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Effect of Elevated CO_2 on Carbon Pools and Fluxes in a Brackish Marsh

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ABSTRACT: The effects of long-term exposure to elevated atmospheric CO_2 (ambient + 340 ppmv) on carbon cycling were investigated for two plant communities in a Chesapeake Bay brackish marsh, one dominated by the C_3 sedge *Schoenoplectus americanus* and the other by the C_4 grass *Spartina patens*. Elevated CO_2 resulted in a significant increase in porewater concentrations of DIC at 30 cm depth ($p < 0.1$). The CO_2 treatment also yielded increases in DOC (15 to 27%) and dissolved CH_4 (12–18%) in the C_3 marsh (means for several depths over the period of June 1998 and June 1999), but not at a significant level. Elevated CO_2 increased mean ecosystem emissions of CO_2 (34–393 g C m^{-2} yr^{-1}) and CH_4 (0.21–0.40 g C m^{-2} yr^{-1}) in the C_3 community, but the effects were only significant on certain dates. For example, CO_2 enrichment increased C export to the atmosphere in the C_3 community during one of two winter seasons measured ($p = 0.09$). In the C_4 community, gross photosynthesis responded relatively weakly to elevated CO_2 (18% increase, $p > 0.1$), and the concomitant effects on dissolved carbon concentrations, respiration, and CH_4 emissions were small or absent. We concluded that elevated CO_2 has the potential to increase dissolved inorganic carbon export to estuaries.

Introduction

The carbon dioxide (CO_2) content of the atmosphere rose by 30% during the 20th century and it is presently increasing by approximately 1% yr^{-1}, a pace that will double the concentration from 365 to 730 ppmv by the end of the 21st century. Experiments in a variety of terrestrial ecosystems have reported that CO_2 enrichment almost always increases net ecosystem CO_2 assimilation (Curtis 1996). Enhanced CO_2 uptake by vegetation has the potential to influence future rates of atmospheric CO_2 increase, provided a portion of the new carbon uptake is sequestered in slow-cycling pools such wood or soil organic matter (VEMAP Members 1995).

The additional carbon also may be allocated to fast-cycling pools where it will be consumed in ecosystem metabolism and rapidly transferred to adjacent ecosystems and the atmosphere. If the response of tidal marshes to elevated CO_2 is similar to some graminoid-dominated grasslands, much of the additional CO_2 assimilated by the C_3 marsh will be exported from the system rather than stored (Hungate et al. 1997). It is the fast cycling pools that are the focus of this work.

Kirkpatrick marsh on the Chesapeake Bay, Maryland, has been the site of an ongoing CO_2 enrichment study since 1987, a sufficient period for short-term carbon pools to respond. Plant communities dominated by a species with C_3 photosynthesis (*Schoenoplectus americanus*) responded to twice-ambient CO_2 levels with increased rates of photosynthesis and net ecosystem CO_2 uptake (Drake et al. 1996). Increased photosynthesis stimulated a variety of short-term carbon sinks including fine root productivity (Drake et al. 1996), N_2 fixation (Dakora and Drake 2000), and methanogenesis (Dacey et al. 1994). These effects were largely absent in a plant community dominated by a C_4 species (*Spartina patens*), demonstrating that the effects are due to the response of C_3 plants to elevated CO_2. The C_4 genus *Spartina* dominates large areas of coastal wetlands in the United States; C_3 species dominate extensive areas of tidal freshwater wetlands on the U.S. Atlantic coast, but also occur in saline wetlands (e.g., *Juncus spp., Baccharis halimifolia, Iva frutesans*).

We previously showed that elevated CO_2 increased photosynthesis in a brackish marsh community dominated by a C_3 sedge (Drake et al. 1996). Since the third year of the experiment (1989) elevated CO_2 has increased net ecosystem exchange by an average of 35% (Rasse et al. 2005). Here we consider the fate of this carbon. Our objective was to quantify the effects of elevated CO_2 on dissolved concentrations of porewater inorganic carbon (DIC), organic carbon (DOC), and methane (CH_4) and direct gaseous emissions of CO_2 and CH_4 to the atmosphere. We hypothesized that elevated CO_2 would increase pool sizes and fluxes in the C_3-dominated community, but not the C_4-dominated community.

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Methods and Materials

The study was conducted in two brackish marsh communities on the Rhode River subestuary of Chesapeake Bay, U.S. (38°51′N, 76°32′W; for site map see Curtis et al. 1989a). The C_3 community is dominated by a C_3 sedge, *S. americanus*, while the C_4 community is dominated by the C_4 grass *S. patens* (Curtis et al. 1989a,b). Since 1987, these communities have been exposed to elevated concentrations of atmospheric CO_2 using open-top chambers. In each community, five replicate 0.47-m^2 plots are treated with either ambient CO_2 concentrations (360 ppm) or elevated CO_2 concentrations (ambient + 340 ppm). The chambers are normally removed from the plots after senescence of the plants in November and returned at the onset of growth in April. For the present study, the chambers were left in place throughout the winters of 1997–1998 and 1998–1999 to measure ecosystem respiration. Descriptions of the communities, the study's block design, and the chamber technology are found in Curtis et al. (1989a,b), Arp (1991), and Leadley and Drake (1992). The soils contain approximately 80% organic matter (39% carbon) to 4.5 m depth and the bulk density of the surface 30 cm is 0.12 gdw cm^{-3}.

Gas Flux Measurements

CO_2 and CH_4 emissions from the C_3 and C_4 communities were measured between December 1997 and September 1999. There were a total of 14 CO_2 flux measurements during the growing season and 17 CO_2 flux measurements during the winter in each community. C_3 and C_4 measurements were taken on the same day. Over the 2-yr period, CH_4 flux was measured during the growing season 14 times in the C_3 community and 7 times in the C_4 community, over the same period of time.

Ecosystem respiration was measured in a closed mode by capping the open-top chamber, disconnecting and sealing the through-flow blower, and measuring changes in CO_2 or CH_4 concentration over time. The circulating blower in each chamber was left on to ensure adequate mixing of the chamber atmosphere. Because CO_2 flux measurements were taken during the day, a reflective blackout cover was used during the growing season to prevent photosynthetic CO_2 assimilation. Changes in CO_2 concentrations were measured over a 5-min period (6 measurements at 1-min intervals) with either a Licor 6200 or Licor 6400 infrared gas analyzer. A linear regression was then used to determine the CO_2 emission in each plot on a given date ($n = 6$). The CO_2 fumigation treatment stopped in the winters of 1997–1998 and 1998–1999, but the chambers were left in place in order to determine whether the growing season stimulation in soil respiration observed by Ball and Drake (1998) carried into the nongrowing season. The atmosphere inside the chambers subjected to elevated CO_2 remained at ~2-times ambient during the CH_4 emission measurements. CO_2 flux from the elevated chambers was always measured at ambient CO_2 concentrations, even during the growing season, in order to minimize diffusive losses via leaks. It was not necessary to measure respiration rates at elevated CO_2 because the dark respiration of plants CO_2 is not instantaneously influenced by CO_2 concentration (Janke 2001). Respiration measurements taken during the growing season using the air-tight lid design were not significantly different from those taken using the open-top system used since 1987 ($p = 0.49$, *t*-test).

CH_4 emission measurements were taken during the late afternoon and at night to minimize the disruption caused by long incubation periods. Long incubations were necessary because fluxes were low, as expected in a brackish marsh, and the chamber headspace (483 L) was relatively large for this type of measurement. Gas samples for CH_4 analysis were taken at 15-min intervals over a 60-min period. Five chambers were measured simultaneously. Samples were stored in gas-tight syringes and analyzed within 24 h on a Hewlett Packard 5890 gas chromatograph with an flame ionization detector (FID) detector and a Porapak Q 80/100 mesh column. The result of leakage tests on the same syringes over a comparable period of time was 94 ± 4% retention. A linear regression was then used to determine the CH_4 emission in each plot on a given date ($n = 5$).

Because CH_4 flux did not correlate well with temperature, we integrated emissions across time by multiplying the average of fluxes measured on consecutive sampling dates by the elapsed time. A different approach was used for CO_2 flux because it correlated well with temperature. The Lloyd and Taylor (1994) equation was used to model the relationship between closed-chamber respiration (measurements described above) and noon soil temperature at 10 cm depth for each community-CO_2 treatment combination. Separate equations were generated for 1998 and for the winter of 1998–1999. These equations were driven by hourly soil temperature data (10 cm depth) from the two communities to predict annual and winter respiration.

Porewater Measurements

In May 1998, 9 wells (i.e., sippers) were placed in each C_3 chamber and 6 wells in each C_4 chamber. Wells in the C_3 community were placed at 10, 30, and 75 cm (3 per depth). Due to the shallow rooting depth of the C_4 community, wells were

placed at only 10 and 30 cm (3 per depth). The wells were made of teflon tubing (9 mm od × 6 mm id) sealed at the bottom with silicone caulk and capped at the top with a 3-way stopcock. Holes in the well extended 2.5 cm above and below the sampling depth. The 15 cm-long aboveground portion of the well was covered in black tape to inhibit algal growth in the well. Stopcocks on the top of the wells prevented outgassing during sampling. To test whether surface water was pulled down the sides of the wells during sampling, a BaCl tracer was introduced to surface water around identical wells placed outside of the study plots. No tracer was found in well samples as determined by a Perkin Elmer Optima 3000DV inductively coupled plasma atomic emission spectrometer.

The wells equilibrated for 2 mo before the first samples were taken, then were sampled on a monthly basis for 1 yr (July 1998–June 1999). Porewater was taken by clearing the well of water with a 60 ml syringe, then drawing a 30 ml sample. A 10 ml syringe was used to draw a 5 ml sample for analysis of dissolved CH_4 in porewater. Five cm^3 of air was added to each CH_4 syringe and the sample was shaken vigorously for 2 min in the field. The water was drained and the remaining gas was stored at 4°C. The samples were warmed to room temperature and analyzed for CH_4 within 24–48 h of sampling.

DIC and DOC samples were filtered in the field with preleached millipore filters (0.45 μm pore) and stored without headspace in glass vials with Teflon caps. Samples were placed on ice until they could be stored at 4°C, then analyzed for DIC within 24 h on a Shimadzu TOC 5050. Samples from the same chamber and depth were pooled, then frozen at −10°C for later analysis of DOC. Frozen samples were thawed at 4°C and analyzed for DOC using the nonpurgeable organic carbon procedure on a Shimadzu TOC 5050 because of the samples' high DIC content. DOC measurements represent only nonvolatile organic carbon. DOC measurements were made within 2 mo of sampling, a period of time over which DOC is quite stable in frozen samples (Qualls et al. 1991).

Root Biomass

A pair of piston cores 5.1 cm diameter × 100 cm deep were removed from each chamber over a 3-wk period in August 1999, and refrigerated for up to 6 mo until processing. The cores were divided into increments ranging from 2 to 5 cm thick and washed through a 2-mm mesh sieve. Dead and living roots (and rhizomes) were separated on the basis of color and consistency, as were the roots of C_3 and C_4 species. Our subjective assessment is that refrigerated storage did not substantially affect root color or consistency. We used the fact that C_3 and C_4 plants discriminate differently against the light isotope of carbon in CO_2 (Schlesinger 1997) to validate our separation of C_3 and C_4 roots. Roots from C_3 plants were dark in color with $\delta^{13}C$ values under ambient conditions that ranged from −24‰ to −26‰, while C_4 plants were light in color with $\delta^{13}C$ that ranged from −12‰ to −15‰ (Megonigal unpublished data). These values are typical of C_3 and C_4 emergent plants.

Statistical Analyses

The CO_2 treatment effect on gas emissions (CO_2 and CH_4) and dissolved compounds in the porewater (DIC, DOC, and CH_4) was analyzed using the PROC MIXED model of SAS version 8.01 (SAS Institute Inc., Cary, North Carolina). A mixed linear design was used in which CO_2 was a fixed factor and the block × treatment interaction was a random factor. The CO_2 treatment effect was analyzed independently by date and depth (for porewater) using individual contrasts as well as by repeated measures models where date was a repeated factor.

As the DIC, DOC, and dissolved CH_4 data were from 3 depths and 12 equally-spaced (monthly) sampling dates, we used first-order linear autoregressive (AR(1)) models in which space and time were discrete (Littell et al. 1996). To avoid psuedoreplication when analyzing porewater samples, we used the mean of the three samples in each chamber at each depth and at each sampling date. In the C_4 community, porewater data from one of the blocks was not used because of the presence of C_3 stems in the plot. In the C_3 community, a mixed linear model was also used to analyze the relationship between mean annual concentrations of DIC in porewater ($n = 12$) and root mass from the August 1999 piston cores (by depth).

The 2 yr of gas emissions data were analyzed separately because both CO_2 and CH_4 showed a strong interannual pattern. As time was a continuous variable in both CO_2 and CH_4 data sets, exponential autocorrelation models were used (Littell et al. 1996). There were typically 9 sampling dates for the analysis of CO_2 and CH_4 emissions (fewer for the second year and some *Spartina* measurements, see Table 1). Belowground biomass in August 1999 was compared with a Student's *t*-test. For all analyses significance levels were set at $p < 0.1$ unless otherwise indicated. Bonferroni's correction was used to correct for multiple comparisons. One-sided tests were used for analyzing multiple comparisons, as treatment effect was only expected to increase gaseous flux or porewater carbon pools.

TABLE 1. Significance level (p) of the CO_2 treatment effect on carbon emissions in the air (CO_2 and CH_4) and dissolved porewater carbon (DIC, DOC, CH_4) during different seasons and years (w = winter, gs = growing season) or at different depths. p values are from repeated measures analyses. n = 12 for porewater carbon. [a] n = 9, [b] n = 8, [c] n = 10, [d] n = 4, [e] n = 5, [f] n = 3, * = $p < 0.1$ for gaseous carbon.

		Schoenoplectus (C_3)			*Spartina* (C_4)		
Gaseous carbon							
	Season and year	CO_2	CH_4		CO_2	CH_4	
Treatment	w1	0.36[a]			0.77[a]		
Treatment	w2	0.09[b,*]			0.55[b]		
Treatment	gs1	0.58[c]	0.40[a]		0.83[c]	0.51[d]	
Treatment	gs2	0.61[d]	0.26[e]		0.10[d]	0.91[f]	
Porewater carbon							
	Soil depth (cm)	DIC	DOC	CH_4	DIC	DOC	CH_4
Treatment	all depths	0.32	0.35	0.57	0.83	0.84	0.89
Treatment × depth	10	0.38	0.58	0.68	0.76	0.69	0.46
Treatment × depth	30	0.08*	0.36	0.51	0.89	0.97	0.65
Treatment × depth	75	0.57	0.14	0.50			

Mechanistic Modeling

Fluxes of CO_2 between the C_3-dominated marsh ecosystem and the atmosphere were simulated with a recently developed mechanistic model specifically adapted to *S. americanus* (C_3) communities (Rasse et al. 2003). The mathematical structure of the model is similar to that of the ASPECTS model (Rasse et al. 2001). Canopy photosynthesis was computed according to the theoretical model of de Pury and Farquhar (1997). Photosynthesis and light interception parameters needed by the model were specifically determined for *S. americanus*, as described in Rasse et al. (2003).

Autotrophic maintenance respiration costs were computed for each plant organ according to the total carbon content of the plant organ, tissue nitrogen concentration, and tissue temperature, according to the formula presented by Rasse et al. (2001). This equation was calibrated on plant tissue respiration data collected at the research site (Drake et al. 1996). Studies have suggested that foliage maintenance respiration is decreased by exposure to light (Kirschbaum and Farquhar 1984; Atkin et al. 2000). Although these studies suggest that the response is somewhat species specific, we used the conservative estimate that leaf respiration is reduced by a factor of 50% from 0 to 100 μmol photons m^{-2} s^{-1}.

Heterotrophic soil respiration was described according to the temperature-dependency relationship of Lloyd and Taylor (1994). The marsh organic soil was assumed to be consistently at saturation throughout the year, which implies that litter decomposition rates were not modified by soil water contents. Litter decomposition rates were calibrated on an annual basis on the data presented in Matamala (1997). Decomposition of litter carbon was simulated as generating 67% CO_2 and 33% stable soil organic carbon. This ratio of mineralization to soil carbon accumulation was derived from marsh soil and vegetation data presented by Nyman et al. (1995) and is consistent with the fact that our site is keeping pace with sea level rise (Megonigal unpublished data).

Model calibration was conducted on the value of the maximum leaf are index (LAI) for the growing season. Although LAI is estimated inside the experimental chambers each year in August, it does not necessarily represent the absolute maximum LAI for the growing season. Over the 13 yr of the experiment, some non-sedge plants have started to invade the pure *S. americanus* stands initially present in the chambers. These observations imply that the true chamber LAI is slightly above the average measured value for *S. americanus* stems alone. Our model calibration suggested that the LAI needed to be increased by 0.3 to fit the ambient respiration data. Simulation runs were started in 1990 so that results for 1998 and 1999 would not be affected by our estimate of the initial conditions. A daily time step was used.

Results

Measured CO_2 Emissions

Winter emissions of CO_2 in the C_3 community were stimulated by elevated CO_2 on 15 of 17 sample dates (Fig. 1). The difference between treatments was only significant on December 21, 1998 and April 26, 1999 ($p < 0.1$ with Bonferonni correction). When the entire period of measurement was considered there was a significant CO_2-induced increase in winter ecosystem respiration in 1998–1999 of 12% ($p = 0.09$). In the C_4 community, elevated CO_2 increased mean winter ecosystem respiration on just 5 of 17 sample dates, and the difference was never significant (Fig. 1, Table 1).

In contrast to the evidence for a CO_2-induced stimulation of ecosystem respiration in the C_3 community during the winter, there was no evidence of such an effect during the summer. Mean

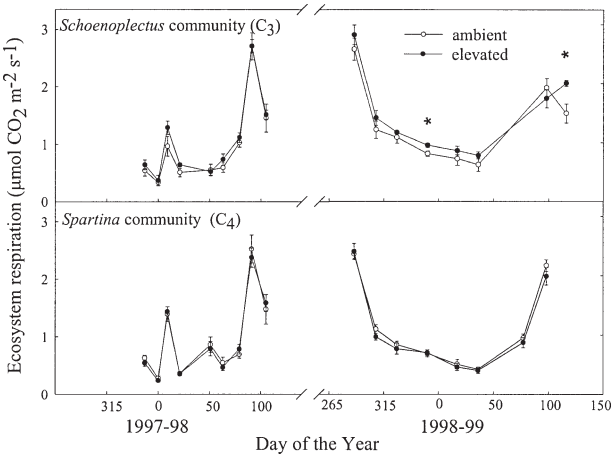

Fig. 1. CO_2 flux during the winter from *Schoenoplectus americanus* and *Spartina patens* marshes exposed to ambient and elevated (ambient + 340 ppm) concentrations of atmospheric CO_2 during the growing season. All measurements were taken at ambient CO_2 concentrations. Bars are standard errors (n = 4 or 5) and * indicates $p < 0.1$ in between treatment comparisons.

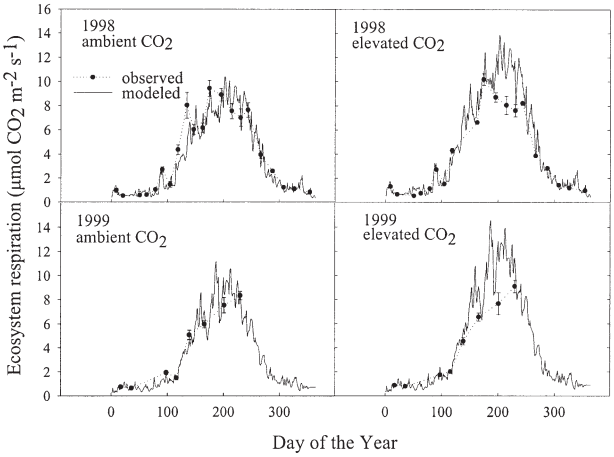

Fig. 2. Measured and simulated CO_2 flux during 1998 and 1999 from a *Schoenoplectus americanus* (C_3) marsh exposed to ambient and elevated (ambient + 340 ppm) levels of atmospheric CO_2. Measurements in both treatments were made at ambient CO_2 concentrations. Error bars as in Fig. 1.

ecosystem respiration was higher in the elevated CO_2 versus ambient CO_2 chambers on 8 of 12 dates, but there were no significant differences on any single date ($p > 0.1$). Repeated measures models did not show a difference between the growing season respiration rates of ambient and elevated chambers in either the C_3 or the C_4 community (Table 1).

TABLE 2. Model estimates of annual and winter CO_2 emissions (g C m^{-2} yr^{-1}) from the C_3 community using the Lloyd and Taylor regression and ASPECTS models, and changes in the components of ecosystem gas exchange as predicted by the ASPECTS model (C_3 community only).

	Lloyd and Taylor		ASPECTS
Community	C_4	C_3	C_3
1998			
Elevated	1448	1527	1719
Ambient	1520	1493	1326
Stimulation	72	34	393
1999			
Elevated	—	—	1584
Ambient	—	—	1222
Stimulation	—	—	363
1998–1999 winter			
Elevated	160	199	241
Ambient	174	177	192
Stimulation	−14	22	49

Average stimulation for 1998–1999 predicted by ASPECTS model

	g C m^{-2} yr^{-1}	%
Autotrophic respiration	241	27
Shoots	−14	−9
Roots	255	34
Heterotrophic respiration	137	35
Gross primary production	460	33
Net primary production	219	42

The Lloyd and Taylor (1994) regression model was fit to predict the ecosystem CO_2 respiration measurements from temperature and the output was generally consistent with in situ measurements (Table 2). Elevated CO_2 stimulated winter respiration by 12% in the C_3 community, but decreased winter respiration in the C_4 community. On an annual basis, elevated CO_2 caused a small increase in ecosystem CO_2 respiration in both communities.

Mechanistic Simulations of Carbon

The mechanistic model produced accurate simulations of the fluctuations in ecosystem respiration at ambient CO_2 levels throughout the year (Fig. 2). Although the absolute magnitude of ecosystem respiration was correctly described by the model, it is not an independent prediction because it is a direct consequence of calibrating (i.e., fitting) the respiration data to maximum leaf area index. The model fit was particularly good for 1998, which had many more data points than 1999. These simulations allowed us to derive an estimate of the annual ecosystem respiration at ambient CO_2 concentrations of 1,326 and 1,222 g C m^{-2} yr^{-1} in 1998 and 1999, respectively (Table 2).

At elevated CO_2 concentrations the mechanistic model tended to overestimate the measured ecosystem respiration, particularly at peak photosynthesis (Fig. 2). On average, over the 2-yr period, the model predicted that ecosystem respiration was increased by 378 g C m^{-2} yr^{-1} in the elevated CO_2 treatment. This increase in simulated ecosystem respiration is less than the average increase in simulated gross primary production, which was 460 g C m^{-2} yr^{-1} (Table 2).

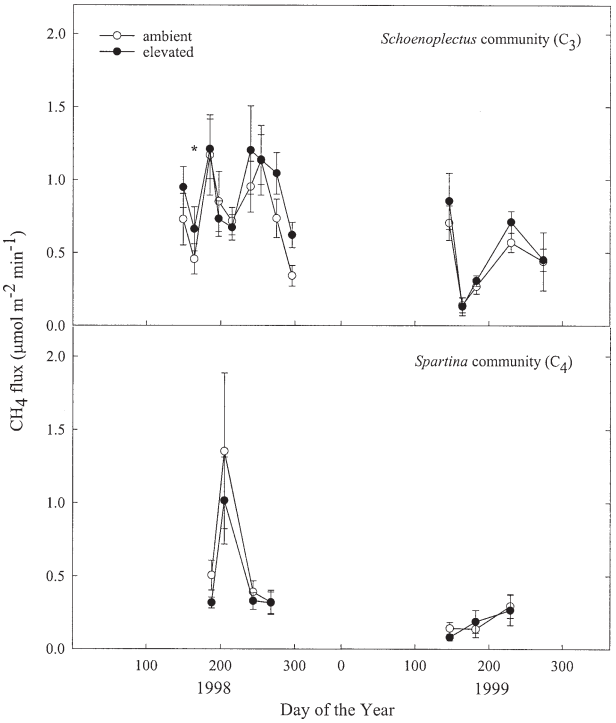

Fig. 3. CH_4 flux from *Schoenoplectus americanus* and *Spartina patens* marshes exposed to ambient and elevated (ambient + 340 ppm) concentrations of atmospheric CO_2. Error bars and * as in Fig. 1.

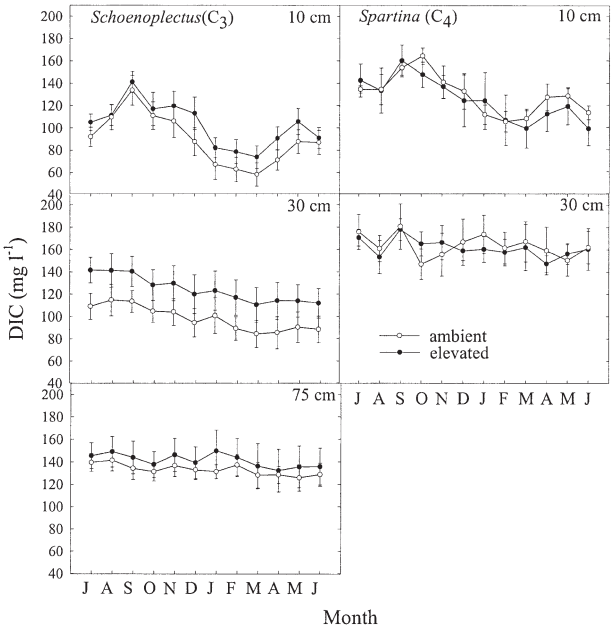

Fig. 4. Concentrations of dissolved inorganic carbon in the soil pore space of *Schoenoplectus americanus* and *Spartina patens* marshes exposed to ambient and elevated (ambient + 340 ppm) concentrations of atmospheric CO_2. Error bars are standard errors (n = 4 or 5).

The mechanistic model predicted that the response to elevated CO_2 was larger for the heterotrophic components of ecosystem respiration (+35%) than for their autotrophic counterpart (+27%). All of the autotrophic response to elevated CO_2 was predicted to occur in the root system, while shoots displayed a slight negative response due to the observed decrease in shoot nitrogen concentration under elevated CO_2.

CH_4 Emissions

Elevated CO_2 caused a significant increase in CH_4 flux in the C_3 community on one sampling date during the 1998 growing season (June 12; $p = 0.01$), but did not stimulate CH_4 flux when considered throughout the 1998 growing season ($p = 0.40$, Fig. 3). There was no CO_2 effect on CH_4 flux in the C_3 community during the 1999 growing season or on CH_4 flux in the C_4 community in the 1998 or 1999 growing season (Table 1).

Dissolved Inorganic Carbon

In a series of 12 monthly samples from the C_3 community, the average DIC concentration of porewater was always higher in the elevated CO_2 treatment than the ambient CO_2 treatment (Fig. 4). Elevated CO_2 increased mean annual DIC concentrations by 15% at 10 cm, 27% at 30 cm, and 6% at 75 cm. This effect was significant at the 30 cm depth ($p = 0.08$, Table 1). The sampling date had a significant effect on DIC concentrations, with peak concentrations at the end of the growing season ($p = 0.0001$). The C_4 community did not change in porewater DIC concentrations under elevated CO_2 (Table 1).

At 30 cm depth, the mass of C_3 roots in the C_3 community was a significant covariate of mean annual DIC concentrations ($p = 0.008$); plots with more C_3 roots had higher concentrations of DIC (Fig. 5). To date, we cannot explain the low root density in the elevated chamber of block five relative to the ambient chamber, and this is a factor that may have contributed to the variability of porewater DIC in our study. In the C_3 community, there was no relationship between DIC concentration and C_4 root mass at 30 cm depth, or between DIC concentrations and C_3 root mass at 10 or 75 cm depth.

Dissolved Organic Carbon and CH_4

Mean DOC and interstitial porewater CH_4 concentrations in the C_3 community were higher in the elevated CO_2 treatment than the ambient CO_2 treatment, but the differences were not significant ($p > 0.1$, Figs. 6 and 7, Table 1). The differences in

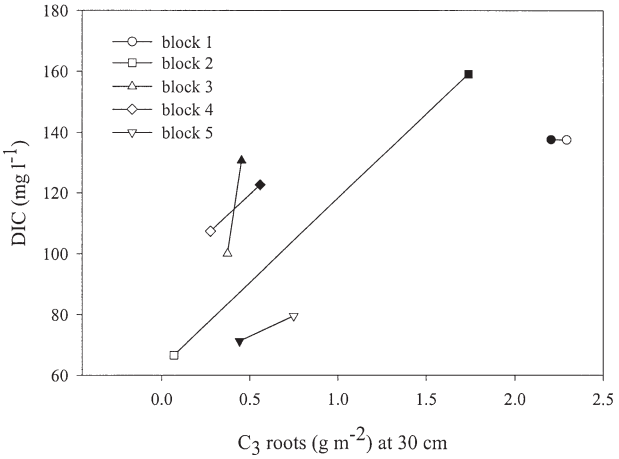

Fig. 5. Relationship between dissolved inorganic carbon concentrations in the soil pore space and C_3 root mass at 30 cm depth in a *Schoenoplectus americanus* community exposed to atmospheric CO_2 concentrations that were ambient (open symbols) or elevated (ambient + 340 ppm, closed symbols). Each symbol shape represents a different block in the experiment.

mean DOC concentrations were 15%, 27%, and 27% at 10, 30, and 75 cm, respectively; mean CH_4 was higher by 12%, 18%, and 16%. In the C_4 community, there was no evidence of a CO_2-treatment effect on DOC (Table 1). Absolute concentrations of DIC, DOC, and CH_4 in the C_4 community were consistently higher than in the C_3 community (Figs. 4, 6, and 7).

Root Biomass

In August 1999, belowground biomass in the C_3 community was significantly higher in the elevated CO_2 than ambient CO_2 chambers of the C_3 community ($p = 0.02$, Table 3). There was less stimulation in the C_4 community, and the effects were not statistically significant.

Discussion

Elevated CO_2 stimulated net ecosystem exchange (NEE) of CO_2 in this temperate brackish marsh by an average of 35% from 1989 to 2003 (Rasse et al. 2005), due mainly to enhanced rates of photosynthesis. Interannual variability in NEE stimulation, which is correlated with variation in rainfall and salinity, can be dramatic (Rasse et al. 2005). During the years spanned by this study the NEE stimulation ranged from about 20% (1999) to 40% (1998). Our primary objective was to determine whether enhanced photosynthesis due to elevated CO_2 stimulates other components of the carbon cycle.

The evidence presented here suggests that elevated CO_2 stimulated carbon cycling in the C_3 plant community. In the C_3 community, treatment means were consistently higher in the elevated CO_2

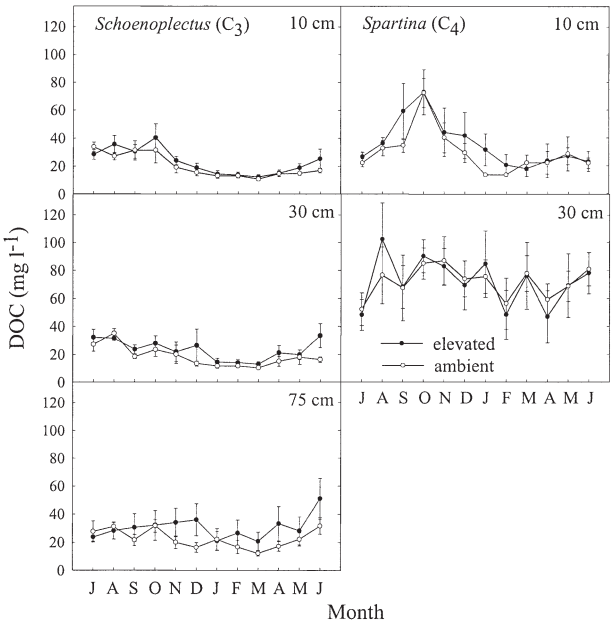

Fig. 6. Concentrations of dissolved organic carbon in the soil pore space of *Schoenoplectus americanus* and *Spartina patens* marshes exposed to ambient and elevated (ambient + 340 ppm) concentrations of atmospheric CO_2. Error bars are standard errors (n = 4 or 5).

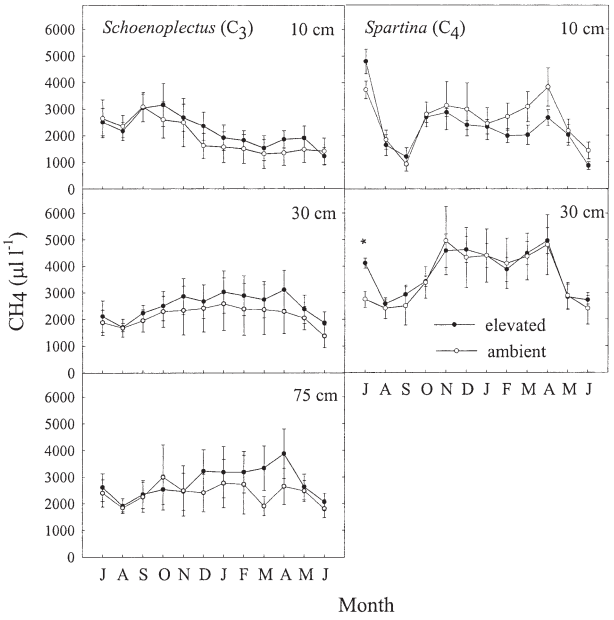

Fig. 7. Concentrations of CH_4 in the soil pore space of *Schoenoplectus americanus* and *Spartina patens* marshes exposed to ambient and elevated (ambient + 340 ppm) concentrations of atmospheric CO_2. Error bars are standard errors (n = 4 or 5) and * indicates $p < 0.1$ in between treatment comparisons.

TABLE 3. Summary of changes in carbon assimilation, biomass, and export from the C_3 and C_4 communities due to elevated CO_2. ** = significantly different at $p \leq 0.05$. nd = not determined because the rate of groundwater flux is unknown. Percent change calculated as (elevated−ambient)/ambient.

	C_3 Community		C_4 Community	
	g C m^{-2} yr^{-1}	%	g C m^{-2} yr^{-1}	%
Gross primary production[a]	600**	31	235**	12
Fine root production[b]	47**	83	8	13
Belowground biomass[c]	379**	42	106	20
CO_2 emissions	34–393	2–30	−72	−5
CH_4 emissions	0.2–0.4	15–17	−0.2–0.01	−20–3
CH_4 pool in groundwater	nd	12–18	nd	−12–5
DOC pool in groundwater	nd	15–27	nd	1–20
DIC pool in groundwater	nd	6–27	nd	−3–1
Rhizome export	0	0	nd	nd

[a] *Schoenoplectus americanus* (C_3) community from Drake et al. (1996), average of 1993 and 1994; *Spartina* (C_4) community from Drake (unpublished data) for the same time interval.
[b] From Curtis et al. (1990). Roots only (not rhizomes) from 0–15 cm depth.
[c] Roots and rhizomes based on piston cores taken in August 1999; units are g C m^{-2} to 15 cm.

treatment than the ambient CO_2 treatment for several response variables including interstitial porewater concentrations of DIC, winter emissions of CO_2, and summer emissions of CH_4. Root biomass was a significant covariate of DIC in the C_3 community, and there was a significant CO_2-induced increase in both root productivity and root biomass ($p = 0.05$, Table 3). Both the temperature-driven regression model and the mechanistic model simulated the observed increase in ecosystem respiration due to elevated CO_2 in the C_3 community (Table 2). Although the magnitude of modeled stimulation of ecosystem respiration was larger than the observed stimulation, the result was qualitatively consistent with the soil respiration work of Ball and Drake (1998). Most importantly, all of these comparisons suggested little or no effect of elevated CO_2 in the C_4 plant community, where CO_2 enrichment has little effect on photosynthesis because of the high carboxylation efficiency of C_4 plants (Hopkins 1995). Elevated CO_2 has a greater effect on C_3 photosysnthesis because it increases carboxylation efficiency by raising the $CO_2 : O_2$ ratio at the Rubisco enzyme. The most parsimonious explanation for our results is that the well-documented stimulation of photosynthesis in the C_3 community enhanced the size of several carbon pools and the magnitude of fluxes by 2–30% (Table 3).

Our experimental design ($n = 5$) at times lacked the statistical power required to resolve differences at the $p < 0.1$ level. The power (i.e., $1 - ß$, where ß is the probability of a type II error) to resolve differences in porewater concentrations of DIC, DOC, and CH_4 ranged from 0.3 to 0.5. We estimate that elevated CO_2 would need to raise DIC concentrations at 30 cm by 45%, rather than the observed 27%, in order for the significant difference ($p < 0.1$) we detected to have a power of 0.8. About 12 samples would be needed to achieve a power of 0.8 at the $p < 0.1$ level; fewer samples would be required if the standard deviation decreased with increasing sample size as might be expected. Because such large sample sizes are typically not possible in elevated CO_2 experiments, it is useful to have other sources of inference such as the C_4 community responses in the present study.

Marsh-atmosphere Gas Fluxes

A previous study in 1994 at this site reported a 15% stimulation in soil respiration in the C_3 community between June and August (Ball and Drake 1998); we observed an increase in mean summer ecosystem respiration of 4% using another method and the difference was not significant. We cannot adequately explain the difference between the two studies. Because our measurements of ecosystem respiration included CO_2 from both shoots and soils, the difference could be explained by a CO_2-induced decrease in shoot respiration. Our model suggests that shoot respiration in the two CO_2 treatments was similar. NEE stimulation was greater during the years of the present study (1998, 1999) than in 1994 (Rasse et al. 2005). In the present study, a CO_2 treatment effect on ecosystem respiration rates was most apparent in the winter. This may reflect decomposition of senescent root biomass, which previous work at the site showed was more abundant in the elevated CO_2 treatment (Curtis et al. 1990).

The CH_4 emission data collected for the present study showed a small and sporadic increase due to CO_2 enrichment. A significant stimulation in flux was only noted on one date, and the mean stimulation was <0.5 μmol m^{-2} min^{-1}. This stimulation in flux is lower than the 1.09 μmol CH_4 m^{-2} min^{-1} mean stimulation noted by Dacey et al. (1994) in the same CO_2-enriched C_3 marsh

during four nights in July 1991. The stimulation of CH_4 emissions by elevated CO_2 in this brackish marsh may be sporadic because of temporal changes in sulfate reduction (Megonigal unpublished data), which inhibits methanogenesis (Megonigal et al. 2004). We estimate that elevated CO_2 chambers in the C_3 community release 0.21–0.40 g more C m^{-2} yr^{-1} as CH_4 than ambient chambers, a flux that is at least 3 orders of magnitude less than the stimulation in CO_2 respiration (Table 2).

Porewater Carbon Pools

The significant increase in root biomass observed in this study must necessarily have stimulated root respiration and DIC production. Correlations between soil respiration and root mass have been reported in several other studies (Vose et al. 1995; Edwards and Norby 1999). Soil respiration was positively related to root biomass in elevated CO_2 mesocosms planted with *Populus tremuloides* (r^2 = 0.87; Pregitzer et al. 2000). Elevated CO_2 has stimulated soil respiration in every study where it has been measured (Allen et al. 2000; see list of citations in Pregitzer et al. 2000; Billings et al. 2002), with the exception of some agricultural studies (Prior et al. 1997; Ineson et al. 1998). In the present study, there was a significant increase in root and rhizome production and biomass (Table 3). The mechanistic model of the C_3 community predicted that elevated CO_2 increased ecosystem respiration due mainly to the response of roots and soil heterotrophs (Table 2). Much of the CO_2 emitted from roots in these saturated soils would be expected to dissolve and accumulate in the DIC pool. It is plausible that elevated CO_2 stimulated soil respiration, which in turn increased the DIC pool. Elevated CO_2 significantly increased the DIC pool in peat cores from a northern fen by 168% (Kang et al. 2001). Because the soil solution pH (approximately 6) does not favor deposition of carbonate minerals, the excess DIC produced in an elevated CO_2 atmosphere has the potential to be exported to the adjacent estuary or atmosphere. Wang and Cai (2004) reported that the Duplin River Estuary on Sapelo Island, Georgia, released as much as 109 g C m^{-2} of DIC to adjacent coastal waters, and much of it from the surrounding salt marshes.

The effects of elevated CO_2 on the DOC pool can be expected to be less dramatic than for the DIC pool because microorganisms efficiently consume labile DOC. Mean concentrations of DOC were 15–27% greater in the elevated CO_2 treatment than the ambient CO_2 treatment of the C_3 community, but the differences were not significant ($p > 0.14$). Elevated CO_2 significantly increased the DOC pool in peat cores from a northern fen by 41% (Kang et al. 2001). Freeman et al. (2004) reported evidence that contemporary elevated CO_2 is contributing to increased dissolved organic carbon output from rivers draining peatlands. Any direct effect of elevated CO_2 on DOC export would be in addition to enhanced DOC export caused by increased precipitation and runoff, as predicted in Canada (Clair et al. 1999). If elevated CO_2 does increase DOC export from wetlands, it could have important effects on carbon metabolism and ultraviolet radiation stress in aquatic ecosystems (Wetzel et al. 1995; Williamson et al. 2001).

A notable pattern in the DIC concentration data is the depth variation in the difference between ambient and elevated CO_2 treatment means (Fig. 4). This pattern reflects the influence of root depth distributions, hydrologic export of DIC, and CO_2 evasion rates to the atmosphere. Root biomass peaks at 10 cm below the soil surface and microbial respiration is expected to decrease sharply with depth due to changes in carbon quality. DIC may accumulate in the porewater at 30 cm depth because of relatively slow rates of evasion to the atmosphere. At 10 cm depth, the proximity to the surface and the movement of flood waters results in greater soil gas evasion. This effect and relatively large changes in soil temperature explain the large seasonal variations in DIC at 10 cm depth. At 75 cm depth, porewater is largely below the rooting zone (1 mg root biomass cm^{-3} compared to 15–30 mg cm^{-3} near the surface), so inputs of CO_2 from root respiration are relatively low.

Implications

Exposure to elevated CO_2 increased net ecosystem carbon uptake in the C_3 community of Kirkpatrick marsh, but it appeared that some of this additional carbon was allocated to relatively fast-cycling carbon pools where it may be returned to the atmosphere, or potentially exported to adjacent aquatic systems as DIC, DOC, and CH_4. It remains to be determined how much of the additional photosynthate produced at elevated CO_2 is exported in groundwater from this particular study site. It is likely that all tidal wetlands will be bathed in an atmosphere of >700 ppmv CO_2 by the year 2100. Because carbon export from wetlands to adjacent estuaries is quantitatively important at ambient CO_2 levels (Cai et al. 2000; Wang and Cai 2004), it is possible that carbon export may increase from marshes dominated by C_3 plant species. Many of the effects reported here such as increased photosynthesis, root biomass, and CH_4 production, have been reported for C_3 species found in tidal freshwater marshes, such as *Orontium aquaticum*, *Peltandra virginica*, and *Taxodium distichum* (Megonigal and Schlesinger 1997; Vann 2000; Vann and Megonigal 2003; Megonigal et al. 2005), suggesting

that such effects may extend to oligohaline and freshwater reaches of the estuary where DIC and CH_4 exports are particularly high (Cai and Wang 1998; Cai et al. 2000; Neubauer et al. 2000). Although it is important to consider estuarine wetlands in assessing the direct effects of elevated CO_2 on water quality and metabolism in the Chesapeake Bay, a full assessment of the issue must take into account the responses of forests, agricultural lands, and other upland ecosystems that also export water and carbon to the estuary.

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