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Elevated CO_2 affects porewater chemistry in a brackish marsh

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Abstract As atmospheric CO_2 concentrations continue to rise and impact plant communities, concomitant shifts in belowground microbial processes are likely, but poorly understood. We measured monthly porewater concentrations of sulfate, sulfide, methane (CH_4), dissolved inorganic carbon and dissolved organic carbon over a 5-year period in a brackish marsh. Samples were collected using porewater wells (i.e., sippers) in a *Schoenoplectus americanus*-dominated (C_3 sedge) community, a *Spartina patens*-dominated (C_4 grass) community and a mixed (C_3 and C_4) community within the marsh. Plant communities were exposed to ambient and elevated (ambient + 340 ppm) CO_2 levels for 15 years prior to porewater sampling, and the treatments continued over the course of our sampling. Sulfate reduction was stimulated by elevated CO_2 in the C_3-dominated community, but not in the C_4-dominated community. Elevated CO_2 also resulted in higher porewater concentrations of CH_4 and dissolved organic carbon in the C_3-dominated system, though inhibition of CH_4 production by sulfate reduction appears to temper the porewater CH_4 response. These patterns mirror the typical divergent responses of C_3 and C_4 plants to elevated CO_2 seen in this ecosystem. Porewater concentrations of nitrogen (as ammonium) and phosphorus did not decrease despite increased plant biomass in the C_3-dominated community, suggesting nutrients do not strongly limit the sustained vegetation response to elevated CO_2. Overall, our data demonstrate that elevated CO_2 drives changes in porewater chemistry and suggest that increased plant productivity likely stimulates microbial decomposition through increases in dissolved organic carbon availability.

Keywords Elevated CO_2 · Methane · Nutrients · Porewater · Sulfate reduction · Wetland

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Introduction

Wetland soils store an estimated 523 Pg of carbon, roughly one-third of the total global soil carbon pool (Bridgham et al. 2006). The accumulation of this soil carbon is the result of a persistent imbalance between

inputs of carbon, primarily from plant productivity, and outputs through microbial respiration and leaching. Future global changes, including elevated atmospheric concentrations of carbon dioxide (CO_2), can influence the size of the wetland soil carbon pool by inducing changes in plant productivity, soil microbial processes, or both. The response of plants to rising CO_2 concentrations has been studied in a number of systems and is generally well understood (Ainsworth and Long 2005). However, the effects of elevated CO_2 on soil microbial processes, and the linkages between plant and microbial responses, are still being elucidated.

The Kirkpatrick Marsh is a brackish tidal marsh on the Chesapeake Bay, Maryland. Since 1987, open-top chambers have been used to elevate CO_2 concentrations by $\sim$340 ppm above ambient in a *Schoenoplectus americanus*-dominated (C_3 sedge) community, a *Spartina patens*-dominated (C_4 grass) community and a $C_3 + C_4$ mixed species community. The responses of these plant communities to elevated CO_2 have been well studied (e.g., Rasse et al. 2005; Erickson et al. 2007 and references cited therein), and generally follow patterns expected based on the dominant photosynthetic pathway. Most recently, Erickson et al. (2007) showed that elevated CO_2 stimulated *S. americanus* shoot and root biomass in both the C_3-dominated community and the mixed community. In contrast, there was no significant effect of elevated CO_2 on *S. patens* biomass in either the mixed or C_4-dominated communities. While the magnitude of these responses varied annually, the overall direction of elevated CO_2 effects were consistent across 18 years of treatment.

There is a growing body of evidence showing that CO_2-enhanced plant productivity indirectly stimulates heterotrophic soil microbial processes as a result of increased organic carbon in the form of recent plant photosynthate (Megonigal et al. 1999). For example, higher rates of CH_4 emissions at elevated CO_2 have been observed in a number of wetland ecosystems (e.g., Megonigal and Schlesinger 1997; Vann and Megonigal 2003; Megonigal et al. 2004; Cheng et al. 2006). Similar plant-microbial linkages have been observed previously at Kirkpatrick Marsh. Ball and Drake (1998) reported a stimulation of soil respiration (as CO_2 flux) in response to elevated CO_2 in the C_3-dominated, C_4-dominated and mixed communities, but it was unclear whether this effect was due to changes in root respiration, microbial respiration, or both. The influence of elevated CO_2 on aerobic microbial respiration was isolated in a greenhouse study by Wolf et al. (2007), who observed an increase in soil organic matter decomposition rates in *S. americanus* mesocosms. Increased CH_4 production in response to elevated CO_2 has also been described at Kirkpatrick Marsh over a period of days (Dacey et al. 1994) and a single year (Marsh et al. 2005). However, the longer term response of anaerobic decomposition to elevated CO_2 remains unknown at this site, as does the response of sulfate reduction, which is likely to play an important role in the response of brackish marsh microbial communities to elevated CO_2.

Increased plant growth at elevated CO_2 also has the potential to influence soil nutrient dynamics. For example, storage of nitrogen as biomass under elevated CO_2 conditions may induce progressive nitrogen limitation (Hungate et al. 2003; Luo et al. 2004; van Groenigen et al. 2006). It has been observed that elevated CO_2 stimulates canopy-level nitrogen uptake in the *S. americanus* community at Kirkpatrick Marsh (Erickson et al. 2007). However, stimulation of *S. americanus* biomass by elevated CO_2 was sustained from 1987 through 2004, suggesting that nitrogen has not progressively limited plant production, possibly due to the 'open' nitrogen cycling (e.g., through tidal exchange) in this marsh (Erickson et al. 2007). In addition to directly influencing nutrient dynamics through increased plant uptake, elevated CO_2 could also influence nutrient dynamics through indirect plant-mediated effects on microbial mineralization (Wolf et al. 2007) and/or immobilization. Despite the potential for elevated CO_2 to alter nutrient availability, porewater nutrient dynamics have not been previously explored in Kirkpatrick Marsh.

In this study we explore the effect of elevated CO_2 on porewater chemistry, which serves as an indicator of anaerobic heterotrophic metabolism and nutrient dynamics, in Kirkpatrick Marsh. Porewater samples were collected using 'sipper' wells from 2002 to 2006 following 15–20 years of continuous prior CO_2 treatment. We hypothesized that increased *S. americanus* biomass and productivity under elevated CO_2 in the C_3-dominated community would (1) stimulate anaerobic decomposition through increased organic carbon availability and (2) decrease porewater nutrient availability as a result of increased plant nutrient uptake. In contrast, these elevated CO_2 effects on

porewater chemistry would not be present in the C_4-dominated community due to a lack of CO_2 stimulation of *S. patens* biomass and productivity.

Methods

Study site

This study was conducted in an ongoing elevated CO_2 experiment in a brackish marsh on the Rhode River subestuary of the Chesapeake Bay, USA (38°51′N, 76°32′W). The dominant plant species at this marsh are the C_3 sedge *Schoenoplectus americanus* (Pers.) Volk Ex Schinz & R. Keller and the C_4 grasses *Spartina patens* (Aiton) Muhl and *Distichlis spicata* (L.) Greene. *S. americanus* was formerly known as *Scirpus olneyi* (A.) Gray, and many previous publications from this site have used this nomenclature. Since 1987, open-top chambers have been used to elevate atmospheric CO_2 in a *Schoenoplectus*-dominated community ("C_3"), a *Spartina*-dominated community ("C_4"), and a mixed assemblage community containing *S. americanus*, *S. patens* and *D. spicata* ("Mixed"). Within each community, 0.47-m^2 circular plots were established using a randomized block design. One plot within each block was ventilated with ambient air ("Ambient") and a second plot was ventilated with ambient air + 340 ppm CO_2 ("Elevated") during May through October in each year. There were five replicates of each treatment per community. Additional descriptions of the experimental design are found in Curtis et al. (1989), Leadley and Drake (1992) and Arp et al. (1993).

Climate data were recorded at the Smithsonian Environmental Research Center, less than 1 km from the experimental site (Table 1). We present cumulative rainfall and average temperatures from the growing season (March–July) because these values were previously demonstrated to mediate the magnitude of the plant response to elevated CO_2 at this site (Erickson et al. 2007). Growing season precipitation and salinity are negatively correlated (Erickson et al. 2007), reflecting the importance of freshwater inputs to regulating salinity at Kirkpatrick Marsh. Thus, monthly measurements of porewater chloride concentrations likely provide insights into seasonal precipitation patterns (e.g., chloride concentrations were higher during the dry months of 2002, Fig. 1).

Table 1 Seasonal climate data

Year	Growing season rain (cm)[a]	Annual rain (cm)	TAir (°C)[b]
2002	37.6	109.0	17.7
2003	72.6	170.5	15.9
2004	54.2	109.0	17.7
2005	62.3	110.0	16.4
2006	55.6	128.3	17.3

[a] Cumulative precipitation received from March to July

[b] Average hourly air temperature measured from March to July

Porewater sampling

Porewater measurements were made using porewater wells (i.e., 'sippers') installed in each plot (Marsh et al. 2005). In May of 1998, nine wells were placed in each chamber in the C_3-dominated community at 10, 30, and 75 cm below the soil surface (three wells per depth) and six wells were placed in each chamber in the C_4-dominated community at 10 and 30 cm (three wells per depth). Originally, 75-cm wells were included in the C_4-dominated chambers; however, porewater samples could not be consistently collected and sampling was not continued. In May of 2003, six wells were also placed in the Mixed community chambers at 10 and 30 cm (three wells per depth). All wells were constructed as described previously (Marsh et al. 2005). Briefly, wells were made of Teflon tubing (9 mm o.d. × 6 mm i.d.) with holes in each well extending 2.5 cm above and below the sampling depths. Wells were sealed at the bottom with silicone caulk and capped at the top with a 3-way stopcock. For the present study, porewater was collected from all wells approximately monthly from January 2002 through December 2006 (for the C_3- and C_4-dominated communities) and from May 2003 through December 2006 (for the Mixed community). Porewater was not sampled in December–March in some years.

On each sampling date, two 30-mL syringes of porewater were collected from each sampling depth in each chamber. The sample in the first syringe was used to measure dissolved CH_4 and phosphorus, and the sample in the second syringe was used to measure sulfide, sulfate (SO_4^{2-}), chloride (Cl^-) and ammonium (NH_4^+) as described below. Initially wells were flushed using a 60-mL syringe to withdraw at least

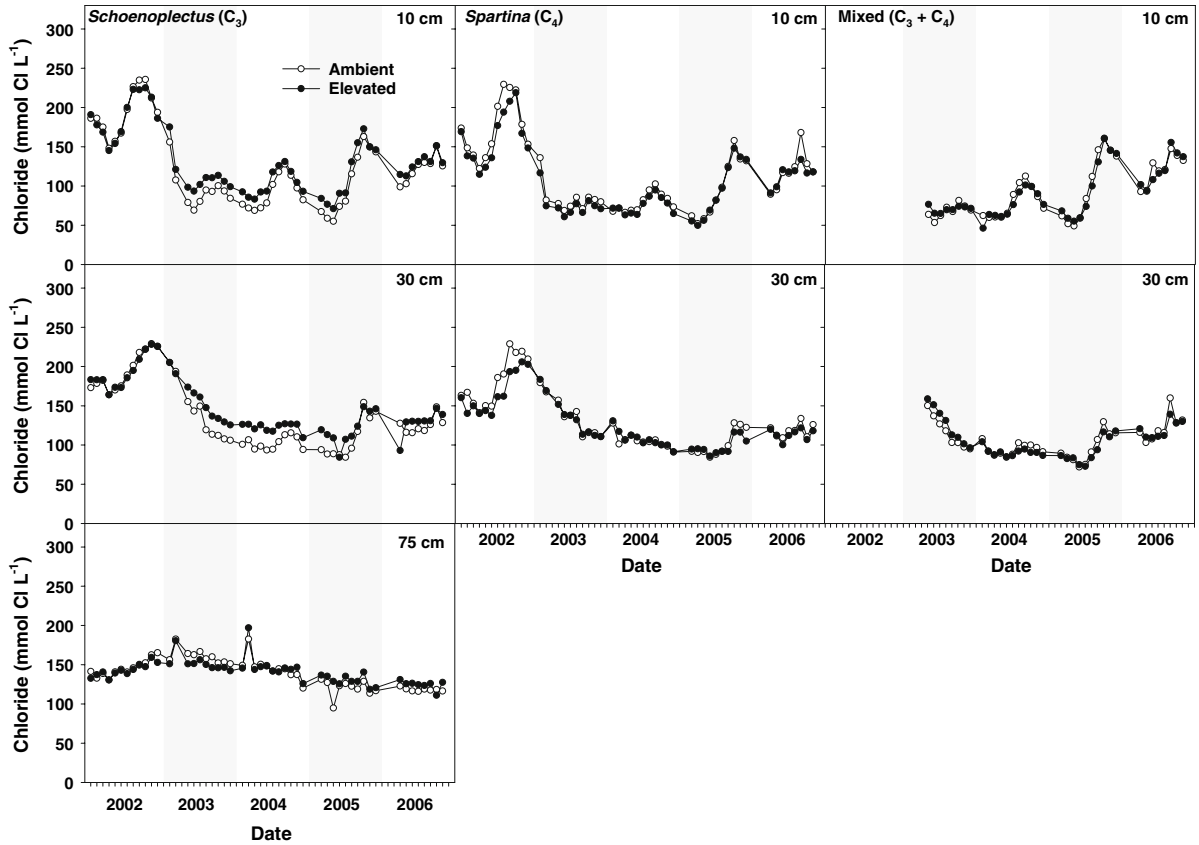

Fig. 1 Monthly averages of porewater chloride concentrations in a C_3-dominated (*Schoenoplectus*), a C_4-dominated (*Spartina*) and a $C_3 + C_4$ mixed community in a brackish marsh exposed to ambient and elevated (ambient + 340 ppm) concentrations of CO_2 during the growing season since 1987. Samples were collected between 2002 and 2006 using porewater 'sippers' at depths of 10, 30 and 75 cm in the C_3-dominated community and at depths of 10 and 30 cm in the C_4-dominated and mixed communities. *Shading* indicates odd years and *error bars* are omitted for clarity

30 mL of porewater. Subsequently a second 30-mL sample was collected from each well. Ten milliliter volumes of this sample were transferred to two 30-mL syringes using 3-way stopcocks to minimize exposure to the ambient atmosphere. The remaining two wells in the chamber were sampled in a similar manner, and the samples from the three wells of a given depth from each chamber were composited in the two 30-mL syringes. On dates when dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were measured, an additional ~30 mL of porewater was collected from each depth and analyzed as described below.

The sample in the first syringe was used to measure dissolved CH_4 using a headspace equilibration technique. From each syringe, 15 mL of porewater was filtered through preleached Millipore syringe filters (0.45 μm pore) into glass scintillation vials and stored in the dark at 4°C for subsequent phosphorus analysis (see below). Dissolved CH_4 was stripped from the remaining 15 mL of porewater by introducing a 15 mL headspace of ambient atmosphere followed by vigorous shaking for 30 s to release trapped CH_4. The remaining porewater was then expelled from the syringe so that 15 mL of headspace (containing the stripped CH_4) remained in each syringe. CH_4 concentrations were measured using a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (Shimadzu Corporation, Kyoto, Japan), typically within 48 h of collection. CH_4 concentrations were corrected for syringe leakage by measuring the decrease in CH_4 concentration of standards stored in syringes concurrently with headspace samples.

The second syringe from each depth in each chamber was used to measure concentrations of porewater sulfide, Cl^- and SO_4^{2-}. Syringes were transported to the laboratory on the day of collection and fixed with 3.5 mL of 5% zinc acetate to precipitate porewater sulfide. Fixed samples were shaken vigorously to homogenize the precipitate and then filtered through preleached Millipore syringe filters (0.45 μm). Two milliliter of fixed porewater was used to condition the filters, and subsequently ~11 mL of porewater was filtered into plastic scintillation vials. Filtered and fixed samples were frozen until analysis. SO_4^{2-} and Cl^- were measured on a Dionex DX 500 ion chromatography system equipped with an ASRS suppressor (Dionex Corporation, Sunnyvale, California, USA). Samples were diluted into 5-mL vials with filter caps and analyzed using an AG15 guard column, an AS15 column and 15 mM KOH eluent. The remaining 20 mL of each fixed sample was titrated for total sulfide concentration using the iodometric method (Clesceri et al. 1989). One milliliter of 0.1 M iodine was added to the 20 mL of porewater and diluted to a final volume of 50 mL in an Erlenmeyer flask. Samples were acidified with 2 mL of concentrated hydrochloric acid and incubated for 20 min in a fume hood. Titrations with 0.01 M sodium thiosulfate were run using a starch indicator. Titrations were corrected for the volume of sodium thiosulfate required to titrate distilled water blanks for each titration set.

Based on measured values of SO_4^{2-} and Cl^-, sulfate depletion was calculated as:

$$\text{Sulfate}_{\text{Dep}} = \left[\left(\text{Cl}^-_{\text{PW}}\right) \times \left(R_{\text{SW}}\right)^{-1}\right] - \text{SO}^{2-}_{4\,\text{PW}}$$

where $\text{Sulfate}_{\text{Dep}}$ is the SO_4^{2-} depletion in mmol S L^{-1}, (Cl^-_{PW}) and $(SO^{2-}_{4\,PW})$ are the concentrations of porewater Cl^- and SO_4^{2-} (in mM) measured by ion chromatography, and R_{SW} is the constant molar ratio of Cl^- to SO_4^{2-} in surface seawater ($R_{SW} = 19.33$; Bianchi 2006). $\text{Sulfate}_{\text{Dep}}$ therefore corrects for any confounding effects of elevated CO_2 on hydrology (e.g., through changes in transpiration). Positive values of $\text{Sulfate}_{\text{Dep}}$ represent a net depletion of SO_4^{2-} compared to the value predicted by the $Cl{:}SO_4^{2-}$ ratio in surface seawater, presumably due to sulfate reduction (Weston et al. 2006a). In a few cases, negative values of $\text{Sulfate}_{\text{Dep}}$ indicate an increase in SO_4^{2-} compared to the theoretical amount found in surface seawater, presumably due to reoxidation of reduced forms of sulfur to sulfate. While rates of sulfate reduction were not measured in this study, $\text{Sulfate}_{\text{Dep}}$ data fit well with other indicators of this important microbial process. There was a significant positive relationship between $\text{Sulfate}_{\text{Dep}}$ and sulfide concentrations across all communities and depths ($P < 0.0001$; $r^2 = 0.36$; data not shown). Sulfide concentrations were only ~30% of $\text{Sulfate}_{\text{Dep}}$ values, which may indicate that additional forms of reduced sulfur were sequestered in solids (particularly the organic fraction in this organic soil) or lost as H_2S gas, and thus were not accounted for by our porewater sampling. Further, the overall patterns seen in the $\text{Sulfate}_{\text{Dep}}$ analyses (described below) are consistent with the patterns seen when the analyses were run with dissolved sulfate concentrations alone (results not shown). Thus we are confident that the $\text{Sulfate}_{\text{Dep}}$ data are as effective as dissolved SO_4^{2-} as an indicator of sulfate reduction, and have the additional benefit of controlling for hydrological effects.

Samples for DOC and DIC analysis were collected in conjunction with other porewater samples and were filtered in the field with preleached Millipore filters (0.45 μm pore) into glass scintillation vials. DOC and DIC were analyzed as described previously (Marsh et al. 2005) using a Schimadzu TOC 5050 (Shimadzu Corporation, Kyoto, Japan). For logistical reasons, DOC and DIC samples were collected every 3 months from April 2004–September 2005 in the C_3- and C_4-dominated communities.

Porewater ammonium (NH_4^+) was analyzed on the same frozen samples used to measure SO_4^{2-} and Cl^- concentrations that had been fixed with zinc acetate and subsequently filtered. Tests on a subset of fixed versus unfixed control samples indicated that the presence of zinc acetate did not interfere with measurement of ammonium. Ammonium samples were analyzed on an Astoria-Pacific Analyzer (Astoria-Pacific International, Clackamas, Oregon, USA) using method A303-S021 calibrated with standards made in a 0.5% zinc acetate matrix. Given the anaerobic status of saturated wetland soils, we assumed that ammonium dominated the dissolved inorganic nitrogen pool in porewater and did not measure porewater nitrate.

Porewater phosphorus as orthophosphate was measured using the ascorbic acid method (Kuo 1996) on a spectrophotometer. Preliminary testing

revealed that zinc acetate interfered with measurements of orthophosphate (data not shown), so phosphorus was not measured in the same samples used to measure ammonium and other ions. Instead, orthophosphate was measured in porewater samples which had been filtered into glass scintillation vials and stored in the dark at 4°C. Porewater phosphorous measurements were made in 2007 for all samples collected 2002–2006. A subset of samples collected in May of 2003 were analyzed for orthophosphate and subsequently analyzed for total dissolved phosphorus using a perchloric acid digestion method (Clesceri et al. 1989). There was a good relationship between values measured using these two methods ($r^2 = 0.89$; slope = 0.92; data not shown) suggesting that the long storage time did not cause dramatic changes in orthophosphorus concentrations. While we are cautious in interpreting absolute concentrations of phosphorous presented here, relative comparisons among CO_2 treatments are meaningful.

Statistical analyses

The effects of elevated CO_2 on porewater constituents were analyzed using a mixed model repeated measures ANOVA (Proc Mixed, SAS version 9.1, SAS Institue Inc., Cary, North Carolina). Within each plant community, mixed linear models, in which CO_2 treatment was a fixed factor and the block × treatment interaction was a random factor, were used for analysis. Data were analyzed by depth using first-order linear autoregressive (AR(1)) models. In the C_3- and C_4-dominated communities, data from one of the blocks were not used because of the dominance of C_3 stems in a C_4 chamber and the dominance of C_4 stems in a C_3 chamber (cf., Marsh et al. 2005). Given the high variability associated with elevated CO_2 effects on microbial processes, $P < 0.10$ was considered significant for these analyses consistent with previous work in this system (Marsh et al. 2005; Erickson et al. 2007).

Results

In the C_3-dominated plant community, 5 years of porewater data suggest that elevated CO_2 increased anaerobic heterotrophic metabolism. Across all depths, sulfate depletion ($Sulfate_{Dep}$), CH_4 concentrations, and sulfide concentrations were higher in the elevated CO_2 treatment on most sampling dates (Figs. 2, 3, 4; Table 2), and these effects were most pronounced at greater depths. The median increase in $Sulfate_{Dep}$ across all depths was 38%, and there were comparable median increases of 33% and 20% for CH_4 and sulfide concentrations, respectively (Table 2). The difference in $Sulfate_{Dep}$ between treatments was significant at the 30-cm depth ($P = 0.10$) and a treatment effect was strongly suggested at the 75-cm depth ($P = 0.11$; Table 2). Increases in CH_4 concentrations were also significant at the 30-cm depth ($P = 0.08$); however, despite consistent trends, they were not significant at the 10-cm ($P = 0.18$) or the 75-cm depths ($P = 0.15$; Table 2). Increases in porewater sulfide in the elevated CO_2 treatment were not significant at any depth.

Consistent with our initial hypothesis, this stimulation of anaerobic decomposition was coincident with an increase in porewater carbon concentrations. Elevated CO_2 increased DOC by a median value of 21% across all depths in the C_3-dominated community (Fig. 5). Despite the decreased sampling frequency, this stimulation of DOC by elevated CO_2 was significant at both the 30-cm ($P = 0.02$) and the 75-cm depths ($P = 0.07$; Table 2). DIC concentrations were higher at elevated CO_2 in the C_3-dominated community on all sampling dates (Fig. 6), although these increases were not significant (Table 2). While alkalinity was generally higher at elevated CO_2, the difference was not significant in the C_3-dominated community (data not shown).

In contrast, porewater chemistry in the C_4-dominated plant community suggests that elevated CO_2 did not stimulate anaerobic heterotrophic metabolism (Figs. 2, 3, 4). Elevated CO_2 decreased $Sulfate_{Dep}$ by a median value of −9% across all depths and only slightly increased CH_4 and sulfide concentrations (median values of 1 and 3%, respectively). These changes were never significant ($P \geq 0.27$; Table 2). Consistent with these patterns, elevated CO_2 had no effect on DOC concentrations in the C_4-dominated community (Fig. 5; Table 2). Elevated CO_2 decreased DIC significantly at the 10-cm depth ($P = 0.08$; Table 2) although there was no discernable treatment pattern at the 30-cm depth (Fig. 6).

Elevated CO_2 generally stimulated anaerobic heterotrophic metabolism in the Mixed community. This stimulation was most consistent at the 10-cm depth where median increases in the elevated CO_2

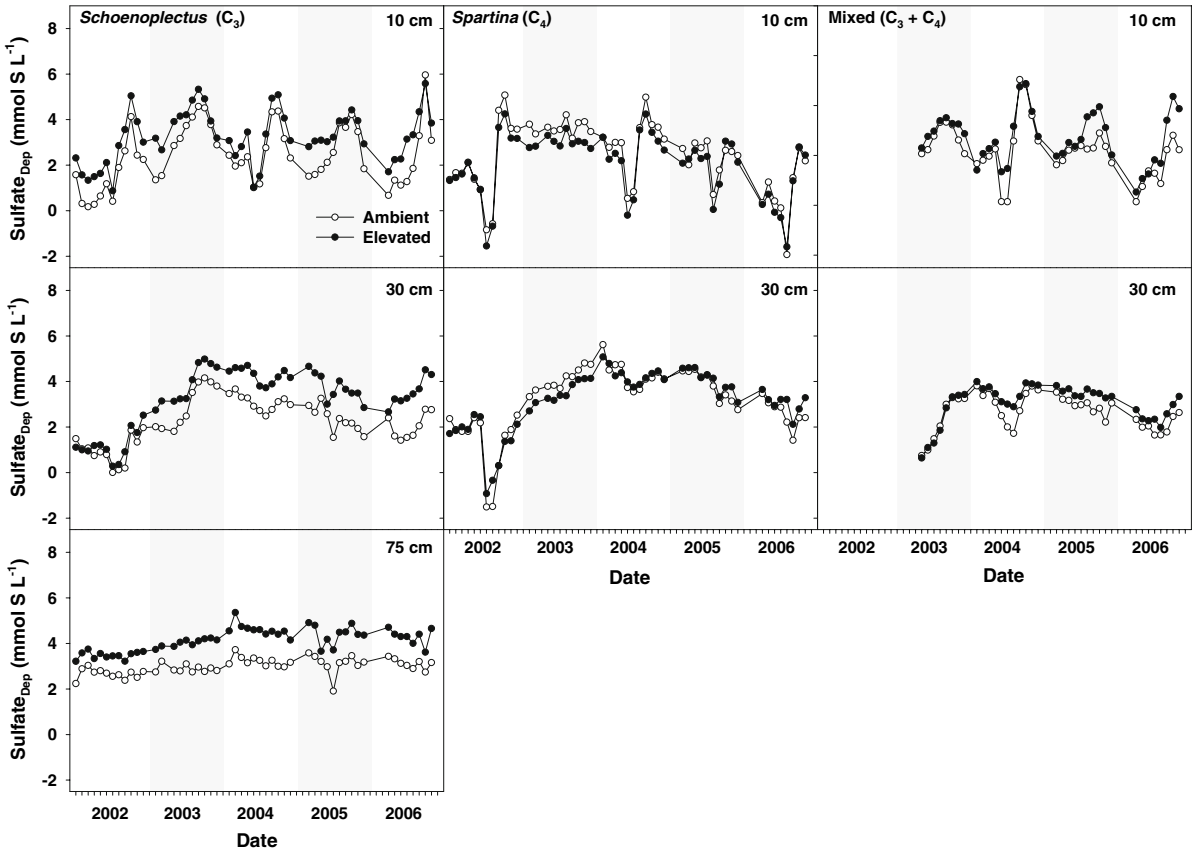

Fig. 2 Effects of elevated CO_2 on $Sulfate_{Dep}$ in three brackish marsh plant communities exposed to ambient and elevated concentrations of CO_2 since 1987. Details follow Fig. 1

treatment were 19, 31 and 7% for $Sulfate_{Dep}$, CH_4 and sulfide concentrations, respectively. Median increases at the 30-cm depth were 12, 5, and 4% for $Sulfate_{Dep}$, sulfide concentrations and CH_4 concentrations (Figs. 2, 3, 4; Table 2). The overall CO_2 treatment effect was only significant for $Sulfate_{Dep}$ at the 10-cm depth ($P < 0.01$; Table 2).

Patterns in porewater nitrogen and phosphorus concentrations were less pronounced (Figs. 7, 8). In the C_3-dominated community, porewater nitrogen and phosphorus were consistently higher in the elevated CO_2 treatment at the 75-cm depth, although this effect was not significant (Table 2). Similarly, nutrient concentrations in the C_4-dominated community were typically higher at elevated CO_2, but this effect was only significant for porewater nitrogen at the 30-cm depth ($P = 0.10$; Table 2).

Elevated CO_2 had no significant effect on chloride concentrations at any depth in any plant community ($P \geq 0.23$; Table 2). In the C_3-dominated community, chloride concentrations were higher in the elevated CO_2 treatment on a majority of sampling dates (Fig. 1); however, the median value of this increase was lower than for other porewater constituents (Table 2). Taken together, this suggests that effects of elevated CO_2 on porewater indicators of heterotrophic metabolism and nutrient dynamics could not be caused simply by changes in hydrology (e.g., through increased transpiration) which would have also influenced chloride concentrations.

Discussion

It is likely that all ecosystems will experience a doubling of current CO_2 concentrations by the year 2100 (Meehl et al. 2007). In the current study, porewater samples were collected approximately

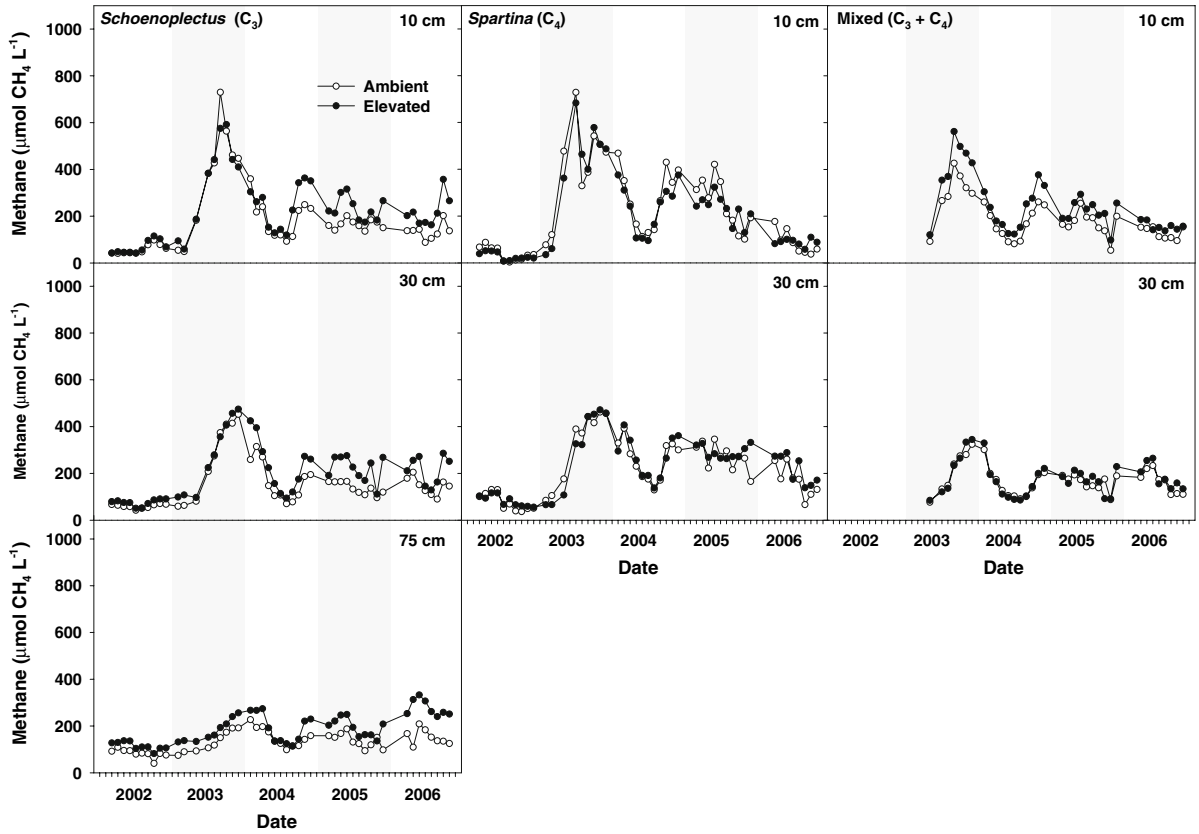

Fig. 3 Effects of elevated CO_2 on porewater CH_4 concentrations in three brackish marsh plant communities exposed to ambient and elevated concentrations of CO_2 since 1987. Details follow Fig. 1

monthly from three different plant communities exposed to elevated CO_2 in situ since 1987. These long-term data offer a unique opportunity to explore how anaerobic carbon mineralization and porewater nutrient dynamics will respond to this important global change.

The effects of elevated CO_2 on porewater chemistry are closely linked to the response of plant productivity to elevated CO_2. Specifically, biomass and productivity in the C_3-dominated community increased in response to elevated CO_2, and this plant response likely translated into a stimulation of anaerobic metabolism in this community. In contrast, the response of C_4 plant biomass to elevated CO_2 has been relatively weak and there were correspondingly limited effects of elevated CO_2 on porewater chemistry. The response of the Mixed community was similar in direction to the C_3-dominated community, but the median changes were generally smaller. The differential responses of these plant communities highlight the importance of considering different wetland types when predicting the future impacts of elevated CO_2 in wetland ecosystems.

Anaerobic heterotrophic metabolism

As we hypothesized, elevated CO_2 stimulated anaerobic heterotrophic metabolism in the C_3-dominated community. Measurements of $Sulfate_{Dep}$ and sulfide concentrations suggest that rates of sulfate reduction were stimulated by elevated CO_2 (Figs. 2, 4; Table 2). Similarly, higher concentrations of porewater CH_4 are indicative of increased rates of methanogenesis (Fig. 3; Table 2).

Previous work at Kirkpatrick Marsh has explored the effects of elevated CO_2 on short-term CH_4 dynamics in this system. Marsh et al. (2005) demonstrated that elevated CO_2 increased porewater concentrations of CH_4 by 12–18%. Our long-term data show the magnitude of this effect varies considerably

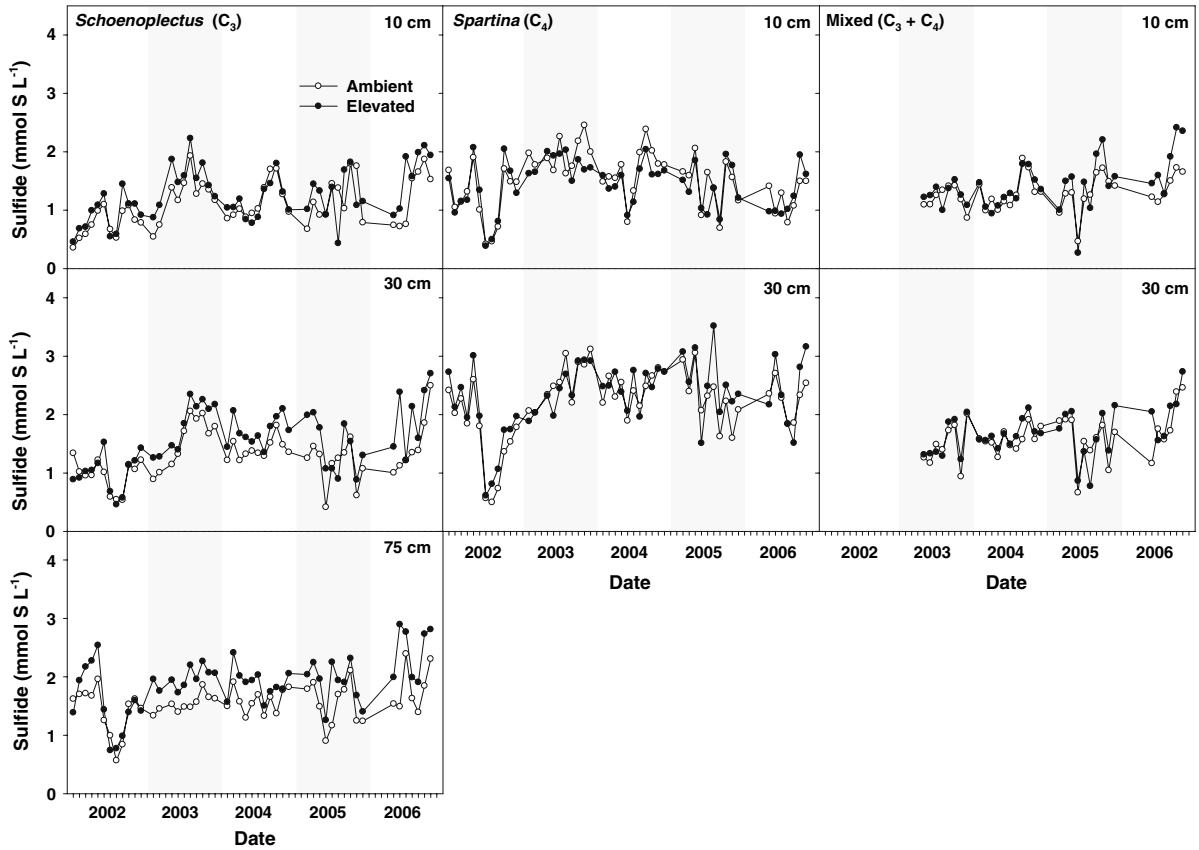

Fig. 4 Effects of elevated CO_2 on porewater sulfide concentration in three brackish marsh plant communities exposed to ambient and elevated concentrations of CO_2 since 1987. Details follow Fig. 1

(median changes from −5 to 124%), likely due to interannual variability during our extended sampling. While we did not measure net CH_4 emission in the current project, previous work demonstrated that elevated CO_2 can stimulate CH_4 flux in the C_3-dominated community. Dacey et al. (1994) reported an ~80% increase in CH_4 flux over a 1 week period in 1991 and Marsh et al. (2005) showed a more modest stimulation of 15–17% in CH_4 emission across a full year. These results are consistent with a growing body of literature that demonstrates elevated CO_2 can stimulate methanogenesis in wetland ecosystems (e.g., Megonigal and Schlesinger 1997; Ziska et al. 1998; Vann and Megonigal 2003; Megonigal et al. 2004; Cheng et al. 2006). It should be noted that while hydrogenotrophic methanogens produce CH_4 by the coupled reduction of CO_2 and oxidation of H_2 (Megonigal et al. 2004), it seems unlikely that increased atmospheric CO_2 could have directly stimulated this process. DIC concentrations in vegetated wetland soils are higher than in ambient air due to autotrophic and heterotrophic respiration (Fig. 6), while H_2 concentrations are typically at the nanomolar level. Thus, hydrogenotrophic methanogenesis is thought to be limited by H_2 (i.e., electron donor) availability rather than CO_2. As CH_4 has 21-times the global warming potential of CO_2 (over a 100 year period; Forster et al. 2007), an increased CH_4 flux from wetlands in response to elevated CO_2 has the potential to serve as a potent positive feedback to ongoing global warming given the large amount of organic carbon currently stored in wetland soils (Bridgham et al. 2006).

However, CH_4 is a minor component of net ecosystem respiration in this brackish marsh. For example, between 1998 and 1999, elevated CO_2

Table 2 Changes in porewater chemistry in response to elevated CO_2 in a C_3-dominated (*Schoenoplectus*), a C_4-dominated (*Spartina*) and a mixed $C_3 + C_4$ community in a brackish marsh

	Schoenoplectus (C_3)			*Spartina* (C_4)		Mixed ($C_3 + C_4$)	
	10 cm	30 cm	75 cm	10 cm	30 cm	10 cm	30 cm
$Sulfate_{Dep}$							
P-value[a]	0.29	**0.10**	0.11	0.27	0.98	**<0.01**	0.32
Frequency[b]	50 of 51	48 of 51	51 of 51	8 of 51	27 of 51	33 of 37	32 of 37
Median (%)[c]	34	41	39	−15	1	19	12
Methane							
P-value[a]	0.18	**0.08**	0.15	0.71	0.72	0.17	0.80
Frequency[b]	42 of 48	47 of 48	46 of 48	19 of 48	32 of 48	35 of 36	22 of 36
Median (%)[c]	20	32	39	−7	6	31	4
Sulfide							
P-value[a]	0.38	0.21	0.23	0.66	0.67	0.16	0.57
Frequency[b]	40 of 50	41 of 50	45 of 50	22 of 50	34 of 50	27 of 35	24 of 35
Median (%)[c]	17	18	23	−7	6	7	5
DOC							
P-value[a]	0.41	**0.02**	**0.07**	0.38	0.80	ND	ND
Frequency[b]	6 of 7	7 of 7	6 of 7	2 of 6	4 of 6		
Median (%)[c]	21	23	13	−11	6		
DIC							
P-value[a]	0.50	0.24	0.36	**0.08**	0.67	ND	ND
Frequency[b]	7 of 7	7 of 7	7 of 7	0 of 6	5 of 6		
Median (%)[c]	34	45	25	−18	2		
Nitrogen							
P-value[a]	0.55	0.54	0.38	0.56	**0.10**	ND	ND
Frequency[b]	13 of 48	31 of 48	46 of 48	36 of 48	45 of 48		
Median (%)[c]	−16	39	33	10	18		
Phosphorus							
P-value[a]	0.23	0.90	0.39	0.72	0.73	ND	ND
Frequency[b]	11 of 37	15 of 37	31 of 37	34 of 37	32 of 37		
Median (%)[c]	−29	−7	23	19	12		
Chloride							
P-value[a]	0.23	0.33	0.55	0.36	0.51	0.77	0.87
Frequency[b]	42 of 51	38 of 51	26 of 51	10 of 51	20 of 51	20 of 37	19 of 37
Median (%)[c]	10	10	0	−6	−2	1	0

ND not determined

[a] Overall elevated CO_2 treatment *P*-value from mixed model repeated measures ANOVAs. Significant treatment effects ($P < 0.10$) are in bold

[b] Frequency refers to the number of sampling dates between 2002 and 2006 where means were higher in the elevated CO_2 treatment than in the ambient treatment. For example, elevated CO_2 increased $Sulfate_{Dep}$ at the 10-cm depth in the C_3-dominated community on 50 out of 51 sampling dates

[c] Median values of the percent increase in response to elevated CO_2. The percent increase in response was calculated as: $[(Mean_{elevated} - Mean_{ambient})/Mean_{ambient}] \times 100$. Negative values represent lower means in the elevated CO_2 treatment than in the ambient treatment

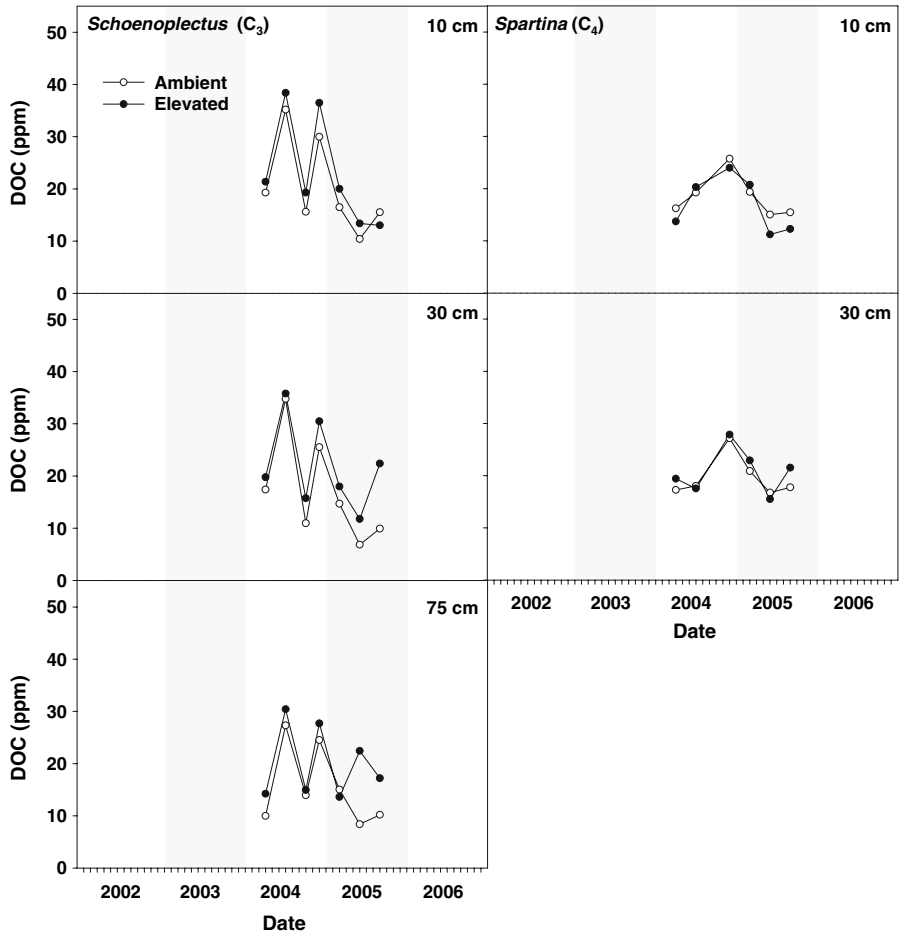

Fig. 5 Effects of elevated CO_2 on porewater DOC concentrations in three brackish marsh plant communities exposed to ambient and elevated concentrations of CO_2 since 1987. Details follow Fig. 1

stimulated CH_4 emissions in the C_3-dominated community by 0.2–0.4 g C m^{-2} $year^{-1}$ compared to a stimulation of CO_2 emissions by 34–393 g C m^{-2} $year^{-1}$ over the same time period (Marsh et al. 2005). Low rates of CH_4 production in this brackish system are likely the result of sulfate inputs from seawater driving the competitively dominant process of sulfate reduction (Kelley et al. 1990; Megonigal et al. 2004; Weston et al. 2006b). Our data show that CH_4 concentrations were generally low in both treatments in the C_3- and C_4-dominated plant communities until SO_4^{2-} concentrations were depleted (Fig. 9). Higher concentrations of CH_4 were most frequent in 2003 (Fig. 3), a particularly wet year (Table 1). This suggests that the reoxidation of reduced sulfur during drier periods could be an important source of sulfate further suppressing methanogenesis in this brackish system. Indeed at the 10- and 30-cm depths of the C_4-dominated community in July and August of 2002, a very dry year (Table 1), average $Sulfate_{Dep}$ was negative, indicating that SO_4^{2-} concentrations were higher than expected based on the ratio of SO_4:Cl in seawater (Fig. 2). Less precipitation leads to surface desiccation in this wetland and increases the likelihood that atmospheric oxygen frequently penetrates the soil profile. While we cannot distinguish between autotrophic and heterotrophic respiration, stimulation of sulfate reduction by elevated CO_2 is likely to explain a portion of the increased DIC concentrations in our data (Fig. 6; Table 2), and a similar increase seen by Marsh et al. (2005). Increased sulfate reduction, which releases two moles of CO_2 for every mole of SO_4^{2-} reduced (Megonigal 2004), could also be an important component of the increased soil respiration observed in this system previously (Ball and Drake 1998).

A major mechanism hypothesized to explain the increase in methanogenesis in wetlands exposed to

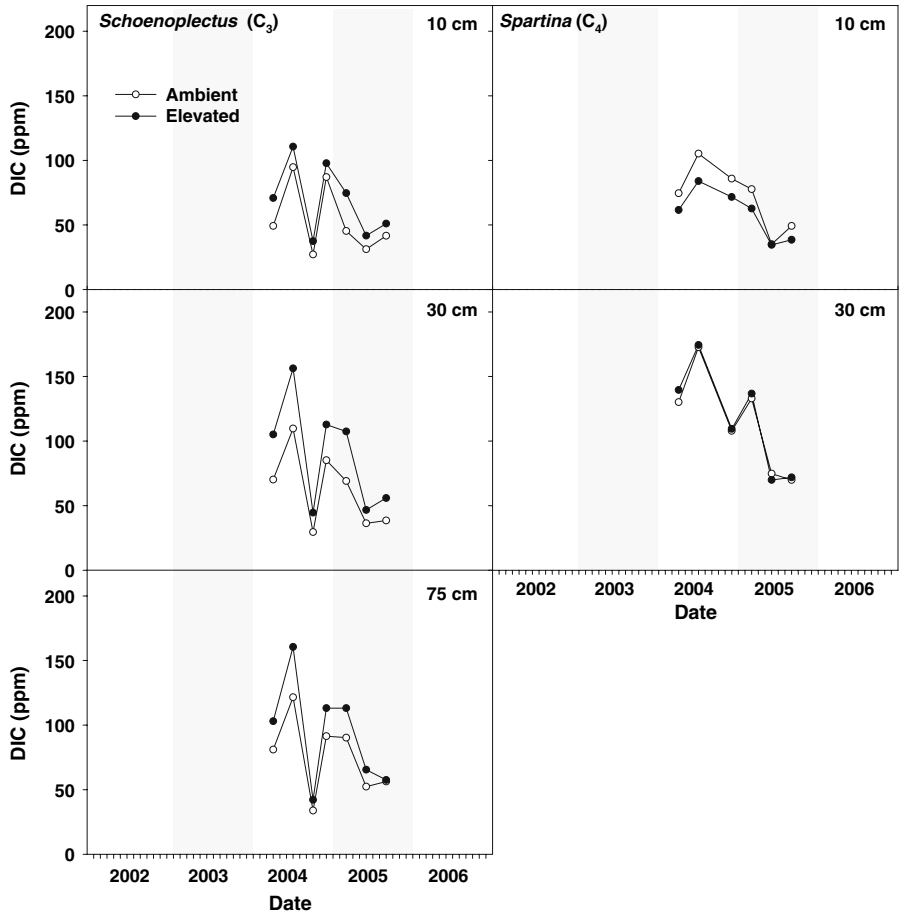

Fig. 6 Effects of elevated CO_2 on porewater DIC concentrations in three brackish marsh plant communities exposed to ambient and elevated concentrations of CO_2 since 1987. Details follow Fig. 1

elevated CO_2 is an increase in labile carbon availability as a consequence of increased plant productivity and biomass (Megonigal et al. 1999, 2004; Vann and Megonigal 2003; Cheng et al. 2006). Elevated CO_2 has been shown to increase DOC export from peatland systems (Freeman et al. 2004; Fenner et al. 2007a) as a result of increased DOC from recent plant productivity (Fenner et al. 2007b). In this brackish system, a similar mechanism may explain observed increases in rates of sulfate reduction. The C_3-dominated community had a long-term average increase in net ecosystem exchange of $\sim$35%, driven primarily by increased photosynthesis (Rasse et al. 2005), and similar increases in above- and below-ground biomass (Erickson et al. 2007). Higher porewater DOC concentrations (Fig. 5; Table 2) suggest that a portion of this extra plant production is available to support heterotrophic metabolism. In a greenhouse experiment, Wolf et al. (2007) demonstrated that elevated CO_2 can increase O_2 loss from roots of a C_3 sedge (*S. americanus*), collected from Kirkpatrick Marsh, and dramatically stimulate the decomposition of soil organic matter. This 'priming' effect suggests that wetland plants can stimulate microbial decomposition both through inputs of carbon and/or O_2. The results from the present study suggest that in response to elevated CO_2, excess organic carbon is made available for anaerobic metabolism. However, it is unclear if this DOC is a result of increased plant activity directly (e.g., root exudates) or the result of aerobic microbes metabolizing complex organic carbon substrates that would remain inaccessible to anaerobic processes in the absence of root-derived O_2.

The stimulation of anaerobic heterotrophic metabolism in the C_3-dominated community was most pronounced at the 30- and 75-cm depths (Table 2; Figs. 2, 3, 4, 5, 6). Living roots have been found as deep as 65 cm in this system, but the majority of belowground biomass is in the upper 30 cm of the soil

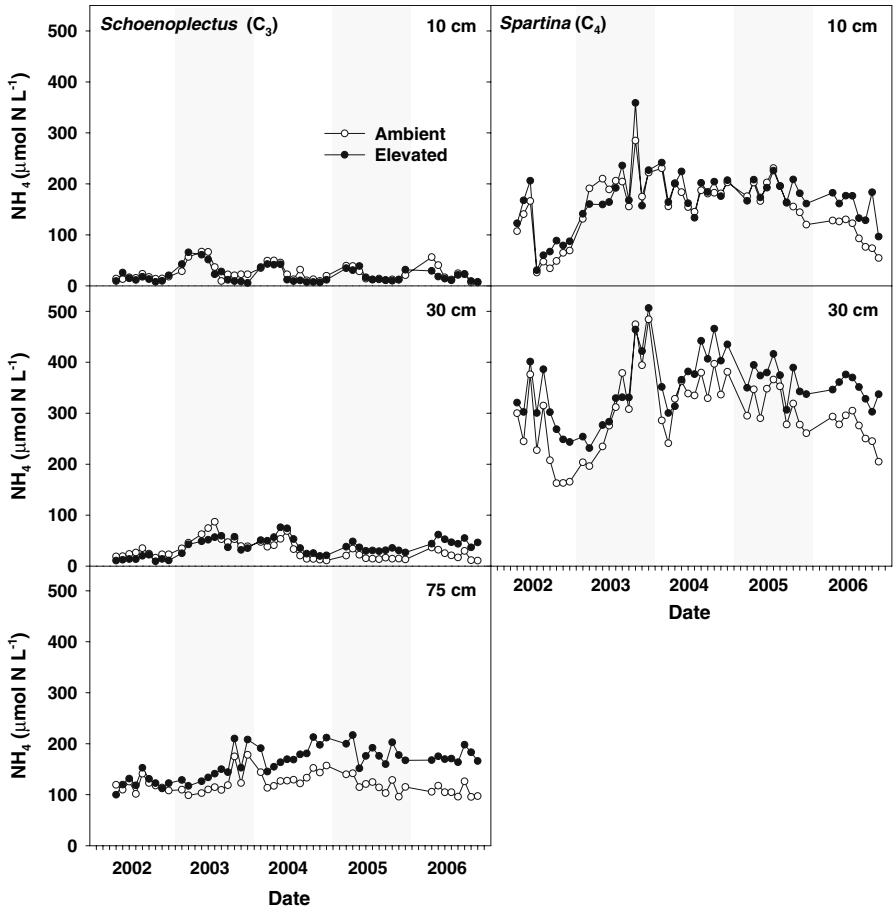

Fig. 7 Effects of elevated CO_2 on porewater nitrogen concentrations in three brackish marsh plant communities exposed to ambient and elevated concentrations of CO_2 since 1987. Details follow Fig. 1

profile (Saunders et al. 2006). The manifestation of elevated CO_2 effects at depths >30 cm implies that the influence of plant-derived carbon may extend well below the dominant rooting zone. Given the low hydrologic conductivity of the sapric peat in this system and stable water depths, it is likely that mass flow and diffusion away from the rhizosphere towards deeper soil depths are slow processes. However, deeper depths may effectively integrate the effects of excess carbon on porewater chemistry over long periods of time compared to shallower depths which are more dynamic in terms of temperature, plant activity, hydrologic exchange and microbial activity.

In contrast to the general stimulation of anaerobic decomposition by elevated CO_2 in the C_3-dominated community, there was no effect of elevated CO_2 on anaerobic heterotrophic metabolism in the C_4-dominated community. Neither $Sulfate_{Dep}$, sulfide nor CH_4 concentrations were significantly increased by elevated CO_2, suggesting that rates of sulfate reduction and methanogenesis were comparable in ambient and elevated CO_2 treatments. These results are consistent with the general lack of stimulation of CH_4 concentrations and CH_4 flux in the C_4-dominated community observed previously at Kirkpatrick Marsh (Marsh et al. 2005). This pattern further highlights the potential importance of excess plant-derived carbon in fueling increased anaerobic metabolism in response to elevated CO_2. As expected based on the physiology of the C_4 photosynthetic pathway (Barbour et al. 1998), the C_4-dominated plant community does not respond strongly to elevated atmospheric CO_2. There was no significant stimulation of above- or belowground biomass from 1987 to 2004 in response to the elevated CO_2 treatment (Erickson et al. 2007), and we observed no increase in DOC concentrations, suggesting that no additional carbon was made available for microbial respiration.

The effects of elevated CO_2 in the $C_3 + C_4$ Mixed community were less clear. In general, indicators of

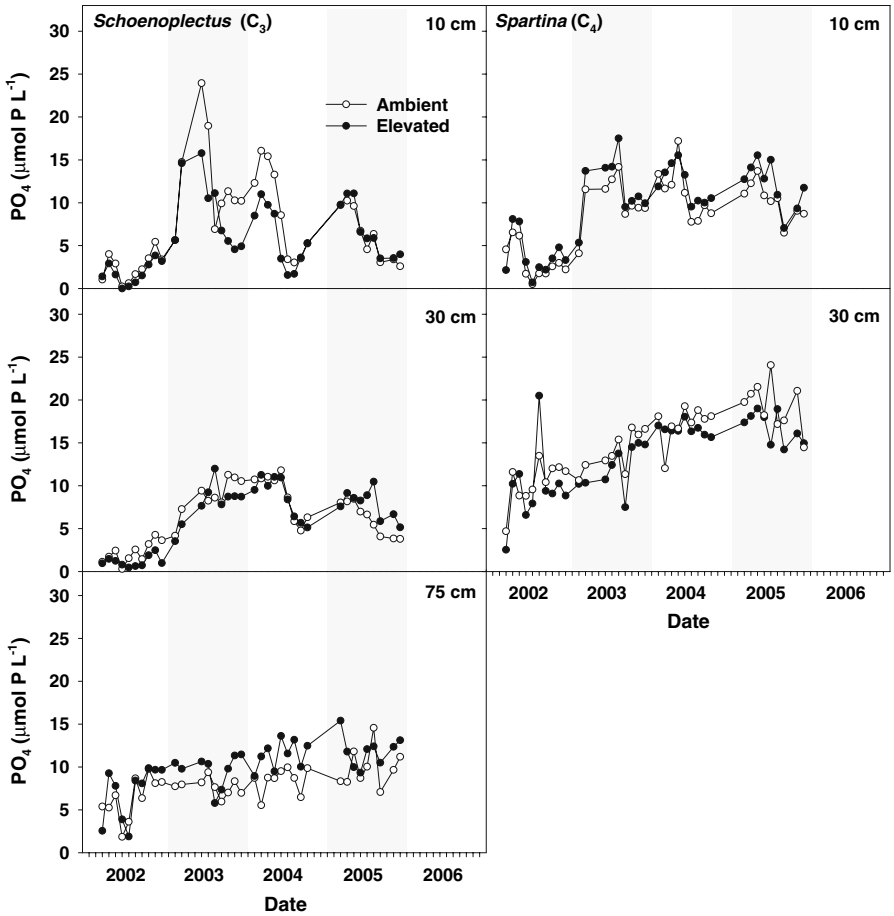

Fig. 8 Effects of elevated CO_2 on porewater phosphorus concentrations in three brackish marsh plant communities exposed to ambient and elevated concentrations of CO_2 since 1987. Details follow Fig. 1

anaerobic carbon mineralization (i.e., $Sulfate_{Dep}$, porewater CH_4 and porewater sulfide) were higher in the elevated CO_2 treatment, especially at the 10-cm depth. C_3 root and shoot biomass have been stimulated by elevated CO_2 in the mixed community (Erickson et al. 2007), and it is possible that excess carbon was made available to anaerobic heterotrophic processes similar to the response observed in the C_3-dominated community. DOC data were not collected in the mixed community to more fully evaluate this hypothesis.

Nutrient dynamics

In contrast to our initial hypothesis, there was no decrease in porewater nitrogen or phosphorus in the C_3-dominated community despite increased above- and belowground biomass in response to elevated CO_2. It appears that nitrogen availability in the C_3-dominated community is sufficient to support the increase in total canopy nitrogen content observed in the elevated CO_2 treatment (Erickson et al. 2007). Little information is available on the total canopy phosphorus content in this system, but the porewater data suggest that CO_2-enhanced plant uptake is not depleting this phosphorus pool. We can only speculate on the reasons that differences in canopy nutrient pools do not translate into differences in porewater nutrient pools. One possibility is that hydrologic allothonous inputs of nitrogen and phosphorus are sufficient to obscure differences in plant demand between the CO_2 treatments. However, there is evidence to suggest that plant production in some tidal marshes is not regulated by allothonous nutrient inputs (Neubauser et al. 2005). A second possibility is that soil organic matter mineralization rates are relatively high at elevated CO_2 due to increased decomposition or 'priming' effects (Wolf et al. 2007), causing a simultaneous increase in nitrogen and phosphorus mineralization. Indeed, porewater

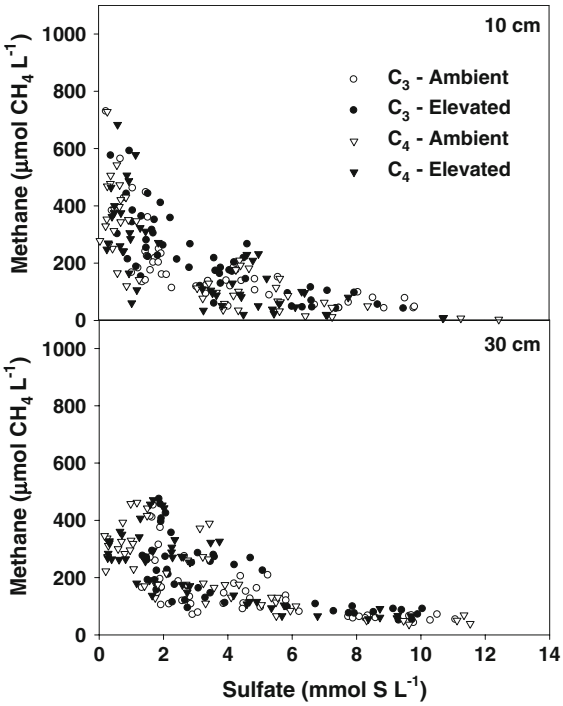

Fig. 9 Relationship between porewater CH_4 and porewater sulfate concentrations in a C_3-dominated (*circles*) and a C_4-dominated (*triangles*) community in a brackish marsh exposed to ambient (*open symbols*) and elevated (ambient + 340 ppm; *closed symbols*) concentrations of CO_2 during the growing season since 1987. Each point represents a monthly porewater sample collected between 2002 and 2006. Samples were collected with porewater 'sippers' at depths of 10 and 30 cm in both plant communities

nutrients tended increase rather than decrease following CO_2 enrichment. Elevated CO_2 significantly increased porewater nitrogen concentrations at the 30-cm depth in the C_4-dominated community (Table 2) and there was a consistent, but non-significant, pattern of increased nitrogen at the 75-cm depth in the C_3-dominated community (Fig. 7). Finally, plants exposed to elevated CO_2 may be meeting their nutrient demands through internal translocation from perennial rhizomes, which increase in the elevated CO_2 treatment (Megonigal, personal communication). In any case, these results suggest that progressive nutrient limitation was not occurring in this brackish marsh ecosystem in years 15–20 of the experiment. It is possible that such effects occurred at the beginning of the study and have since dissipated, highlighting the importance of long-term experiments.

Interestingly, porewater nitrogen concentrations were dramatically higher in the C_4-dominated plant community than in the C_3-dominated plant community (Fig. 7). One possible explanation for this pattern is that shoot nitrogen concentrations tend to be higher in the C_3 sedge *S. americanus* than in the C_4 grass *S. patens* (Erickson et al. 2007). If differences in plant uptake explain this pattern, rather than pre-existing differences in nitrogen availability, it indicates that species-specific differences in plant uptake affect nutrient availability more than changes due to elevated CO_2 within a species.

Implications for marsh ecosystems

Increased atmospheric concentrations of CO_2 will impact all ecosystems in the future. This study demonstrates that elevated CO_2 will differentially influence porewater chemistry in C_3- and C_4-dominated marsh communities, and suggests that these influences will be related to the biomass response of the dominant plant community. Specifically, the effects of elevated CO_2 on microbial processes are likely to be most pronounced in C_3-dominated ecosystems where plants respond positively to elevated CO_2 (Rasse et al. 2005; Erickson et al. 2007).

Anaerobic heterotrophic microbial decomposition will likely increase in C_3-dominated ecosystems due to elevated CO_2, and this has the potential to release additional CO_2 and CH_4 to the atmosphere, augmenting anthropogenic emissions of these important greenhouse gases. Although the net flux of CH_4 is likely to be minimal in brackish ecosystems where sulfate reduction is the dominant microbial process, it nevertheless could be significant in freshwater wetland ecosystems where methanogenesis is frequently a dominant metabolic pathway (Megonigal and Schlesinger 1997; Vann and Megonigal 2003). DOC and DIC were both elevated in the C_3-dominated community, and the export of these ecologically important carbon compounds from marshes to adjacent aquatic systems may increase in response to elevated CO_2. Accumulating evidence from this and other studies (Erickson et al. 2007) suggests that progressive nutrient limitation will not decrease the elevated-CO_2 response of C_3-dominated communities through time in systems with 'open' nutrient cycles.

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