ effect reported for the first 15 days after planting (Jitla *et al.*, 1997). In 1998, the seedlings were transplanted on May 27 and O₃ exposures started 1 week later. Once established, the transplanted seedlings were thinned to one plant per pellet.

For both years, seedlings were transplanted when 5 cm tall to 15 L pots (29 cm diameter) at five densities. The densities ranged from 1 to 5 plants pot⁻¹ (14–70 plants m⁻², D1–5), spanning current management practices. The increased density in the restricted pot volume insured competition for belowground resources, be it space, water and/or nutrients while also inducing aboveground competition for light. Except for D1, plants were placed at the same distance from the edge of the pot and from each other. Density was replicated four times within each chamber for a total of 20 pots. Plants were grown in a 2 : 1 : 1 (volume) mixture of clay-loam topsoil, sand, and vermiculite-sphagnum-perlite horticultural mix (MetroMix 220; W. R. Grace Co., Cambridge, MA, USA). Plants were fertilized with N–P–K (10–30–20; Peters Blossom Booster, W. R. Grace Co.) once a week and with soluble trace elements and

Table 1 Major phenological events for rice over two growing seasons

	1997				1998			
	Ambient CO ₂		Elevated CO ₂		Ambient CO ₂		Elevated CO ₂	
	CF	+O ₃	CF	+O ₃	CF	+O ₃	CF	+O ₃
<i>Vegetative stage</i>								
Seeding	April 28	April 28	April 28	April 28	May 4	May 4	May 4	May 4
Transplantation	May 21	May 21	May 21	May 21	May 27	May 27	May 27	May 27
<i>Reproductive stage</i>								
Panicle initiation	76	72	73	75	85	84	84	84
Heading	83	81	81	82	94	93	92	93
Flowering	87	84	84	85	97	96	96	96
<i>Ripening stage</i>								
Milking	95	93	92	96	106	103	104	103
Panicle harvest	118	118	118	118	111	111	111	111

The rice was grown in open-top field chambers at five densities (14–70 plants m⁻²; D) either in charcoal-filtered (CF) or O₃-fumigated (+O₃) air at ambient or elevated CO₂. All densities were seeded and transplanted at the same time and calendar dates are used. Days after transplanting (DAT) are used afterwards for ease of comparison. Dates are representative of the lowest density (D1).

micronutrients (Peters S.T.E.M.; W. R. Grace Co.) at the beginning of each month. In 1997, each macronutrient application consisted of full-strength fertilizer (2.5 g L⁻¹ pot⁻¹) until the heading stage (Table 1) and half-strength afterwards (total N 49 g m⁻², total P 148 g m⁻²); the first micronutrient application was full-strength (0.31 g L⁻¹ pot⁻¹) and half-strength thereafter. In 1998, the nutrient application was kept at full-strength past the milking stage (112 DAT; total N 56 g m⁻², total P 169 g m⁻²). All pots, sitting in saucers, were drip-irrigated to flooding daily with overflow water remaining in the saucers.

Experimental design

Combinations of two [O₃]s and two atmospheric [CO₂]s were used as the main treatments. The O₃ treatments consisted of charcoal-filtered air (CF) or CF air to which O₃ was added to reach 1.5 × the average ambient concentrations at the field site from June through August of the previous 4 years (+O₃). CO₂ treatments consisted of ambient (A; ca. 367 μmol mol⁻¹) and elevated CO₂ (+CO₂; ca. 700 μmol mol⁻¹). Delivery of O₃ and maintenance of [CO₂]s in the chambers was as described in Booker *et al.* (1997). The main treatments were replicated three times for a total of 12 OTCs. Treatments were assigned randomly within each of the three blocks. Plant density was used as a split-plot treatment with pots in each chamber arranged in a stratified random design. Within each chamber, the 20 pots were arranged in circles alternating from D1 to D5. Arrangements of the pots, equidistant by 25 cm, consisted of 12 pots on the outside circle and seven pots around a center pot such that all densities were mixed in the chamber. The first pot was placed on the southern edge starting with density 1, 3, and 5, respectively in each of the three replicated blocks.

Yield and biomass measurements

Total biomass, including roots, and seed yield were determined at the end of the growing season. Visible O₃ damage such as necrosis or stipple was not assessed prior or during the harvest. First, the panicles were harvested. In 1997, individual panicles that dried early were harvested before the final harvest to avoid grain loss. In 1998, all panicles were left on the plants until final harvest. After harvest, panicles were dried at 32 °C for a week, then weighed and pooled by pot. After weighing, they were threshed. All seeds were hulled manually after panicle threshing, and seeds were weighed. The remaining aboveground

standing biomass and roots were harvested. The root system was washed of soil before drying by soaking the root mass in large volumes of water and using screen mesh to recover loose roots. All vegetative material was oven-dried to constant weight at 50 °C.

Biomass and yield model

The total biomass data were analyzed according to a simple competition model we developed that is based on biomass at harvest time. For simplicity, we assumed that the production of one biomass unit required one arbitrary resource unit by viewing the plant as the integrating mechanism. An arbitrary resource unit (arbitrary unit) is composed of all the various types of resources needed allowing for CO₂, nutrients, light, and space that includes the root volume. We further assumed that when multiple plants were present they were able to compete equally for the available resources. Estimates of total dry biomass at harvest (B_t g m⁻²) were calculated from the total available resources (R_t , units m⁻²) as

$$B_t = B_p D \text{ if } R_p D \leq R_t, \text{ or as} \quad (1)$$

$$B_t = B_p D F_a \text{ if } R_p D > R_t, \quad (2)$$

where B_p is the total biomass per plant (g plant⁻¹) when competition is not limiting productivity; D is the plant density (# plants m⁻²); R_p is the total of the available resources used per plant when resources are *in excess*, that is, the resource requirement for each plant (unit plant⁻¹); F_a is the fraction of the resource requirement available to each plant when resources are limiting and is expressed as $R_t D^{-1} R_p^{-1}$. This simple competition model was rearranged as a function of available area per plant (m² plant⁻¹) so that the model slope would represent the competition-limited productivity per unit land area, that is, when multiple plants compete for limited resources. The rearranged Eqns (1) and (2) and conditions follow as Eqns (3) and (4), respectively, where $1/D$ is the area per plant.

$$\frac{B_t}{D} = B_p \text{ if } 1/D \geq R_p/R_t, \text{ or as} \quad (3)$$

$$\frac{B_t}{D} = \left(B_p \frac{R_t}{R_p}\right) \frac{1}{D} \text{ if } 1/D < R_p/R_t. \quad (4)$$

Thus, in the absence of competition, B_t/D is constant, representing the maximum growth or yield potential of individual plants, while the rate of change of individual

plant productivity (biomass) with area per plant, that is, the productivity slope under competitive conditions is $[B_p(R_t/R_p)]$ and the transition between the two segments of the model is given by $1/D = R_p/R_t$.

Using this model first required setting a value for R_t that was estimated from the upper limit of measured total biomass production for all treatments for each year. The rearranged model was run with means using TABLE CURVE 2D (Systat Software, Point Richmond, CA, USA), which provided estimates of R_p and B_p . The model was also estimated with all data using a segmented method in SAS (SAS Institute Inc., 1986, *SAS/STAT™ User's Guide, Release 6.03 Edition*, Cary, NC, USA): B_p was estimated for plants without competition ($D1$), the productivity slope was estimated for densities greater than $D1$ with the intercept through 0, and R_p was calculated from the slope estimate. Both methods provided similar values for all parameters. Pairwise comparisons of treatments for slope and B_p were also carried out in the segmented method. The vegetative biomass was calculated as total plant biomass minus the seed and panicle mass per plant. Vegetative biomass and yields were modeled and analyzed the same way as total biomass using vegetative biomass per plant (V_p) or seed yield per plant (Y_p), respectively, instead of B_p .

Statistical analysis

The treatment effects on biomass and yield were analyzed using a multifactorial split-plot ANOVA design (SAS Institute Inc., 1986, *SAS/STAT™ User's Guide, Release 6.03 Edition*, Cary, NC, USA). Year, CO_2 , O_3 , and block were treated as the main effects and density was used as a split-plot effect. To correct for heterogeneity of the variances among densities, the natural log of the individual plant biomass variables was used to perform the analyses. In addition, to estimate the effect of competition on individual plant biomass, vegetative biomass, and seed yield, the heterogeneity of the productivity slopes between treatments was tested using generalized linear models (Kleinbaum & Kupper, 1978; SAS Institute Inc., 1986, *SAS/STAT™ User's Guide, Release 6.03 Edition*, Cary, NC, USA) for the slope estimates in the segmented method described above. The CO_2 ERs were calculated from treatment means and standard deviation. We generated the 95% confidence intervals for the ERs by parametric bootstrap (Efron & Tibshirani, 1993). The ratio of 2000 resamples from normal distributions having means and variances obtained from elevated and ambient treatments means were used to obtain the 95th percentile. The resulting 95% confidence intervals were compared with t -test in pairwise comparisons.

Results

Environmental data and fumigation treatment

Environmental conditions differed between the 1997 and 1998 growing seasons. Following seedling transplant in 1997, June was cool and often overcast, and seedling establishment was slow. In contrast, June 1998 mean temperature averaged 3 °C warmer than in 1997 with few cloudy days. July and August 1997 maximum temperatures were above 35 °C for 10 and 7 consecutive days, respectively. The August period coincided with flowering in the + CO_2 but not in the A treatments because phenological development was accelerated in the CO_2 treatments (Table 1). In contrast, the maximum temperature exceeded 35 °C only twice throughout the 1998 growing season.

The CO_2 and O_3 treatments were comparable between years (Table 2). The ambient seasonal $[CO_2]$ s averaged 368 and 364 $\mu\text{mol mol}^{-1}$ in 1997 and 1998, respectively, and the elevated $[CO_2]$ averaged 712 and 701 $\mu\text{mol mol}^{-1}$ in 1997 and 1998, respectively. The 12 h average daily $[O_3]$ s in the CF chambers averaged 27 and 28 nmol mol^{-1} in 1997 and 1998, respectively, and 73 and 77 nmol mol^{-1} in 1997 and 1998, respectively in the + O_3 chambers. The peak-weighted O_3 index (SUM06), which is the sum of hourly average $[O_3] \geq 60 \text{ nmol mol}^{-1}$, was also used to represent $[O_3]$ (Lee *et al.*, 1991). The monthly SUM06 was highest in July 1997 closely followed by July and August 1998. The seasonal SUM06 (mid-June to mid-September) averaged 1.3 $\mu\text{mol mol}^{-1} \text{ h}$ in the CF chambers for both years and 68.2 ± 0.4 and $74.7 \pm 0.8 \mu\text{mol mol}^{-1} \text{ h}$ in 1997 and 1998, respectively, in the + O_3 chambers.

Biomass and yield response to density, O_3 and CO_2

For all biomass parameters examined, the plant response to density varied when treated with different O_3 and CO_2 concentrations (Table 3, Figs 1 and 2). Although increasing density significantly decreased all individual plant parameters, the relative effects varied depending on main treatments as shown by significant interactions on an individual plant basis for total biomass, vegetative biomass, and seed yield (Table 3, Figs 1 and 2). When biomass and yield were expressed per ground area, the density interactions were reduced or eliminated. In general, O_3 significantly reduced total biomass via vegetative biomass but only in A, as shown by the significant $CO_2 \times O_3$ interactions. In contrast, O_3 had no significant effect on seed yield. Also, the impact of O_3 was the same regardless of density, as shown by the consistent lack for $D \times O_3$ interaction. Elevated CO_2 generally affected growth and yield although the effects varied among densities and between years (Table 3,

Table 2 Mean monthly daytime CO₂ and O₃ concentrations and monthly sum of the daily peak-weighted O₃ index (SUM06) for rice grown in open-top field chambers during the summers of 1997 and 1998

	Treatment*	Year	June	July	August	September
Daily [CO ₂] ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)	A	1997	374 \pm 6	366 \pm 4	362 \pm 8	369 \pm 5
		1998	368 \pm 1	362 \pm 3	360 \pm 6	367 \pm 0
	+CO ₂	1997	723 \pm 8	698 \pm 4	717 \pm 4	708 \pm 2
		1998	697 \pm 5	695 \pm 5	706 \pm 1	701 \pm 3
Daily [O ₃] ($\text{nmol O}_3 \text{ mol}^{-1}$)	CF	1997	27.0 \pm 2.0	31.5 \pm 2.4	26.7 \pm 4.3	23.2 \pm 6.1
		1998	30.8 \pm 1.0	31.0 \pm 1.0	26.7 \pm 0.7	22.9 \pm 0.9
	+O ₃	1997	76.8 \pm 1.2	77.8 \pm 2.8	69.7 \pm 1.6	67.3 \pm 1.3
		1998	74.8 \pm 0.9	76.2 \pm 0.6	79.0 \pm 0.2	76.6 \pm 0.7
Sum06 ($\mu\text{mol O}_3 \text{ mol}^{-1} \text{ h}$) [†]	CF	1997	0.0 \pm 0.0	0.7 \pm 0.2	0.1 \pm 0.1	0.5 \pm 0.1
		1998	0.2 \pm 0.1	0.7 \pm 0.2	0.3 \pm 0.1	0.7 \pm 0.4
	+O ₃	1997	9.3 \pm 0.4	26.3 \pm 0.3	21.4 \pm 0.4	18.5 \pm 0.2
		1998	12.9 \pm 0.4	24.3 \pm 0.2	25.6 \pm 0.1	22.1 \pm 0.4

*The CO₂ was added to ambient air (A) 24 h day⁻¹ for the elevated CO₂ treatment (+CO₂). The O₃ was added 12 h day⁻¹ to charcoal-filtered (CF) air at 1.5 times the average ambient O₃ concentrations of four previous years (+O₃). Numbers are mean \pm standard error. There were six chambers per gas treatment.

[†]The SUM06 is the sum of hourly average O₃ concentrations between 08:00 and 20:00 hours $\geq 0.06 \mu\text{mol mol}^{-1}$.

Table 3 Probability values from analyses of variance for biomass, yield, and root to shoot ratio (R/S) of rice grown in open-top field chambers at five densities (14–70 plants m⁻²; *D*) either in charcoal-filtered or O₃-fumigated air at ambient or elevated CO₂

Component	Total biomass		Vegetative biomass		Seed yield		R/S g g ⁻¹
	g plant ⁻¹	g m ⁻²	g plant ⁻¹	g m ⁻²	g plant ⁻¹	g m ⁻²	
O ₃	0.0003	0.02	0.001	0.001	0.10	0.7	0.17
CO ₂	0.0001	0.001	0.0001	0.0001	0.06	0.03	0.11
Year	0.0007	0.03	0.8	0.5	0.0005	0.005	0.007
O ₃ \times CO ₂	0.0007	0.03	0.003	0.005	0.13	0.5	0.07
Year \times O ₃	0.5	0.4	0.9	0.4	0.9	0.4	0.6
Year \times CO ₂	0.008	0.005	0.03	0.0006	0.2	0.3	0.17
Year \times O ₃ \times CO ₂	0.09	0.6	0.05	0.04	0.9	0.3	0.14
<i>D</i>	0.0001	0.0001	0.0001	0.0001	0.0001	0.03	0.001
<i>D</i> \times year	0.02	0.01	0.15	0.10	0.004	0.7	0.5
<i>D</i> \times O ₃	0.4	0.7	0.4	0.9	0.7	0.7	0.3
<i>D</i> \times CO ₂	0.04	0.13	0.07	0.8	0.08	0.5	0.9
<i>D</i> \times O ₃ \times CO ₂	0.7	0.9	0.7	0.8	0.8	0.5	0.4

The plants were grown for two consecutive field seasons. The general ANOVA model used year, O₃, CO₂ and block as the main effects and density as the split-plot effect. In all analyses, the block effect and block interactions were found not significant and are not reported. ANOVAs for individual plant responses were done on natural log-transformed data.

Figs 1 and 2). The year and year \times CO₂ effects reflect the enhanced total biomass in 1998 compared with 1997 that was associated with enhanced seed yield rather than vegetative biomass. These patterns show the complexity of resource limitation on the CO₂ and O₃ interactive effects for rice biomass.

Modeled biomass and yield response to O₃ and CO₂

Our simple competition models were used to assess the impact of CO₂ and O₃ on biomass and seed yield. The

model estimate of R_t was chosen to accommodate the highest average total biomass observed across all treatments within a year and was about 8% less in 1997 than 1998 (Table 4). If we had opted to accommodate all individual observations rather than the average, R_t would be 6250 g m⁻² in 1998 resulting in a difference of 14% fewer resources available in 1997. The choice was arbitrary and had little impact on the competitive outcome (Table 4). The model outcomes represent accurately the empirical biomass and seed yield data at all densities (Figs 1 and 2, Table 4). The plateaus in

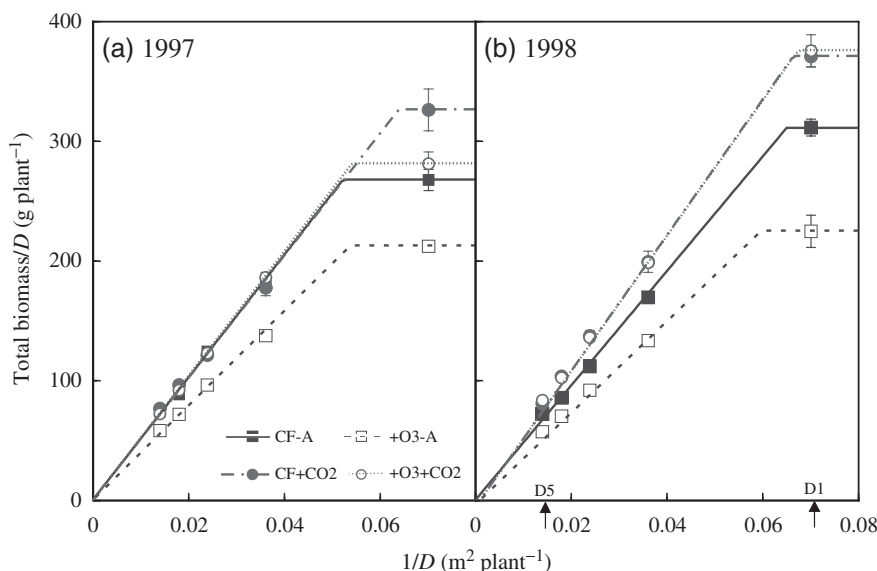


Fig. 1 Effect of plant density on individual plant biomass of rice grown at different $[O_3]$ and $[CO_2]$. For ambient atmospheric CO_2 (A, $365 \mu\text{mol } CO_2 \text{ mol}^{-1}$), and elevated CO_2 (+ CO_2 , $700 \mu\text{mol } CO_2 \text{ mol}^{-1}$), plants were grown in charcoal-filtered (CF) or O_3 -fumigated air (+ O_3 , $1.5 \times$ ambient $[O_3]$) in 1997 (a) and in 1998 (b) at five densities (from 14–70 plants m^{-2}). Cultural conditions were similar between years except for a 14% increase in fertilizers in 1998. A competition model was used to fit the response of plant biomass to density $^{-1}$, that is, area available per plant. Symbols represent average \pm standard error ($n = 12$).

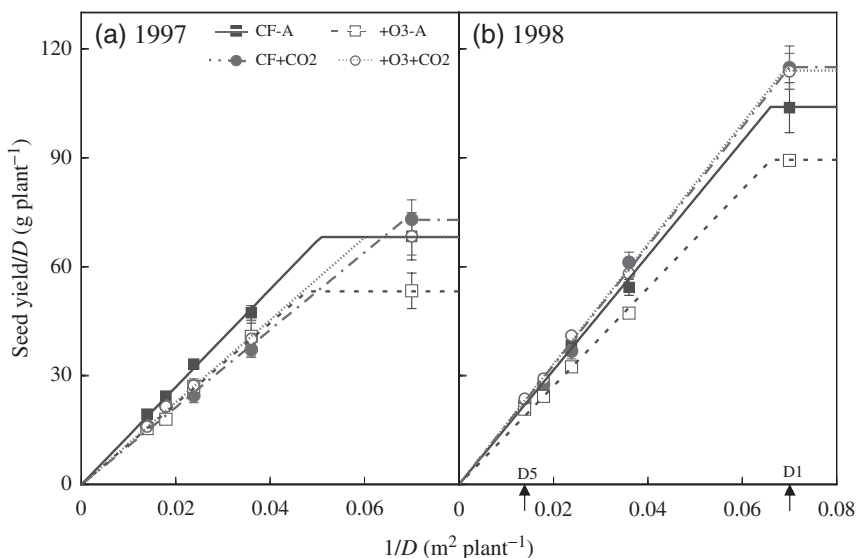


Fig. 2 Effect of plant density on seed yield per plant for rice grown at different $[O_3]$ and $[CO_2]$. For ambient atmospheric CO_2 (A, $365 \mu\text{mol } CO_2 \text{ mol}^{-1}$), and elevated CO_2 (+ CO_2 , $700 \mu\text{mol } CO_2 \text{ mol}^{-1}$), plants were grown in charcoal-filtered (CF) or O_3 -fumigated air (+ O_3 , $1.5 \times$ ambient $[O_3]$) in 1997 (a) and in 1998 (b) as described in Fig. 1. Model fit and averages are as described in Fig. 1.

the model represent the density-independent biomass and yield potential, that is, the result of the total resources used for the plant biomass when resources are in excess. The slopes of the models represent the density-dependent biomass and yield, that is, the efficiency of each plant to use its share of available resources into biomass when resources are limited.

Using model parameters, the alteration of CO_2 and O_3 effects on biomass and yield can be contrasted with and without competition.

Density-dependent biomass and yield response. For all treatments, individual plant biomass decreased with increasing competition for resources (Figs 1 and 2,

Table 4 Model parameters from a simple plant competition model for rice grown in open-top field chambers for 2 years at five densities (14–70 plants m⁻²; D) either in charcoal-filtered (CF) or O₃-fumigated (+O3) air at ambient or elevated CO₂

	1997				1998			
	Ambient CO ₂		Elevated CO ₂		Ambient CO ₂		Elevated CO ₂	
	CF	+O3	CF	+O3	CF	+O3	CF	+O3
<i>R_t</i> (g m ⁻² *)	5500	5500	5500	5500	6000	6000	6000	6000
<i>Total biomass</i>								
<i>R_p</i> (g plant ⁻¹)†	287.6 ± 6.6	295.9 ± 5.9	351.7 ± 8.3	298.1 ± 3.8	390.3 ± 5.6	352.5 ± 6.7	392.6 ± 4.9	398.7 ± 5.2
<i>B_p</i> (g plant ⁻¹)‡	^A267.9 ± 17.5	C212.6 ± 10.5	B326.4 ± 9.0	^A281.4 ± 9.9	B311.0 ± 8.9	C225.0 ± 3.7	D371.0 ± 7.1	D375.9 ± 13.4
Slope (g m ⁻²)	^A5123 ± 83	B3949 ± 72	^A5104 ± 109	^A5191 ± 70	^A4778 ± 58	B3825 ± 46	C5669 ± 54	C5658 ± 46
<i>r</i> ²	0.991	0.988	0.984	0.994	0.994	0.994	0.996	0.997
<i>F_p</i> (% of <i>R_t</i>)‡	68.2	54.1	83.1	71.6	72.6	52.5	86.6	87.7
<i>Vegetative biomass</i>								
<i>R_v</i> (g plant ⁻¹)	282.5 ± 9.0	307.0 ± 13.7	335.7 ± 6.8	287.9 ± 0.7	379.7 ± 10.6	314.6 ± 5.8	379.4 ± 13.6	386.2 ± 7.4
<i>V_p</i> (g plant ⁻¹)‡	^A157.0 ± 14.3	C126.7 ± 8.1	B206.2 ± 7.8	^A173.0 ± 5.4	^A168.0 ± 8.1	C103.5 ± 2.9	B213.9 ± 11.9	B215.6 ± 10.8
Slope (g m ⁻²)	^A3058 ± 63	B2268 ± 59	^A3379 ± 87	^A3305 ± 50	C2651 ± 47	B1971 ± 48	^A3378 ± 57	^A3351 ± 38
<i>r</i> ²	0.986	0.978	0.977	0.992	0.987	0.974	0.987	0.994
<i>F_v</i> (% of <i>R_t</i>)§	40.0	32.2	52.5	44.0	39.2	24.2	49.9	50.3
<i>Seed yield</i>								
<i>R_p</i> (g plant ⁻¹)	279.2 ± 9.1	263.0 ± 9.3	376.5 ± 15.7	331.0 ± 10.3	396.1 ± 15.3	396.5 ± 8.0	420.2 ± 14.6	412.8 ± 7.1
<i>Y_p</i> (g plant ⁻¹)‡	^A68.3 ± 6.0	B53.3 ± 5.5	^A73 ± 5.3	^A68.3 ± 5.2	D104.9 ± 5.8	E89.4 ± 1.5	D114.9 ± 6.5	D113.9 ± 4.9
Slope (g m ⁻²)	^A1347 ± 39	BC1114 ± 27	C1067 ± 31	C1136 ± 55	D1573 ± 45	^A1351 ± 20	B1643 ± 29	B1654 ± 39
<i>r</i> ²	0.973	0.979	0.971	0.923	0.965	0.991	0.986	0.976
<i>F_y</i> (% of <i>R_t</i>)§	17.4	13.6	18.6	17.4	24.3	20.9	26.8	26.6

Different letters within a row indicate significant treatment effect at $P < 0.05$.

**R_t* represents the maximum quantity of resources available assuming one resource unit for each biomass unit and is estimated from the maximum observed biomass from all treatments each year.

†*R_p* represents the resources used per plant when density-dependent resources are *not limiting*.

‡*B_p*, *V_p*, and *Y_p* represent the quantity of resources directly used into realized total plant biomass, vegetative biomass, and seed yield, respectively, per unit area when density-dependent resources are *not limiting*.

§*F_p*, *F_v*, *F_y* represent the fraction of total available resources (*R_t*) directly used into total biomass (*B_p*), vegetative biomass (*V_p*), seed yield (*Y_p*), respectively, when density-dependent resources are *not limiting*.

Table 4). However, the rate of change in biomass with decreasing area, as shown by the productivity slope, varied according to treatment and year. For plant total biomass, O₃ significantly reduced the productivity slopes compared with CF in A (Fig. 1, Table 4) for both years (23% and 20% in 1997 and 1998, respectively). Yet, in +CO₂, O₃ had no significant effect on the productivity slopes for plant total biomass (Fig. 1, Table 4) as the significant O₃ by CO₂ interactions showed the alleviation of O₃ damage by +CO₂ (Table 3). Elevated CO₂ significantly increased the productivity slope for total biomass only in 1998 when biomass increased with available ground area at a greater rate in +CO₂ than in A air. For vegetative biomass, responses to density between O₃ and CO₂ treatments were generally similar to total biomass (Table 4, graph not shown). Although density affected the CO₂ response, these patterns of productivity slopes for total and vegetative biomass show the amelioration of O₃ by CO₂ as density increases.

The density-dependent seed yield response to O₃ and CO₂ differed from the response of total and vegetative biomass. In both years, O₃ significantly decreased (17% and 14% in 1997 and 1998, respectively) the productivity slopes for yield compared with CF-A (Table 4) as above. However, in +CO₂, the productivity slopes for seed yield were significantly increased (53%) in 1998 relative to 1997 for CF (Fig. 2, Table 4). This was because +CO₂ significantly enhanced the productivity slopes both in +O₃ and CF in 1998, while, in 1997, the productivity slopes in +CO₂ were similar to the one in +O₃-A and lower than in CF-A. These differences are also shown

by the significant $D \times \text{year}$ and $D \times \text{CO}_2$ interactions (Table 3). Thus, the seed yield response to CO₂ and O₃ was limited by density.

Density-independent biomass and yield responses. In the absence of competition plant biomass and seed yield usually differed from that expected from the productivity slopes of the biomass or yield linear relationship with density. In 1997, B_p was lower than predicted by the productivity slope for all treatments (Fig. 1a, Table 4). In addition, O₃ significantly decreased B_p in both CO₂ treatments. In contrast, in 1998, B_p was not significantly different from the projected productivity slope in both +CO₂ treatments (Fig. 1b), CO₂ ameliorating the O₃ effect on total biomass. For vegetative biomass, the density-independent responses to O₃ and CO₂ were similar to the ones for total biomass as observed by comparing productivity slopes with V_p (Table 4). Likewise, in the absence of competition, the seed yield response to CO₂ and O₃ was consistent with the productivity slopes in 1998 but only for CF + CO₂ in 1997 (Fig. 2). In 1997, Y_p was reached at a lower density than expected from the productivity slope for yield in A. In both years, Y_p was significantly lower in +O₃-A than all other treatments, which were not different from each other within a year (Fig. 2, Table 4). Again, Y_p was higher in 1998 than in 1997 for all treatments. In 1998, when resource availability allowed for more biomass and yield in elevated CO₂, +CO₂ ameliorated losses in O₃-treated plants.

When resources were in excess of individual demand, the amount of available resources used into

Table 5 Root to shoot biomass ratio (g g^{-1}) of rice grown in open-top field chambers at five densities ($D1-5$; 14–70 plants m^{-2}) either in charcoal-filtered (CF) or O₃-fumigated air (+O₃) at ambient or elevated CO₂

Density	Ambient CO ₂		Elevated CO ₂	
	CF	+O ₃	CF	+O ₃
1997				
D1	^A 0.082 ± 0.007 _a	^A 0.065 ± 0.008 _a	^A 0.073 ± 0.003 _a	^A 0.077 ± 0.007 _a
D2	^A 0.079 ± 0.002 _a	^B 0.057 ± 0.002 _a	^A 0.081 ± 0.005 _{ac}	^A 0.073 ± 0.004 _a
D3	^{AB} 0.089 ± 0.007 _a	^C 0.069 ± 0.005 _a	^A 0.100 ± 0.005 _b	^{BC} 0.080 ± 0.006 _a
D4	^{AB} 0.079 ± 0.005 _a	^B 0.068 ± 0.003 _a	^A 0.083 ± 0.006 _{ac}	^{AB} 0.080 ± 0.005 _a
D5	^{AB} 0.085 ± 0.005 _a	^B 0.071 ± 0.005 _a	^A 0.089 ± 0.003 _{bc}	^{AB} 0.080 ± 0.007 _a
1998				
D1	^A 0.105 ± 0.007 _a	^B 0.077 ± 0.004 _a	^A 0.105 ± 0.004 _a	^A 0.106 ± 0.008 _a
D2	^{AB} 0.106 ± 0.006 _a	^A 0.089 ± 0.006 _a	^{AB} 0.108 ± 0.005 _a	^B 0.123 ± 0.011 _a
D3	^A 0.117 ± 0.003 _{ab}	^B 0.082 ± 0.005 _a	^A 0.119 ± 0.006 _{ab}	^A 0.110 ± 0.005 _a
D4	^A 0.123 ± 0.005 _b	^B 0.096 ± 0.001 _a	^A 0.127 ± 0.006 _b	^A 0.118 ± 0.005 _a
D5	^A 0.118 ± 0.004 _{ab}	^B 0.082 ± 0.003 _a	^A 0.116 ± 0.006 _{ab}	^A 0.114 ± 0.004 _a

The plants were grown during two consecutive field seasons. For each year and density, different superscripts within a row show significant treatment effect at $P \leq 0.05$. For each year per treatment, different subscripts within a column show significant density effect at $P \leq 0.05$ using least mean square pairwise comparisons.

Table 6 Comparison of CO₂ enhancement effect for plants grown without or with competition

	1997		1998	
	CF	+O ₃	CF	+O ₃
<i>Total Biomass</i>				
<i>B_p</i> (D1)	^A 21.8 \pm 3.1 _a	^A 32.1 \pm 2.7 _a	^A 19.3 \pm 1.3 _a	^B 67.1 \pm 1.9 _a
Slope (D2–5)	^A –0.3 \pm 0.9 _b	^B 31.4 \pm 1.0 _a	^B 26.6 \pm 0.6 _a	^C 48.0 \pm 0.6 _b
<i>Vegetative Biomass</i>				
<i>V_p</i>	^A 30.8 \pm 4.7 _a	^A 36.6 \pm 3.3 _a	^A 27.2 \pm 2.9 _a	^B 107.9 \pm 3.6 _a
Slope	^A 10.7 \pm 1.3 _b	^B 45.7 \pm 1.4 _a	^C 27.4 \pm 1.0 _a	^D 70.1 \pm 1.3 _b
<i>Seed yield</i>				
<i>Y_p</i>	^A 6.3 \pm 4.1 _a	^{AB} 28.6 \pm 5.9 _a	^A 9.5 \pm 2.6 _a	^B 27.2 \pm 1.7 _a
Slope	^A –20.8 \pm 1.1 _b	^B –0.2 \pm 2.0 _b	^B 4.3 \pm 1.1 _a	^C 22.6 \pm 1.0 _a

The percent enhancement is calculated as $(ER-1) \times 100$ where ER is the ratio of slope of +CO₂/slope of A from the mean slope and biomass per treatment, respectively, for total biomass, vegetative biomass, and seed yield (see Table 4 for means and symbols). For each parameter, different superscripts indicate significant treatment effects (within a row) at $P \leq 0.05$. For each treatment per year, different subscripts indicate significant differences between *B_p* and the slope at $P \leq 0.05$.

biomass, *R_p*, was generally lower in all treatments in 1997 than in 1998 (Table 4). However, similar fractions of total resources *R_t* into total biomass (*F_p*, which is $[B_p/R_t] \times 100$) were used between years in all treatments but +O₃-A (70%, 85%, and 53%, for CF-A, +CO₂, and +O₃-A, respectively), resulting in an average 69% across treatments in 1997 and 75% in 1998 (Table 4). Although consistently lower, the fraction of resources going to vegetative biomass (*F_v*, which is $[V_p/R_t] \times 100$) followed patterns similar to the ones observed for *F_p*. The fraction of resources going to seed yield (*F_y*, which is $[Y_p/R_t] \times 100$) was 22% lower for +O₃-A than for all other treatments even though *F_y* across all treatments averaged 32% less in 1997 than in 1998. The differential amount of resources used in biomass among treatments each year reflects their resource use efficiency.

Root/shoot ratio response to density, O₃, and CO₂

Density generally had a significant effect on root/shoot ratio (R/S; Tables 3 and 5) although the patterns among treatments differed. Larger R/S were generally found at higher rather than lower densities for each treatment combination (Table 5). Ozone decreased R/S ratios in A although the effect was not significant at D1, D4 and D5 in 1997 and for D2 in 1998. O₃ had no effect on R/S in +CO₂ showing the interactive effect of CO₂ and O₃ (Table 3). Also, for each treatment combination, the R/S was consistently higher in 1998 than in 1997 (Tables 3 and 5). No linear pattern emerged between density and R/S and the effect of O₃ on R/S was counterbalanced by elevated CO₂.

CO₂ enhancement ratio for total biomass and seed yield

The effect of density on the CO₂ enhancement ratios varied among treatments between years. The ERs, both

from our competition models (Table 6) and from the empirical data (Fig. 3), showed a reduced effect at higher densities when different. For total biomass, in 1997, the ER in CF air was 22% only for *B_p* (i.e., without competition) whereas no enhancement was found at higher densities (Table 6, Fig. 3a), and the ER in +O₃ was maintained at an average 32%. In contrast in 1998, ER for total biomass was sustained above 20% at all densities in the CF treatments whereas the ER in +O₃ was reduced from 70% for *B_p* to 50% at higher densities (Table 6, Fig. 3a). Although all ERs for vegetative biomass were higher than for total biomass, similar patterns were found (Table 6). However, the ERs for seed yield were significantly reduced by density in 1997 only. In 1997, the seed yield in CF air showed an initial ER of 10% for *Y_p* that was reduced at high densities even to –20% (Fig. 3b, Table 6) while, in +O₃ air, the 28% ER for *Y_p* declined to no enhancement at higher densities. In 1998, the seed yield of O₃-treated plants was enhanced 23–27% by CO₂ treatment at all densities (Fig. 3b, Table 6) while, in CF-air, the ER decreased at higher densities. The seed yield was generally not enhanced by +CO₂ to the same level as the plant biomass. The effect of +CO₂ was generally reduced at higher densities in 1997 while O₃ usually increased the enhancement effect.

Discussion

The magnitude of the CO₂ and O₃ effects on biomass and seed yield of rice was contingent on plant competition at increasing density. As hypothesized, the competition for resources with increased density limited the CO₂ enhancement of biomass and especially seed yield. For plants grown in competition in 1997, a year with

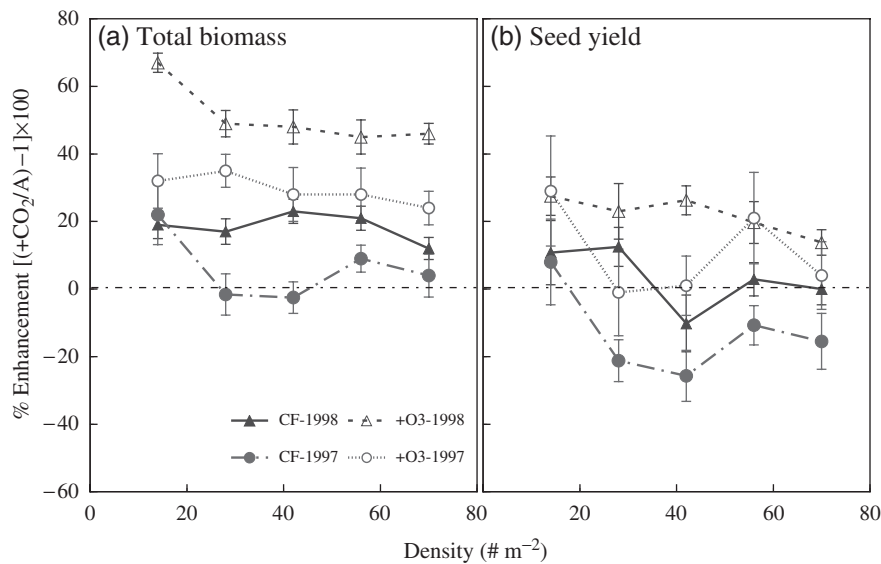


Fig. 3 Effect of plant density on the CO₂ enhancement ratio for plant biomass and seed yield of plants grown at different [O₃]s. Using average data, the ratio of biomass at elevated CO₂ to biomass at ambient CO₂ was compared for total biomass (a) and seed yield (b).

lower nutrient availability during reproduction, the CO₂ effect in CF air was reduced for total biomass because of a decline in seed yield. Compared with CF-A, the efficiency of resource use was unchanged by +CO₂ for total biomass and was reduced for seed yield. In 1998, +CO₂ enhanced biomass at all densities due to more efficient resource use but not seed yield. These patterns are well represented by our simple competitive models of total biomass and seed yield response to resource availability that showed more of the resources were going to biomass in +CO₂, hence being depleted faster and enhancing competitive interactions. Although pollutant O₃ reduced biomass and yield in CF air, O₃ did not eliminate the competitive effect in elevated CO₂ as hypothesized because resource use efficiency was similar in CF and +O₃ treatments for biomass production. Likewise, when combined with O₃, seed yield was enhanced by CO₂ at all densities in 1998 when more nutrients were available. The greater ER for total biomass and yield in +O₃ treatments potentially reflects both the protective effect of +CO₂ and the reduced growth due to lower resource use in +O₃-A as shown by our competition model.

Competition for resources

In all gas treatments, the changes in total biomass, vegetative biomass, and seed yield with density are consistent with the law of constant yield in agricultural systems (Barnes, 1977) and indicate increased competition for resources. Seeds yields for this rice hybrid in CF-A were comparable with field tests in the south-

eastern United States (Black *et al.*, 1995). Yield is dependent on resource availability and resource use efficiency rather than the number of plants as shown by the productivity slopes. As competition increases with density, individual plants respond to limiting resources by altering their biomass allocation according to a functional C:N balance model that suggests an adjustment of root biomass and activity to balance the shoot biomass and activity (e.g. Reynolds & Thornley, 1982). In elevated CO₂, the greater carbon uptake and carbohydrate production may result in greater root activity and faster N depletion that would increase competition for belowground resources in a dense stand. Such enhanced competition is consistent with the higher slopes of total biomass and yield and with the R/S observed in 1998 for plants grown at +CO₂ when more nutrients were available (Tables 5 and 7).

The effect of competition for resources is easily demonstrated by comparing the response without competition, the plateaus, vs. the productivity slopes at greater densities. In 1997, the plateaus for density-independent total and vegetative biomass in CF-A were 27% lower than expected from the productivity slope but only 12% for CF + CO₂ suggesting that plants were primarily limited by CO₂ availability in A. In +CO₂, competition for resources other than CO₂ limited the growth response. Navas *et al.* (1999) showed that plants grown individually responded differently to elevated CO₂ than plants grown in dense monotypic stands. For both years, plants grown in +O₃-A also had their B_p lower than expected from the productivity slopes suggesting some limitation by C availability so +CO₂ increased

Table 7 Between years comparisons of productivity slopes and plateaus for rice grown singly (B_p) or in competition (slope)

Treatment		Total		Vegetative		Seed	
CO ₂	O ₃	B_p	Slope	V_p	Slope	Y_p	Slope
Ambient	CF	1997 < 1998	=	=	97 > 98	1997 < 1998	=
	+O ₃	=	=	=	=	1997 < 1998	1997 < 1998
Elevated	CF	1997 < 1998	1997 < 1998	=	=	1997 < 1998	1997 < 1998
	+O ₃	1997 < 1998	1997 < 1998	1997 < 1998	=	1997 < 1998	1997 < 1998

'=' denotes no significant difference between years at the $P < 0.05$ level.

biomass at $D1$ and the slope. However, in 1997 the protective effect of +CO₂ in reducing O₃ flux was not sufficient to raise B_p to the modeled biomass suggesting that significant other costs were associated with the presence of O₃. The large CO₂ enhancement effect observed without competition for biomass was not sustained in seed yield.

Our simple competition models are based on determining density-independent resource use of the individual plant that combines resource uptake and efficiency. Recall that the total resources available to plants, R_t , was set by the upper limit of biomass production at the lowest plant density, the plant biomass being the integrating mechanism. For both years, an average 85% of R_t was used for biomass in our competition model at +CO₂ compared with 70% in CF-A (Table 4). This is partly because 95% of the resources used per plant (R_p) resulted in total biomass in the +CO₂ treatments compared with 87% in CF-A or 69% in +O₃-A. Because more of the resources are used directly in biomass with +CO₂, increased competition for these shared resources potentially limits the CO₂ effect, as we observed at high density. These findings differ from the total vegetative biomass responses reported in greenhouse experiments on annual species that show similar CO₂ enhancements for plants grown individually or at high density (*Brassica kaber*, Wayne *et al.*, 1999; *A. theophrasti*, Thomas *et al.*, 1999) or greater CO₂ enhancements for plants grown at high density than individually (*Cassia obtusifolia*, Thomas *et al.*, 1999). However, the timing of harvest in their studies may explain the discrepancies as greater CO₂ effects are often reported at earlier stages of the plant life cycle (Huxman *et al.*, 1999; Lewis *et al.*, 2003) and our experiment showed limited CO₂ enhancement for seed yield compared with enhancements for total and vegetative biomass.

Pollutant O₃ did not reduce or eliminate resource competition for plants grown at elevated CO₂. Rather, the enhanced growth with +CO₂ alleviated some or all of the negative effects of O₃. Kozovits *et al.* (2005) have reported that competition was important in determin-

ing the CO₂ and O₃ responses of beech and spruce and the outcome differed depending on intra vs. interspecific competition. Here, the lower productivity slopes we observed for total biomass and seed yield in +O₃-A indicate a reduced resource use efficiency that may reduce the competitive effect. Less of the available resources were used because the plants are unable to fully use resources for production of biomass or yield. The competition model further showed that, for density-independent B_p , an average 53% of available resources was used for plant biomass for +O₃-A plants compared with 70% in CF-A or 85% in +CO₂ treatments. Such a lower biomass with O₃ is consistent with more carbon being invested in maintenance respiration at ambient CO₂ (Amthor, 1988), and with higher leaf respiration and reduced biomass for plants exposed to O₃ compared with plants in clean air (Volin & Reich, 1996) as suggested by the lower ratio of B_p to R_p for +O₃-A plants (average 68% vs. 86–94% for CF and +CO₂ treatments, respectively). Of the resources taken up by plants in +O₃, some may be used in repair of the O₃ damage or avoidance of oxidative stress (e.g. Burkey *et al.*, 2000) and are not available for growth (Andersen, 2003). In turn, this reduced growth with +O₃-A limited the capacity for resource exploitation, hence resources harvested by plants, as shown by the lower R_p s in +O₃-A. The lower R/S in +O₃-A-treated plants further suggests limited capacity for exploitation of belowground resources. These resource uses suggest that the negative O₃ effect on biomass and yield in competitive environments might be compensated by increased soil fertility to enhance resource uptake. However, when the model plateau is limited by the inherent resource allocation limitation of the plant, productivity may be increased by enhanced planting density.

Competition and CO₂ effects on biomass vs. seed yield

A discrepancy exists between the CO₂ enhancement effects observed for biomass and for seed yield. The greater vegetative biomass in +CO₂ suggests increased

potential for seed production, possibly because of photosynthetic area. However, the lack of a resultant yield increase in these +CO₂ treatments indicates possible increased competition for light at higher densities. Light limitation was suggested for ragweed plants that grew taller at elevated than at ambient CO₂ to compensate for the lower irradiances in a high-density canopy (Stinson *et al.*, 2006). Furthermore, reduced CO₂ enhancement on reproductive yield was reported for native annual plants grown in 33% shade (Leishman *et al.*, 1999) and for wheat shaded for part of its growth (Mitchell *et al.*, 1996) possibly because of reduced carbohydrate production. In rice, carbohydrates from the second and third leaf often contribute more than the flag leaf to seed filling (Yoshida, 1972), and competition for light may have reduced their photosynthetic rates. Anten *et al.* (2004) has shown asymmetric competition for light acquisition between rice plants in dense stands that was more intense at higher N availability. In such a competitive environment, Lemaire & Millard (1999) suggested that plants have an enhanced requirement for structural support to reach light that would further alter the R/S ratio. Furthermore in elevated CO₂, a higher photosynthetic nitrogen-use-efficiency resulted in lower R/S increases than expected (Zerihun *et al.*, 2000). This combination of structural and functional changes may explain the lack of a clear pattern of R/S with increasing densities.

Although light was possibly limiting, several differences in biomass and seed yield between 1997 and 1998 when nutrient availability differed suggest a below-ground limitation as well. This finding is consistent with significant CO₂ effects reported on biomass only when enough N was supplied for rice (Ziska *et al.*, 1996b; Li *et al.*, 2004) and wheat (Cardoso-Vilhena & Barnes, 2001). Although the N application in this study was comparable with optimal standard cultural practices for rice, the daily flooding of pots likely may have resulted in periodic acute N shortages at critical stages for rice growth. For example, the leaf N contents measured at the end of the 1997 growing season were 10% lower in +CO₂ than in CF-A (data not shown) without competition and were further reduced up to 18% with increasing density in +CO₂. Such N shortage is also consistent with the higher seed productivity slopes and Y_p in 1998, when nutrient application was maintained through early reproduction. Yamakawa *et al.* (2004) suggested that soil N availability was limiting yield of rice grown in FACE because the N and P contents were reduced 27% and 6%, respectively, with +CO₂ at panicle initiation and more at final harvest. Such a lack of CO₂ effect on reproductive mass has also been reported for native annuals grown individually in greenhouses (*Cardamine hirsuta*, *Spergula arvensis*, Leish-

man *et al.*, 1999; *Bromus madritentis*, Huxman *et al.*, 1999; *Xanthium strumarium*, Lewis *et al.*, 2003). This lack of a CO₂ effect on yield while total biomass increased could result from reduced N availability (e.g. greenhouse-grown *Xanthium canadensis* grown at low N; Kinugasa *et al.*, 2003). Competition for belowground resources with increasing density in this study was accompanied by reduced yield per plant.

Available rooting volume may interact with nutrient limitation in our experiment. Although nutrients, primarily N, are most likely limiting in our experiment, rooting volume available for growth and nutrient exploration was also potentially limiting. Nevertheless, McConnaughay *et al.* (1993) showed that total nutrient content rather than pot volume was limiting the growth response to elevated CO₂. Furthermore, comparisons of field- and pot-grown crop species showed similar relative growth and yield response to CO₂ (Heagle *et al.*, 1999), and to combined CO₂ and O₃ (Booker *et al.*, 2005). Here, we have considered rooting volume as another resource.

Alternatively, the lack of a CO₂ effect on seed yield may indicate a limitation in photoassimilate translocation to the seeds. These results are consistent with Rowland-Bamford *et al.* (1990) who showed that more carbohydrates were exported out of the leaf with elevated CO₂ but the increased carbohydrate translocation was to the vegetative tissue and not to the seeds. Our competition model also supported this alternative. Without competition the model shows that, even though a greater fraction of the available resources was used in total biomass with +CO₂, the same small fraction of resources was invested in seed biomass in +CO₂ compared with CF-A. The increased respiratory cost with elevated CO₂ after heading in rice (Sakai *et al.*, 2001) may contribute to the reduced photoassimilate translocation to seed. In addition, although a counter-intuitive finding when one considers that annual species shift their resources to reproduction in the last stages of development, reduced carbon translocation through the phloem was reported with elevated CO₂ for *Echinochloa crus-galli* (Potvin *et al.*, 1984). For *Glycine max* grown in elevated CO₂, a decreased daytime carbohydrate export reported during late seed filling was accompanied by increased predawn leaf carbohydrates (Rogers *et al.*, 2004), also suggesting reduced carbon translocation. Thus, photosassimilate allocation to reproduction was possibly altered by a translocation limitation within the rice plant.

Confounding temperature effects

In addition to atmospheric CO₂, temperature is another density-independent factor likely to affect biomass and

yield. The lower Y_p in 1997 than in 1998 while V_p remained unchanged may reflect the impact of higher temperature during reproduction in 1997 than in 1998 (Table 7). Furthermore, these high temperatures in 1997 may affect resource use when in competition and partly explain the reduced yield measured in +CO₂ than in A. For CF + CO₂, the lower seed yield and seed productivity slope in 1997 than in 1998 are consistent with a high-temperature effect reported for rice (Kim *et al.*, 1996, Ziska *et al.*, 1996a). In 1997, plants experienced maximum temperatures of $\geq 35^\circ\text{C}$ during flowering in +CO₂ because of their accelerated phenology. Baker & Allen (1993) have shown little increase in yield with +CO₂ at 34°C while an increase of 1.3–1.6 times the ambient yield was observed at 28 and 31°C . The southern US rice variety, a parent of the hybrid used here, was more temperature-sensitive than the oriental varieties used in the above studies (Baker, 2004).

CO₂ enhancement ratios and O₃

In this study, the ERs remained above 20% for total biomass and fairly constant at higher densities when examining plants in +O₃ air, and likewise for seed yield in 1998. The lower biomass in +O₃-A air translated to these CO₂ enhancement effects. These results agree with an enhanced CO₂ effect reported at high density on aboveground biomass and bolls for cotton grown at two densities in a FACE system (Derner *et al.*, 2003) only if O₃ was an inadvertent factor. Here, O₃ reduced resource use as indicated by our competition model, as discussed above. With O₃ fumigation, the ER is consistently large because elevated CO₂ reduces the potential O₃ damage by reducing the flux of O₃ into the leaf (Fiscus *et al.*, 1997, 2002; McKee *et al.*, 1995) and provides more carbon resources to repair or prevent the O₃ damage (Kellomäki & Wang, 1998; Cardoso-Vilhena *et al.*, 2004). Elevated CO₂ may shift the photosynthetic limitation toward the substrate RuBP regeneration, which is less sensitive to oxidative damage, hence enhancing photosynthesis and providing more photo-assimilate for use in repair (McKee *et al.*, 1995, 2000). This suggested protective mechanism was supported by an experiment in which an equal O₃ flux into soybean leaves was maintained at ambient and elevated CO₂ (Booker & Fiscus, 2005).

Conclusions

This study demonstrates that, in clean air at increasing density, the CO₂ effect on plant biomass and seed yield was resource limited, be it nutrients, space, and/or light availability. Our simple competition models showed that, without competition, a greater proportion of re-

sources was utilized for biomass in +CO₂ and, in competition, the steeper rate of change in individual plant biomass with density (productivity slopes) suggested increased depletion of resources that limited plant growth and yield. Furthermore, the impact of resource limitation is greater on seed yield than on vegetative biomass. Because of the reduced growth in O₃ at ambient CO₂ coupled with the decreased negative effect of pollutant O₃ at elevated CO₂, a significant CO₂ enhancement was obtained for biomass and yield at all densities in the combined treatment. The effect of projected future atmospheric CO₂ is mediated by pollutant O₃ and planting density as these factors impact resource use in different ways. Thus, in support of Amthor's (1998) assertion that multiple factors be considered in crop management to benefit from enhanced atmospheric CO₂, our study shows that better consideration of pollutant O₃ and other potentially limiting resources is needed in elevated CO₂ studies to modify cultural practices and maximize seed crop production in future scenarios of CO₂ enhancement.

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