

Salinity and sea level mediate elevated CO₂ effects on C₃–C₄ plant interactions and tissue nitrogen in a Chesapeake Bay tidal wetland

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Abstract

Elevated atmospheric carbon dioxide concentrations ([CO₂]) generally increase plant photosynthesis in C₃ species, but not in C₄ species, and reduce stomatal conductance in both C₃ and C₄ plants. In addition, tissue nitrogen concentration ([N]) often fails to keep pace with enhanced carbon gain under elevated CO₂, particularly in C₃ species. While these responses are well documented in many species, implications for plant growth and nutrient cycling in native ecosystems are not clear. Here we present data on 18 years of measurement of above and belowground biomass, tissue [N] and total standing crop of N for a *Scirpus olneyi*-dominated (C₃ sedge) community, a *Spartina patens*-dominated (C₄ grass) community and a C₃–C₄-mixed species community exposed to ambient and elevated (ambient + 340 ppm) atmospheric [CO₂] in natural salinity and sea level conditions of a Chesapeake Bay wetland. Increased biomass production (shoots plus roots) under elevated [CO₂] in the *S. olneyi*-dominated community was sustained throughout the study, averaging approximately 35%, while no significant effect of elevated [CO₂] was found for total biomass in the C₄-dominated community. We found a significant decline in C₄ biomass (correlated with rising sea level) and a concomitant increase in C₃ biomass in the mixed community. This shift from C₄ to C₃ was accelerated by the elevated [CO₂] treatment. The elevated [CO₂] stimulation of total biomass accumulation was greatest during rainy, low salinity years: the average increase above the ambient treatment during the three wettest years (1994, 1996, 2003) was 2.9 t ha⁻¹ but in the three driest years (1995, 1999, 2002), it was 1.2 t ha⁻¹. Elevated [CO₂] depressed tissue [N] in both species, but especially in the *S. olneyi* where the relative depression was positively correlated with salinity and negatively related with the relative enhancement of total biomass production. Thus, the greatest amount of carbon was added to the *S. olneyi*-dominated community during years when shoot [N] was reduced the most, suggesting that the availability of N was not the most or even the main limitation to elevated [CO₂] stimulation of carbon accumulation in this ecosystem.

Keywords: elevated CO₂, net primary productivity, salinity, *Scirpus olneyi*, sea level, *Spartina patens*

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Introduction

Enhanced photosynthetic rates and reduced stomatal conductance (g_s) are common first-order responses to elevated atmospheric carbon dioxide concentration ([CO₂]) in C₃ vegetation (Bazzaz, 1990; Drake *et al.*, 1997; Nowak *et al.*, 2004; Ainsworth & Long, 2005). Although reductions in g_s are commonly reported in C₄ plant species exposed to elevated [CO₂], direct

effects on photosynthesis are relatively modest or absent because CO₂ is concentrated (~3–10 times ambient [CO₂]) at carboxylation sites within C₄ bundle sheath cells (e.g. Owensby *et al.*, 1997; Ghannoum *et al.*, 2000; Leakey *et al.*, 2006). The consequences of these primary CO₂-mediated responses for plant growth and ecosystem processes are complex and elusive because the relationship between photosynthesis and growth is not understood in a generalized way. Short-term responses to elevated [CO₂], largely from glasshouse and controlled environment chamber studies, showed the average stimulation of plant growth in response to a

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doubling of atmospheric [CO₂] was approximately 40% for C₃ plants and about 20% for C₄ plants (Poorter, 1993; Drake *et al.*, 1997). Recent estimates of elevated CO₂ (~1.5 times current ambient [CO₂]) on biomass production using free-air CO₂ enrichment (FACE) technology have also reported a stimulation in growth for both C₃ (~15–20%) and C₄ (~5%) plants (Ainsworth & Long, 2005; Morgan *et al.*, 2005), although Leakey *et al.* (2006) report no measurable effects of elevated [CO₂] on photosynthesis or growth of maize, a C₄ plant, in the absence of drought. Beyond the influence of photosynthetic pathway, much of the variability in the growth response to CO₂ has been attributed to improved water balance and plant nutrition and/or the physical environment (Lloyd & Farquhar, 1996; Owensby *et al.*, 1999; Oren *et al.*, 2001; Nowak *et al.*, 2004). A better understanding of the interactive effects of environmental variability and elevated [CO₂] on ecosystem processes is needed.

Complex interactions between salinity and flooding affect plant growth and distribution in tidal wetland communities (Rozema & Van Diggelen, 1991; Pennings & Callaway, 1992; Broome *et al.*, 1995; Huckle *et al.*, 2000; Pennings *et al.*, 2005). For example, Broome *et al.* (1995) found nearly a 75% decline in aboveground biomass of a C₃ sedge across a salinity gradient from 0 to 20 ppt and a lesser, but significant, decline due to flooding. Experimental data also indicate that biomass production in tidal wetland systems may be limited by N (Gallagher, 1975; Valiela *et al.*, 1978; Bradley & Morris, 1991). Thus, effects of elevated [CO₂] on plant growth and N dynamics depend in part on the extent to which enhanced photosynthesis and reduced stomatal conductance in response to elevated [CO₂] can ameliorate flooding- and salt-induced effects on plant function (Rozema *et al.*, 1991; Ball *et al.*, 1997). It has been hypothesized that increased N- and water-use efficiencies under elevated [CO₂] would increase the relative biomass response to elevated [CO₂] during water stress, although experimental data to support this hypothesis are limited and have shown mixed results (Ball *et al.*, 1997; Owensby *et al.*, 1999; Housman *et al.*, 2006).

Effects of elevated [CO₂] on ecosystems are likely to cause feedbacks that may alter long-term (>10 years) biomass responses to atmospheric [CO₂] enrichment (Mooney *et al.*, 1991). For example, N may increasingly be tied up in organic pools and the available tissue nitrogen concentration ([N]) gradually drawn down by elevated [CO₂] stimulation of growth, inducing progressive N limitation through time (Hungate *et al.*, 2003, 2005; Luo *et al.*, 2004). Consequently, the magnitude of long-term biomass responses to elevated [CO₂] and the extent to which initial stimulations in biomass can be sustained remain unresolved questions that are central to understanding how rising atmospheric [CO₂] will

alter ecosystem carbon balance (Norby *et al.*, 2003, 2005; Long *et al.*, 2004; Körner *et al.*, 2005). Resolving variability in biomass production and N cycling through time and across a range of environmental conditions in response to elevated [CO₂] is important for making accurate predictions of future ecosystem function.

A recent analysis conducted by Rasse *et al.* (2005) on our long-term study of a C₃ wetland sedge community found sustained stimulation of net ecosystem carbon exchange (NEE), aboveground biomass and shoot density in response to long-term atmospheric [CO₂] enrichment. Rasse *et al.* (2005) also reported contrasting interactions between elevated [CO₂] and salinity on NEE and shoot density, whereby relatively greater positive-elevated [CO₂] effects on shoot density were related with higher salinities, but relative enhancement of NEE by elevated [CO₂] was greatest at low salinity. While Rasse *et al.* (2005) identified some key responses of this C₃ wetland sedge community to interactive effects of elevated [CO₂] and salinity, it did not present data on annual root productivity, C₄ wetland plant responses, responses of co-occurring C₃ and C₄ plants, tissue and canopy N dynamics, and the interaction between the effects of elevated [CO₂] and sea level on these processes. Sea level has risen nearly 10 cm during the course of this long-term study and is considered to be an important determinant of tidal wetland function (Morris *et al.*, 2003; Pennings *et al.*, 2005). Thus, in this study, we extend the analyses of Rasse *et al.* (2005) by reporting results on C₃ and C₄ plant interactions, above and belowground biomass and tissue [N] for C₃-dominated, C₄-dominated and mixed C₃–C₄ brackish wetland communities during 18 years of exposure to enriched atmospheric [CO₂]. Our analysis of the data was designed to extract effects of the natural interannual variability in salinity and sea level in order to test the hypothesis that we would see relatively greater CO₂ effects on shoot and root biomass production in both C₃ and C₄ plant species during years of high salinity and/or high mean sea level due to a relative enhancement of CO₂ availability under environmental conditions that induce stomatal closure. We further hypothesized that reduction of tissue [N] under elevated [CO₂] would be inversely correlated with salinity and/or sea level (i.e. relatively greater reduction in tissue [N] associated with reduced stimulation of biomass) in the C₃ plant consistent with the tight coupling of leaf [N] and photosynthetic rates (Reich *et al.*, 1997).

Materials and methods

Site description and experimental design

This study was conducted on a brackish marsh of the Rhode River (38°53'N, 76°33'W), a subestuary on the

western shore of Chesapeake Bay. The study site was located in the high marsh (40–60 cm above mean low water), which is flooded approximately 2% of the time and is representative of brackish high marshes along Mid-Atlantic North America (Jordan & Correll, 1991). Dominant vegetation in the high marsh include the C₃ sedge *Scirpus olneyi* (A.) Gray (a.k.a. *Schoenoplectus americanus* (Pers.) Volk. Ex Schinz & R. Keller), the C₄ grass *Spartina patens* (Aiton) Muhl., and the C₄ grass *Distichlis spicata* (L.) Greene. Within the high marsh, *S. patens* tends to occur at relatively high elevations, *S. olneyi* at relatively low elevations and *D. spicata* occurs throughout the high marsh, although all three species overlap (Arp *et al.*, 1993). As part of an ongoing study in the marsh, a *S. olneyi*-dominated community, a *S. patens*-dominated community and a mixed-assembly community containing *S. olneyi*, *S. patens* and *D. spicata* have been exposed to elevated atmospheric carbon dioxide since 1987 using open-top chambers (Drake *et al.*, 1989).

Within each community, 15 circular plots of 0.47 m² were established according to a randomized block design with three treatments per block (Curtis *et al.*, 1989). One chambered plot per block was ventilated with ambient air (ambient treatment) and another chambered plot per block was ventilated with ambient air + 340 ppm CO₂ (elevated treatment). The remaining plot in each block had no chamber but was otherwise treated like the chambered plots (control treatment). Since 1987, CO₂ exposure began each year when the plants emerged in the spring and continued 24 h day⁻¹ through autumn following total senescence. A survey of all plots was conducted in 1986 before initiation of the treatments and showed no significant differences in biomass assigned to the three treatments in each community (Arp *et al.*, 1993).

Environmental variables

Mean sea level data measured as mean water level relative to a NOAA/NOS tidal gauge were obtained from the US Naval Academy in nearby Annapolis, MD (station 8575512). Mean hourly water level data from 1975 to 2004 were averaged each year for the months of May, June and July to produce a growing season mean. During this 30-year time period, growing season MSL increased at a rate of approximately 0.4 cm yr⁻¹ ($P < 0.01$; $R^2 = 0.69$), similar to the long-term (1928–1999) annual increase in mean sea level of 0.35 cm yr⁻¹ at the same location (http://co-ops.nos.noaa.gov/data_res.html).

Hourly precipitation and tidal creek salinity data were recorded near (<1 km) the site. Precipitation data were summed for the months of March through July to determine a seasonal precipitation value. Salinity data from March through July were averaged to provide a

seasonal estimate of salinity. Similarly, monthly mean air temperatures (T_{AIR}) measured at Baltimore–Washington airport were obtained from the National Climatic Data Center (NCDC) and averaged from March to July to produce a mean growing season temperature (<http://www.ncdc.noaa.gov/>). In contrast to mean sea level, the climate data included the months of March and April because of the importance of early spring precipitation for salinity in Chesapeake Bay (e.g. Gibson & Najjar, 2000). Time periods for all environmental variables ended in July because this was when collection of peak biomass data occurred. However, the period of time that these variables affect vegetation growth and other ecosystem properties may be longer or shorter than the period we used and may also vary from year to year. Nevertheless, the months chosen provide a reasonable time frame for the current analyses (Rasse *et al.*, 2005). Furthermore, an analysis of other time periods did not change the nature of the relationships reported in the current study (data not shown).

Biomass and N estimates

During annual harvests, data were collected on *S. olneyi* shoot properties in the C₃ and mixed communities from 1987 to 2004. At the peak of the growing season, which occurred during the last week of July to the first week in August (Curtis *et al.*, 1989), photosynthetically active (green tissue) *S. olneyi* shoots in each plot were counted and measured for length and width at 1/2 the shoot length. Each year, shoots from random quadrats in each plot were harvested (approximately 10 shoots per plot) and shoot biomass was estimated using census data and allometric relations from harvested subsamples as described in Curtis *et al.* (1989). Because of high shoot densities, aboveground biomass of the C₄ grasses was estimated by subsampling five randomly selected 25 cm² quadrats in each plot. Shoots chosen for subsampling from each of the plots were oven dried at 60 °C to a constant mass, weighed, ground to pass through a 60 mesh screen and analyzed for tissue C and N concentrations (see Drake *et al.*, 1996).

Relative belowground annual net primary production was estimated using root ingrowth cores in each community (Lund *et al.*, 1970; Curtis *et al.*, 1989). Following senescence of plant growth in late autumn (approximately first half of November), three root ingrowth cores, each about 5 cm in diameter and 20 cm in length, were extracted from each plot and refrigerated at 4 °C for processing. After extraction of the cores, the same holes in each of the plots were immediately filled with a sifted commercial peat (Pioneer Peat Inc., Grand Forks, ND, USA) similar to the peat found in the marsh. The extracted ingrowth cores were rinsed free of sediment.

Roots and rhizomes were recovered and dried to a constant mass at 60 °C. Root and rhizome weights from each of the three cores per plot were averaged to give an estimate of root biomass production. Root and rhizome tissues from each plot were pooled, ground to pass through a 60 mesh screen and analyzed for tissue C and N concentrations. Given the wide variability of within plot rhizome biomass production, only root biomass data are presented in the current study.

Data analyses

Significant treatment effects on biomass and [N] collected over 18 consecutive years (1987–2004) were analyzed using the mixed model repeated measures (treating year as a repeated categorical variable) procedure of the SAS statistical software system (e.g. Littell *et al.*, 1998). To assess treatment effects on response variables, elevated [CO₂] plots were compared with ambient chambered plots, and ambient chambered plots were compared with unchambered plots to test for possible chamber effects on response variables. To account for potential dependence among observations through time, a discrete autoregressive correlation model was used to model dependence among the within-group errors. Degrees of freedom were determined using the Satterthwaite approximation. Annual mean relative CO₂ responses, calculated each year as [(mean of elevated plots – mean of ambient plots) / mean of ambient plots] × 100%, were compared across years of high (>7 ppt) and low (<7 ppt) salinity and high (study mean to study mean + 5.7 cm) and low (study mean to study mean – 4.2 cm) sea level to look at relative CO₂ effects on growth parameters under contrasting salinity and water level environments. Salinity of the tidal creek, as well as soil porewater salinity at 10 cm (since 2001, data not shown) were not correlated with sea level, so effects of salinity and sea level were treated independently. A one-tailed *t*-test with equal variance was used to test the significance of relative CO₂ effects on response variables across high and low salinity and high and low water level years. In addition, relations between relative CO₂ effects on N and biomass were analyzed using standard regression procedures for years where all data were available. Given high variability of growth parameters in the marsh, *P* < 0.10 was considered significant for these analyses.

Results

Climate conditions

Throughout the 18-year data collection period variation in the seasonal average of *T*_{AIR} was relatively modest

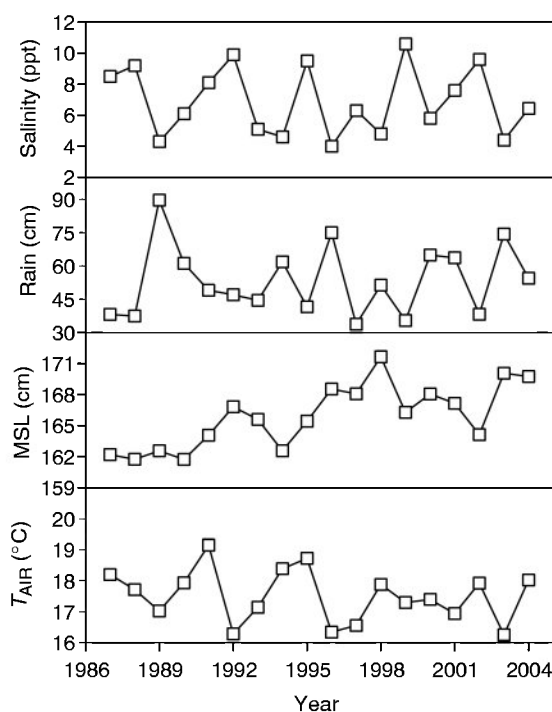


Fig. 1 Seasonal climate data during the 18-year study period. Monthly mean air temperature (*T*_{AIR}) and tidal creek salinity were averaged from March to July, while the sum of all precipitation received during the same period is presented. Mean sea level data averaged from May to July are presented (measured as centimeters above a fixed standard).

(~10–20%), ranging from 16 to 19 °C (Fig. 1). During the same time period variability in tidal creek salinity, water level and precipitation was far greater. Salinity ranged from approximately 4–10 ppt. Over threefold variation in precipitation was observed ranging from 25 cm to over 80 cm. Similarly, mean sea level varied over 10 cm during the course of the study and showed an increasing trend with time. No correlation between mean sea level and precipitation or *T*_{AIR} and precipitation was found. However, precipitation was negatively related to salinity ($R^2 = 0.52$; $P < 0.01$), reflecting the influence of freshwater discharge on the salinity of the tidal creek.

Above and belowground biomass production

Elevated atmospheric [CO₂] significantly enhanced *S. olneyi* shoot biomass production in the *S. olneyi*-dominated and mixed communities (Table 1, Fig. 2. Note: top panel of Fig. 2 modified from Fig. 1 of Rasse *et al.*, 2005). However, C₄ shoot biomass was not significantly affected by elevated atmospheric [CO₂] in either the mixed or *S. patens*-dominated communities. Chambers had no significant effect on *S. olneyi* shoot biomass in

Table 1 *P*-values resulting from repeated measures analysis assessing treatment effects (C, unchambered control treatment; A, ambient [CO₂] chambered treatment; E, elevated [CO₂] chambered treatment) on above and belowground biomass production in three marsh communities (*Scirpus*, mixed and *Spartina*) of a subestuary of Chesapeake Bay

Biomass	<i>Scirpus</i> (C ₃)		Mixed			<i>Spartina</i> (C ₄)	
	Shoot	Root	C ₃ shoot	C ₄ shoot	Root	Shoot	Root
<i>E vs. A</i>							
Trt	0.08 (+)	<0.01 (+)	0.06 (+)	0.81	<0.01 (+)	0.32	0.12
Yr	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
TrtxYr	0.39	0.03	0.12	0.24	<0.01	0.20	0.99
<i>A vs. C</i>							
Trt	0.69	<0.01 (+)	0.04 (+)	<0.01 (-)	0.27	0.01 (-)	0.02 (-)
Yr	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
TrtxYr	<0.01	0.33	0.01	0.02	<0.01	0.02	0.06

(+) denotes a significant increase and (-) a decrease due to elevated CO₂ or chamber. Bold indicates *P* < 0.10.

the *S. olneyi*-dominated community and a significant positive effect in the mixed community, whereas chambers significantly reduced shoot biomass of C₄ grasses in both the mixed and *S. patens*-dominated communities (Table 1). Beyond chamber and CO₂ treatment effects, shoot biomass production varied significantly (over fivefold) in all communities throughout the study, however no significant interactions between CO₂ and year on shoot biomass were found (Table 1, Fig. 2).

In addition to interannual variability, both C₃ and C₄ shoot biomass production declined through time, with the exception of C₃ shoot biomass in the mixed community which increased through time. Despite this trend, *S. olneyi* shoot biomass production was substantially higher in the *S. olneyi*-dominated community compared with the mixed community, although by the end of the study they became more similar due to their opposite trends through time (Fig. 2). In contrast, C₄ shoot biomass production was similar in the mixed and *S. patens*-dominated communities.

Similar to shoot biomass, elevated [CO₂] significantly increased root ingrowth biomass in the *S. olneyi*-dominated and mixed communities, but did not significantly affect root ingrowth biomass in the *S. patens*-dominated community (Table 1). Chambers significantly reduced root ingrowth biomass in the *S. olneyi*-dominated and mixed communities but had no significant effect on root ingrowth biomass in the *S. patens*-dominated community. Root ingrowth biomass also varied significantly from year to year in all communities and CO₂ interacted significantly with year to affect root biomass in the *S. olneyi*-dominated and mixed communities (Fig. 3, Table 1). While exhibiting substantial interannual variability, root biomass in the single-species dominated communities showed no long-term trends with time, but root biomass did increase through time in the mixed community as *S. olneyi* increased in abundance.

Tissue N concentrations

Shoot [N] was generally higher and more variable in the C₃ sedge, ranging from 7 to 14 mg N g⁻¹ plant, compared with the C₄ species which ranged from about 5 to 9 mg g⁻¹ across all communities (Fig. 4). Elevated atmospheric [CO₂] significantly reduced shoot [N] in *S. olneyi* in all cases and also significantly reduced shoot [N] in *S. patens* growing in the *S. patens*-dominated community, but not in the mixed community (Table 2). We found significant interannual variability in shoot [N], but no significant interaction between CO₂ treatment and year. No significant chamber effect on shoot [N] occurred, with the exception of *S. patens* in the *S. patens*-dominated community which had a positive chamber effect on shoot [N].

Root [N] was similar in all communities and varied significantly from year to year, ranging from 6 to 13 mg g⁻¹ (Fig. 5, Table 2). Elevated atmospheric [CO₂] significantly reduced root [N] in all communities throughout the study (Table 2). A significant negative chamber effect on root [N] was found in the *S. olneyi*-dominated and mixed communities. Unlike some of the biomass components, trends in tissue [N] through time were not evident.

Combined shoot and root biomass production in the *S. olneyi*-dominated community was strongly correlated with canopy N content (kg N ha⁻¹) across all treatments, but the slope of this relationship (0.115) was significantly higher (*P* = 0.10) under elevated [CO₂] compared with the slope for the ambient [CO₂] treatment (0.086) (Fig. 6). Moreover, greater production of total biomass occurred for plants grown in elevated [CO₂] at any standing crop of canopy N. Despite depressed shoot [N] in *S. olneyi* (over 25% in some years), elevated [CO₂] stimulated canopy N content because of the stimulation of shoot biomass (Fig. 6).

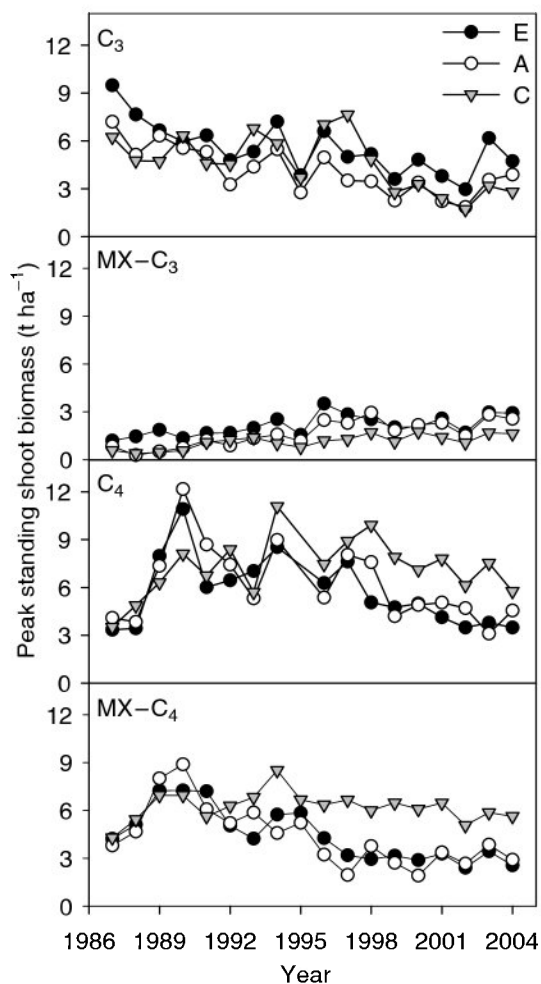


Fig. 2 Annual treatment mean ($n = 5$) aboveground biomass at the peak of the growing season (late July to early August) for *Scirpus olneyi* in the *S. olneyi*-dominated community (C_3) (modified from Fig. 1 of Rasse *et al.*, 2005), *S. olneyi* (MX- C_3) in the mixed-assembly community, *Spartina patens* in the *S. patens*-dominated community (C_4) and *S. patens* and *Distichlis spicata* (MX- C_4) in the mixed-assembly community. Open circles represent ambient [CO_2] chambered plots (A), black-filled circles represent elevated [CO_2] chambered plots (E) and gray-filled triangles represent unchambered control plots (C).

Interestingly, stimulation of total biomass production in the *S. olneyi*-dominated community was strongly and positively correlated with the stimulation of canopy N content, but negatively correlated with shoot [N] (Fig. 6).

Effects of salinity, sea level and elevated [CO_2] on biomass and tissue [N]

Salinity (precipitation) and/or mean sea level were related to biomass and N dynamics in both the C_3 and C_4 species in all communities. For example, during the relatively low salinity years of 1996, 2000 and 2003,

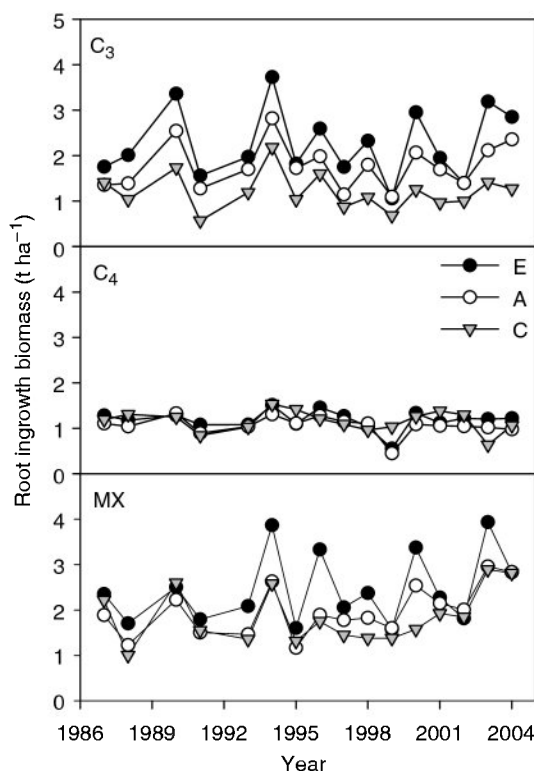


Fig. 3 Total root ingrowth biomass production during the 18-year study period for a *Scirpus olneyi*-dominated community (C_3), a *Spartina patens*-dominated community (C_4) and a mixed-assembly community (MX), which included a mix of C_3 and C_4 species. Open circles represent ambient [CO_2] chambered plots (A), black-filled circles represent elevated [CO_2] chambered plots (E), and gray-filled triangles represent unchambered control plots (C).

S. olneyi shoot biomass averaged about 4.0 t ha^{-1} across unchambered control plots, but only averaged 2.7 t ha^{-1} during the high salinity years of 1995, 1999 and 2002 in the *S. olneyi*-dominated community. Mean sea level was also related to biomass production, which was most evident in C_3 and C_4 shoot production in the mixed community (Fig. 7). An increase in *S. olneyi* biomass was significantly ($P < 0.01$) correlated with mean sea level in the mixed community, while C_4 biomass was negatively ($P < 0.01$) correlated with mean sea level, although this effect was greatly hastened by the presence of chambers. Furthermore, *S. olneyi* shoot biomass response to mean sea level differed significantly ($P = 0.04$) under elevated [CO_2], but elevated [CO_2] did not affect the response of C_4 shoot biomass to mean sea level.

In addition to variability in absolute CO_2 treatment effects on biomass and N, relative effects of elevated [CO_2] were variable, and this variability was related to salinity and sea level in the marsh. For instance, relative effects of elevated [CO_2] often differed between years of

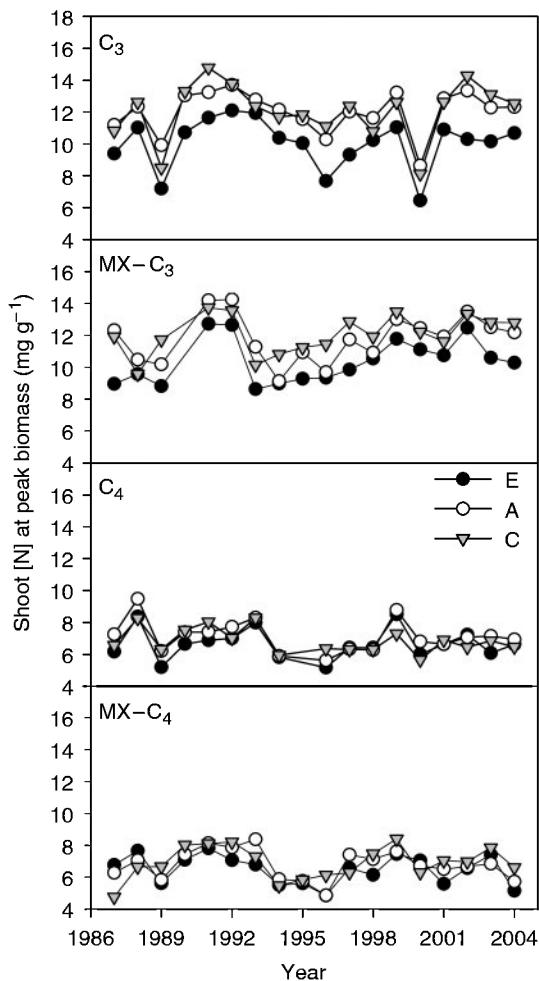


Fig. 4 Mean ($n = 5$) shoot nitrogen concentration [N] data collected during the 18-year study period for *Scirpus olneyi* in the *S. olneyi*-dominated community (C_3), *S. olneyi* (MX- C_3) in the mixed-assembly community, *Spartina patens* in the *S. patens*-dominated community and *S. patens* (MX- C_4) in the mixed-assembly community. Open circles represent ambient $[CO_2]$ chambered plots (A), black-filled circles represent elevated $[CO_2]$ chambered plots (E), and gray-filled triangles represent unchambered control plots (C).

low (<7 ppt) and high (>7 ppt) salinity in the *S. olneyi*-dominated community (Fig. 8). During years of low salinity, relative CO_2 effects on shoot biomass were significantly less, while elevated $[CO_2]$ effects on root biomass were significantly greater compared to high salinity years. Relative declines in *S. olneyi* shoot [N] were significantly greater during years of low salinity, which combined with a relatively lower stimulation of shoot biomass led to a significantly lower elevated $[CO_2]$ effect on canopy N content during years of low salinity compared with high salinity years. Similarly, elevated $[CO_2]$ stimulated shoot biomass production in the *S. olneyi*-dominated community relatively more

during years of high mean sea level (Fig. 8), which also led to a significantly greater elevated $[CO_2]$ effect on canopy N content.

In contrast to the C_3 sedge, relative CO_2 stimulation of C_4 shoot biomass in the *S. patens* dominated community was significantly less during high salinity years compared with low salinity years (Fig. 8). However, elevated $[CO_2]$ effects on root biomass did not differ with salinity. No difference in the relative effect of CO_2 on shoot [N] was found across salinity categories, but elevated $[CO_2]$ reduced canopy N content much more during high salinity years as a result of the reduced shoot biomass production. We found no significant interactions between elevated $[CO_2]$ and mean sea level on any response in the *S. patens*-dominated community (Fig. 8).

Salinity did not significantly alter the CO_2 treatment effect on C_3 or C_4 shoot production in the mixed community, but elevated $[CO_2]$ significantly stimulated root growth in the mixed community more under low salinity conditions compared with high salinity conditions (Fig. 8). Concomitant with the relative stimulation of root growth under low salinity, a significant relative decrease in root [N] was found under elevated $[CO_2]$. Elevated $[CO_2]$ had a significantly greater effect on *S. olneyi* shoot biomass production in the mixed community during years where mean sea level was relatively low compared with years where sea level was relatively high (Fig. 8).

Discussion

Implications of elevated $[CO_2]$ for long-term stimulation of biomass

Elevated atmospheric $[CO_2]$ enhancement of C_3 biomass was sustained through time in the *S. olneyi*-dominated community (Figs 2 and 3), averaging about 40% for shoots and 26% for roots, whereas elevated $[CO_2]$ had no significant overall effect on biomass production in the C_4 grass community. Elevated atmospheric $[CO_2]$ has led to an initial increase of plant growth in many natural ecosystems including the arctic tundra (Oechel & Vourlitis, 1996), brackish wetlands (Arp *et al.*, 1993), deserts (Smith *et al.*, 2000), scrub oak (Dijkstra *et al.*, 2002), forested ecosystems (DeLucia *et al.*, 1999; Isebrands *et al.*, 2003) and grasslands (Owensby *et al.*, 1999; Zavaleta *et al.*, 2003) although responses have diminished in some cases (Oechel & Vourlitis, 1996; Norby *et al.*, 2003; Körner *et al.*, 2005). The results from this study in a tidal wetland ecosystem demonstrate that elevated $[CO_2]$ effects on biomass production can be sustained through time. In contrast to studies where growth responses to elevated $[CO_2]$ have diminished (e.g. Oechel & Vourlitis, 1996; Körner *et al.*, 2005), the

Table 2 *P*-values resulting from repeated measures analysis assessing treatment effects (C, unchambered control treatment; A, ambient [CO₂] chambered treatment; E, elevated [CO₂] chambered treatment) on above and belowground tissue [N] in three marsh communities (*Scirpus*, mixed and *Spartina*) of a subestuary of Chesapeake Bay

	<i>Scirpus</i> (C ₃)		Mixed			<i>Spartina</i> (C ₄)	
	Shoot	Root	C ₃ shoot	C ₄ shoot	Root	Shoot	Root
<i>E vs. A</i>							
Trt	<0.01 (-)	0.01 (-)	<0.01 (-)	0.14	0.09 (-)	<0.01 (-)	<0.01 (-)
Yr	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
TrtxYr	0.96	0.45	0.66	0.57	0.70	0.79	0.97
<i>A vs. C</i>							
Trt	0.93	<0.01 (-)	0.43	0.55	<0.01 (-)	0.03 (+)	0.99
Yr	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
TrtxYr	0.66	0.28	0.15	0.70	0.49	0.04	0.29

(+) denotes a significant increase and (-) a decrease due to elevated [CO₂] or chamber. Bold indicates *P* < 0.10.

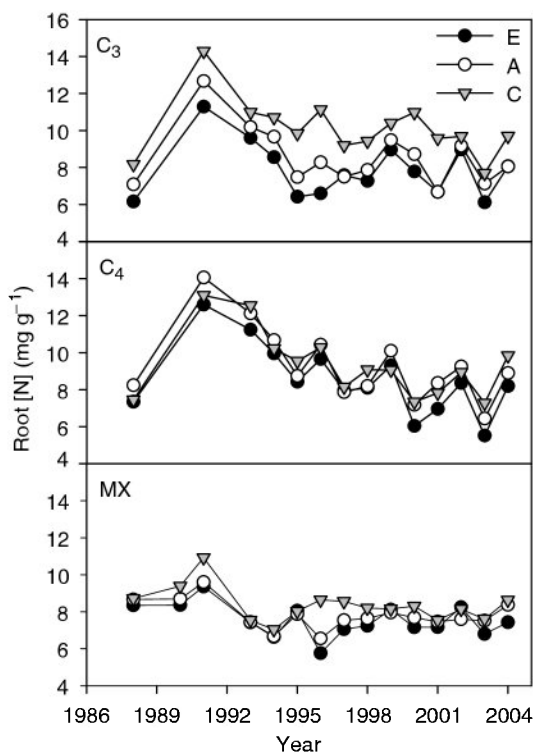


Fig. 5 Mean (*n* = 5) root nitrogen concentration [N] data collected during the 18-year study period for a *Scirpus olneyi*-dominated community (C₃), a *Spartina patens*-dominated community (C₄) and a mixed-assemblage community (MX), which included a mix of C₃ and C₄ species. Open circles represent ambient [CO₂] chambered plots (A), black-filled circles represent elevated [CO₂] chambered plots (E), and gray-filled triangles represent unchambered control plots (C).

sustained growth response of *S. olneyi* in the current study may be related to a relatively 'open' N cycling in tidal wetlands, whereas systems with a more 'closed' N

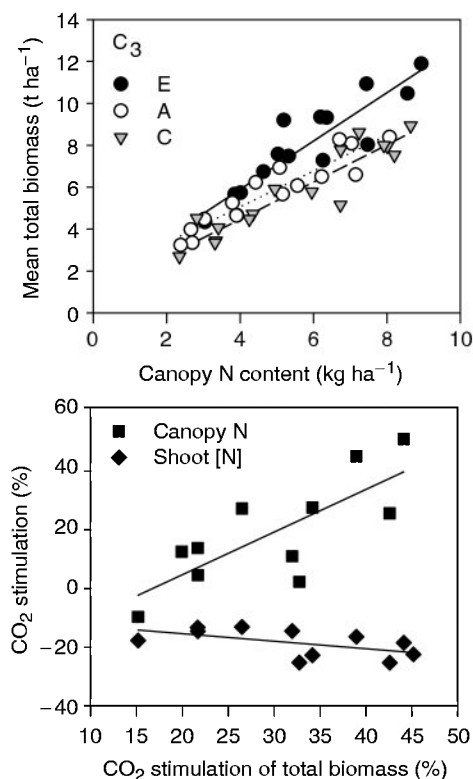


Fig. 6 Relations between annual mean treatment (*n* = 5) total (shoots and roots) biomass production (t ha⁻¹) and canopy N content (kg ha⁻¹) for the *Scirpus olneyi* (C₃)-dominated community (top panel). Regression equations for elevated treatment (solid line): $y = 0.115x + 1.28$ ($R^2 = 0.81$; $P < 0.01$); ambient control (dotted line): $y = 0.086x + 1.63$ ($R^2 = 0.87$; $P < 0.01$); and unchambered control (dashed line): $y = 0.089x + 0.91$ ($R^2 = 0.87$; $P < 0.01$). Relations between the stimulation [(E-A)/A × 100%] of mean total biomass vs. canopy N content [■; $y = 1.45x - 24.4$ ($R^2 = 0.60$; $P < 0.01$)] and vs. shoot [N] [◆; $y = -0.26x - 10.2$ ($R^2 = 0.31$; $P = 0.07$)] for the *S. olneyi*-dominated community (bottom panel).

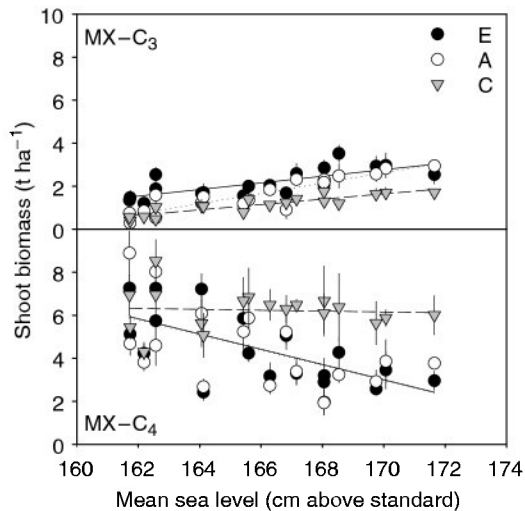


Fig. 7 Relations between mean treatment ($n = 5$) shoot biomass production (t ha^{-1}) and seasonal mean sea level (centimeter above a fixed standard) for (a) C_3 (*Scirpus olneyi*) and (b) C_4 (*Spartina patens* & *Distichlis spicata*) species in the mixed-assembly community. Regression equations are (a) ambient (dotted line): $y = 0.239x - 38.0$ ($R^2 = 0.79$; $P < 0.01$); elevated (solid line): $y = 0.155x - 23.5$ ($R^2 = 0.50$; $P < 0.01$); unchambered control (dashed line): $y = 0.125x - 23.5$ ($R^2 = 0.77$; $P < 0.01$); and (b) ambient (dotted line): $y = -0.352x + 62.83$ ($R^2 = 0.21$; $P < 0.01$); elevated (solid line): $y = -0.355x + 63.30$ ($R^2 = 0.27$; $P < 0.01$); unchambered control (dashed line): $y = 0.029x + 9.71$ ($R^2 = 0.05$; $P = 0.78$). A significant ambient and elevated $\text{CO}_2 \times$ sea level interaction ($P = 0.04$) was found for the C_3 species.

cycle may show a progressive decline in growth (e.g. Luo *et al.*, 2004; Hungate *et al.*, 2005). Nevertheless, sustained enhancement of growth has been found in a scrub oak ecosystem (Dijkstra *et al.*, 2002; Hymus *et al.*, 2002), a tallgrass prairie (Owensby *et al.*, 1999) and several forested ecosystems (Norby *et al.*, 2005) indicating that increased productivity of many ecosystems will follow global increases in atmospheric $[\text{CO}_2]$.

The lack of an overall C_4 growth response to elevated $[\text{CO}_2]$ was somewhat surprising in an environment where salt-induced water stress is common given that other studies have reported increases in C_4 plant performance under elevated $[\text{CO}_2]$ attributable to increased water-use efficiency (Owensby *et al.*, 1999; Wand *et al.*, 1999). However, in this tidal wetland system there may be increased flooding associated with reduced water use under elevated $[\text{CO}_2]$ (Arp *et al.*, 1993; Niklaus *et al.*, 1998), which could negatively impact plant growth for species with limited ability to tolerate flooding (Howes *et al.*, 1986; Magonigal & Schlesinger, 1997).

Thus, the significant and sustained enhancement of biomass production in the C_3 sedge and the corresponding lack of enhancement in the C_4 grasses have implications for enhanced carbon sequestration and potential

shifts in species abundance and distribution. However, elevated $[\text{CO}_2]$ effects on C_3 biomass in the mixed community were temporally variable, as the significant CO_2 enhancement of C_3 shoot biomass in the mixed community declined through time leading to no treatment effect on aboveground biomass after about 10 years of elevated $[\text{CO}_2]$ exposure (Fig. 2). The sustained biomass response of *S. olneyi* to elevated $[\text{CO}_2]$ in the *S. olneyi*-dominated community was likely a result of enhanced photosynthesis (Rasse *et al.*, 2005), which makes the disappearance of the elevated $[\text{CO}_2]$ effect on *S. olneyi* shoot biomass in the mixed community difficult to explain. The most probable explanation involves elevated $[\text{CO}_2]$ effects on biological and physical interactions (i.e. biomass production, marsh surface accretion rates, salinity and sea level) through time.

Biomass production, precipitation, salinity and flooding

Aside from elevated $[\text{CO}_2]$ effects on growth, salinity and flooding affected species distribution and biomass production throughout the study. Salinity and flooding are well-known determinants of vegetation distribution and biomass production in tidal wetlands (Pennings & Callaway, 1992; Pennings *et al.*, 2005). In the high marsh zone where our study site is located, *S. patens* is predominantly found at relatively high elevations, characterized by high salt stress compared with the relatively low elevations where *S. olneyi* is generally found (Arp *et al.*, 1993). Although *S. olneyi* and *S. patens* are salt tolerant, biomass production in both species was influenced by salinity (Flowers & Yeo, 1986). We found that the greatest biomass production occurred during years when precipitation was high and salinity low, ca. 2.9 t ha^{-1} more *S. olneyi* biomass in the elevated $[\text{CO}_2]$ treatment during the three wettest years, and the converse when precipitation was low and salinity high, although even in the driest years, elevated $[\text{CO}_2]$ increased biomass production by an average 1.2 t ha^{-1} . Ambient shoot biomass increased approximately 50% for C_3 and 17% for C_4 species as salinity went from a high of 10.6 ppt in 1999 to a lower salinity of 5.8 ppt in 2000 while seasonal mean water levels were similar. These patterns were observed in both the C_3 and C_4 species across all communities but the interaction between the elevated $[\text{CO}_2]$ treatment and salinity was greater in the more salt-sensitive C_3 species (Rozema *et al.*, 1991; Broome *et al.*, 1995).

In addition to salt-mediated effects on biomass, water level in the marsh affected biomass production during the study. *S. olneyi* shoot biomass declined in the *S. olneyi*-dominated community at the same time it increased in the mixed community, and this was correlated in both cases with rising water levels in the marsh

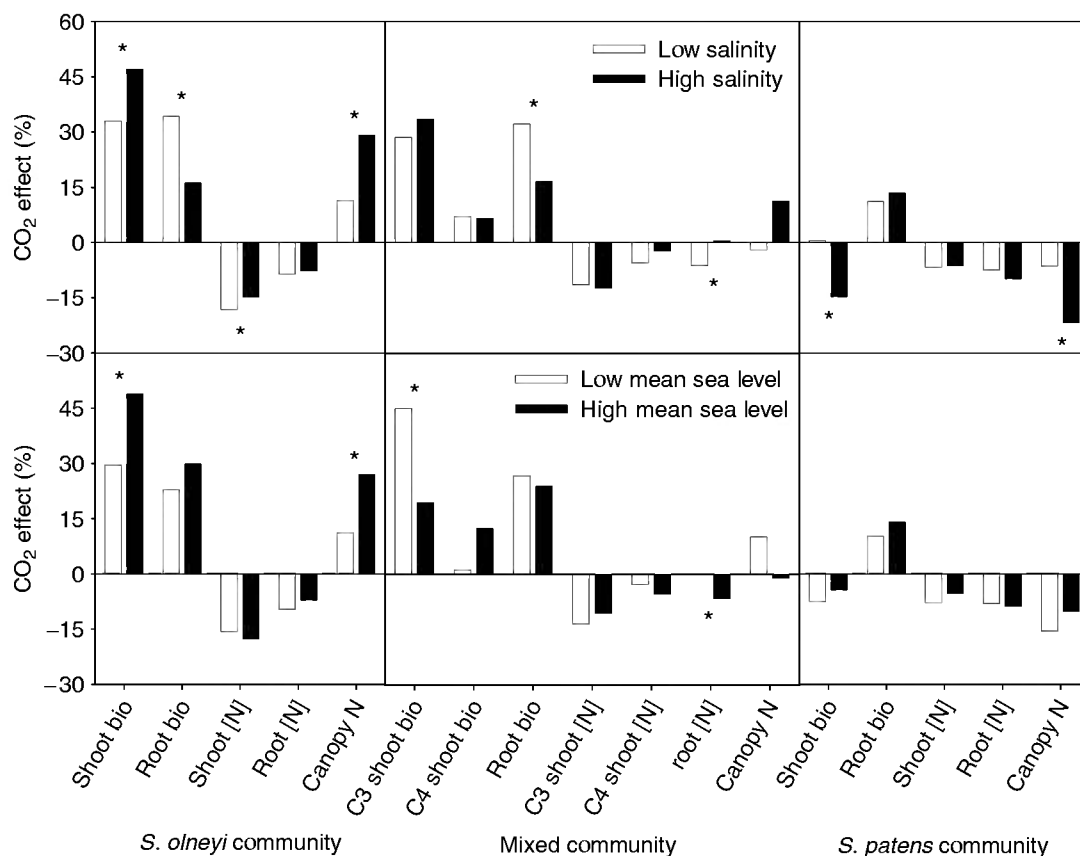


Fig. 8 Mean relative CO₂ stimulation $[(E-A)/A \times 100\%]$ of biomass, tissue [N] and canopy N content of the C₃ sedge (*Scirpus olneyi*) and C₄ grasses (*Spartina patens* and *Distichlis spicata*) across a range of communities for years where seasonal mean salinity of the tidal creek was low (<7 ppt; unfilled bars) and high (>7 ppt; filled bars), and years where seasonal mean sea level was relatively low (mean -4.2 to mean cm; unfilled bars) and high (mean to mean +5.7 cm; filled bars). The relative CO₂ effect was tested to be significantly greater or less than ambient using a one-tailed *t*-test assuming equal variance (**P* < 0.10).

(Fig. 7). Similarly, the decline of the C₄ grasses through time was also strongly related to mean sea level. A pronounced decline in C₄ biomass in both communities occurred during the years from 1994 to 1998, during which time sea level rose dramatically, and since 1998 both C₄ biomass and mean sea level have been relatively stable (Figs 1 and 2).

Sea level determines the frequency and duration of flooding in the marsh, thereby influencing soil pore-water salinity and belowground microbial processes that affect plant production (Morris *et al.*, 1990). As discussed in the 'Materials and methods,' variability in sea level had no discernible effect on soil pore-water salinity, especially when compared with precipitation in this wetland (data not shown). So, given the C₃ and C₄ biomass trends in the current study, we propose that rising sea level affected biomass production through increased flooding, which led to a decline in *S. patens*, a less flood-tolerant species (Anastasiou & Brooks, 2003), and an increase in *S. olneyi* in the mixed community,

with its well-developed aerenchyma system to transport oxygen to the roots (Arp *et al.*, 1993; Broome *et al.*, 1995). This notion is consistent with the findings of Broome *et al.* (1995), who found that *S. olneyi* was more sensitive to salt compared with *S. patens*, and *S. patens* was more sensitive to flooding compared with *S. olneyi*. Increased duration of flooding may also explain the negative chamber effect on *S. patens* biomass production. There is less turbulence within the chambers compared with outside the chambers (Drake & Peresta, 1994) which would reduce evapotranspiration (Bremer *et al.*, 1996). While relations between sea level, salinity and biomass production during the course of this study suggest the importance of these variables, the precise mechanisms through which they affect biomass are not well understood and are deserving of further research. Nevertheless, these mechanisms may have interacted with the effects of elevated [CO₂], contributing to much of the variability found in the biomass response to elevated [CO₂].

Consequences of environmental variability for biomass production under elevated [CO₂]

Our results point to interactions between salinity, sea level and CO₂ in the regulation of plant growth and species interactions on the Chesapeake Bay wetland studied here (Derner *et al.*, 2003). We found greater relative elevated [CO₂] effects on C₃ shoot biomass during high salinity or low precipitation years, but stimulation of root biomass was lower, offsetting the higher stimulation of shoot biomass (Fig. 8). This pattern resulted from an increase in root production relative to shoot production of ambient-grown plants under high salinity (Ball & Farquhar, 1984), while the ratio remained unchanged for plants grown in elevated [CO₂]. We interpret these findings to indicate that elevated [CO₂] at least partially ameliorated salt stress on *S. olneyi* biomass production, although absolute biomass production was low in both treatments during years of high salinity. The C₄ species in our study showed no CO₂ effect on shoot biomass during low salinity years and a negative effect during high salinity years (~–15%), which was not readily explainable, but Rozema *et al.* (1991) reported a similar reduction in growth of *S. patens* under elevated [CO₂] and high salinity.

Our finding of greater *relative* effects of elevated [CO₂] stimulation of *S. olneyi* shoot biomass at high salinity is consistent with Owensby *et al.* (1999), who reported greater *relative* elevated [CO₂] effects on biomass during years of high water stress in a tallgrass prairie. However, *S. olneyi* roots and *S. patens* shoots showed relatively greater CO₂ stimulations during low salinity years, similar to Mojave Desert shrubs, which had a greater photosynthetic and growth response to increased [CO₂] during wet years (Naumburg *et al.*, 2003; Housman *et al.*, 2006). Thus, interactions between increased [CO₂] and water availability have consequences for plant growth and likely other ecosystems processes.

Interactions between mean sea level and elevated [CO₂] were more subtle, affecting primarily *S. olneyi* shoot production. Effects of elevated [CO₂] were greatest during relatively high water level years in the *S. olneyi*-dominated community. In contrast, elevated [CO₂] stimulated *S. olneyi* shoot biomass more during relatively low water level years in the mixed community. Interestingly, the relative effects of elevated [CO₂] for *S. olneyi* biomass production were greatest when absolute biomass production was least in both communities, which may reflect a general pattern of relatively greater CO₂ effects on C₃ biomass growing under stressful conditions (Luo & Mooney, 1999). A possible mechanism for greater relative elevated [CO₂] effects on *S. olneyi* biomass under stress could be related to a relative increase in water-use efficiency (i.e. carbon

assimilated per unit water loss) under stressful environmental conditions (Leymarie *et al.*, 1999), as both increased flooding and increased salinity often decrease stomatal conductance (e.g. Farquhar & Sharkey, 1982, unpublished data).

Alternatively, relatively greater CO₂ treatment effects on *S. olneyi* biomass in response to physical stress could also be explained by indirect effects of elevated CO₂ on soil surface accretion rates. Macrophyte production in tidal wetlands is closely related to soil surface accretion rates (e.g. Morris *et al.*, 2003). Therefore, it is plausible that increased biomass production under elevated [CO₂] has increased soil surface accretion relative to ambient control plots, thereby altering relations between the physical environment, particularly water level and the CO₂ treatments. Although a substantial amount of sequestered carbon remains unaccounted for in the present study (e.g. Marsh *et al.*, 2005) and could be in accreting soils, more detailed data on water levels and plot elevation, which have not been collected to date, are needed to fully resolve the question of whether elevated [CO₂] leads to increased soil surface accretion rates in the marsh.

Relations among elevated [CO₂], N and biomass

Depression of *S. olneyi* shoot [N] and Rubisco content (Jacob *et al.*, 1995) was sustained but variable throughout the study, and varied in a way that was consistent with soil N availability failing to keep pace with accelerated plant growth (Stitt & Krapp, 1999). We found lower shoot [N] during years of high precipitation and low salinity (which supported high biomass production), while the converse was true when precipitation was low and salinity was high (and biomass production was relatively low). Years of low salinity were also years when the *relative* elevated [CO₂] effect on *S. olneyi* shoot biomass and canopy N content were low (Fig. 8), and soil inorganic [N] was also low (Matamala & Drake, 1999), indicating possible constraints on the supply of N: had there been more N available, there may have been even greater [CO₂] stimulation of growth. While this theoretical possibility is admitted, elevated [CO₂] enhancement of growth in the *S. olneyi*-dominated community more than offset reduced tissue [N], resulting in a greater standing crop of plant N (Fig. 6) suggesting that N limitation occurs only when other stress factors are relatively low or absent. In addition, it is important to emphasize that the largest elevated [CO₂] stimulation of *S. olneyi* total biomass production occurred during years when elevated [CO₂] stimulation of shoot [N] was greatest (i.e. greatest relative depressions in shoot [N]), which did not support our hypothesis that the relative stimulation of shoot [N] would be

positively correlated with the stimulation of total biomass (Fig. 6). We did, however, find a strong positive correlation between elevated [CO₂] stimulation of *S. olneyi* total biomass and canopy N content (Green *et al.*, 2003; Ollinger & Smith, 2005). Furthermore, based on these lines of evidence, the relative depression of shoot [N] in *S. olneyi* could not be related to acclimation of photosynthesis (Jacob *et al.*, 1995; Rogers *et al.*, 1996; Drake *et al.*, 1997). The data available to us (e.g. Rasse *et al.*, 2005) indicates that acclimation of photosynthesis was greatest during the most stressful years (i.e. when the reduction of tissue [N] was least) consistent with the stimulation of total biomass.

Photosynthetic downregulation and/or accelerated plant growth with reduced soil N supply under elevated [CO₂] could not, however, explain why significant declines in both shoot and root [N] were found for *S. patens* in the *S. patens*-dominated community (Figs 3 and 4), which showed no significant growth response to elevated [CO₂] (Tables 1 and 2). Reduced stomatal conductance and presumably reduced transpiration, which has been observed for all communities under elevated [CO₂] (Arp *et al.*, 1993), could have contributed to a decline in N acquisition (McDonald *et al.*, 2002) or led to more reduced soil conditions and an inhibition of N uptake by increased hydrogen sulfide production (Koch *et al.*, 1990). Regardless of mechanism, tissue [N] was reduced in roots and shoots in both species, especially the C₃ species, throughout the study.

Conclusions

In this study, elevated [CO₂] stimulated total biomass production (~35%) in the C₃ sedge, *S. olneyi*, and this effect was sustained for 18 years, but had no significant overall growth effect on the C₄ grass, *S. patens*. Although elevated [CO₂] stimulated total biomass production in the C₃ dominated community in all years, interannual variation in the effect of elevated [CO₂] was high and mainly regulated by variation in rainfall, salinity and tidal inundation. Interestingly, elevated [CO₂] significantly reduced shoot and root [N] in both C₃ and C₄ species with no apparent effects for growth in the *S. patens*, but potentially constraining growth responses of *S. olneyi* to elevated [CO₂] at low salinity. The greatest growth occurred during the wettest years and for *S. olneyi* was inversely correlated with a reduction in tissue [N] suggesting that if N limitation of growth occurred, it did so mainly in the absence of other stresses. There was a general decline in productivity of *S. olneyi* in the *S. olneyi*-dominated community and *S. patens* throughout the study, apparently caused by rising sea level. The decline in *S. olneyi* productivity was partially overcome by growth in elevated [CO₂].

Across all environmental conditions elevated [CO₂] favored growth of *S. olneyi* at the expense of *S. patens* and *D. spicata*.

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