An oxygen-mediated positive feedback between elevated carbon dioxide and soil organic matter decomposition in a simulated anaerobic wetland

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Abstract

We examined the effects of elevated atmospheric CO_2 on soil carbon decomposition in an experimental anaerobic wetland system. Pots containing either bare C_4-derived soil or the C_3 sedge *Scirpus olneyi* planted in C_4-derived soil were incubated in greenhouse chambers at either ambient or twice-ambient atmospheric CO_2. We measured CO_2 flux from each pot, quantified soil organic matter (SOM) mineralization using $\delta^{13}C$, and determined root and shoot biomass. SOM mineralization increased in response to elevated CO_2 by 83–218% ($P < 0.0001$). In addition, soil redox potential was significantly and positively correlated with root biomass ($P = 0.003$). Our results (1) show that there is a positive feedback between elevated atmospheric CO_2 concentrations and wetland SOM decomposition and (2) suggest that this process is mediated by the release of oxygen from the roots of wetland plants. Because this feedback may occur in any wetland system, including peatlands, these results suggest a limitation on the size of the carbon sink presented by anaerobic wetland soils in a future elevated-CO_2 atmosphere.

Introduction

Soil carbon is the largest terrestrial C pool, storing almost three times as much C as terrestrial biomass (Schimel, 1995). Understanding how soil processes, and ultimately the soil C sink, are affected by elevated CO_2 is crucial to understanding future C cycling. Because soil C pools are determined by the balance of C input to soils, primarily from plant biomass, and C export from soils through leaching and decomposition (Schlesinger & Andrews, 2000), perturbations to either of these factors can lead to changes in soil C dynamics.

The effect of rising CO_2 on plant growth is well studied and understood for many systems (Owensby *et al.*, 1993; Drake *et al.*, 1997; Norby *et al.*, 1999; Korner, 2000). In contrast, the interaction of rising CO_2 with processes that control C export from soils has received much less study across a broad range of ecosystems.

Studies that have evaluated effects of elevated CO_2 on soil organic matter (SOM) decomposition in upland soils have yielded equivocal results; elevated CO_2 has accelerated, suppressed, or had no effect on SOM decomposition, depending on plant community type, season, nutrient availability, and a host of other factors (e.g. Cheng, 1999; Cardon *et al.*, 2001; Lin *et al.*, 2001; Hoosbeek *et al.*, 2004; Lichter *et al.*, 2005; Carney *et al.*, 2007).

Understanding the effects of elevated CO_2 on SOM decomposition in wetland ecosystems is important for predicting future C dynamics and sequestration. Although wetlands occupy a relatively small percentage of the world's land area, they store a disproportionate amount of soil C – by some estimates up to one-third of the total soil C pool (Gorham, 1991; Jenkinson *et al.*, 1991). As with other systems, it has been demonstrated that C_3 wetland plants are more productive when grown in an elevated CO_2 atmosphere (Drake, 1992; Rasse *et al.*, 2005), but few experimental data describe the responsiveness of wetland SOM decomposition to this global change.

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Mobilization of soil C from wetlands into the atmosphere could have substantial large-scale consequences, including the creation of a positive feedback to rising atmospheric CO$_2$ and the loss of wetland soil-surface elevation, leading to increased inundation and potential wetland loss. The loss of soil-surface elevation is particularly detrimental in coastal wetlands, which must actually increase in elevation to keep pace with sea-level rise and thus avoid inundation (Day et al., 2000).

Two prior studies hint that elevated CO$_2$ may influence SOM decomposition in wetlands. First, Ball & Drake (1998) noted that a natural salt marsh subjected to CO$_2$ enrichment showed increased soil respiration, though they were unable to determine the source of the additional respiration. Second, a field study of wetland microbial metabolism in a salt marsh exposed to elevated CO$_2$ indirectly suggests an increase in sulfate reduction rate (J. P. Megonigal, unpublished data), the primary microbial metabolic pathway in this anaerobic system (Megonigal et al., 2004).

In this study, we conducted a greenhouse experiment exploiting the difference in δ^{13}C natural abundance between C$_3$ and C$_4$ plant material (Cheng, 1996; Rochette et al., 1999; Kuzyakov, 2006) to examine the effects of CO$_2$ enrichment on wetland SOM decomposition.

Materials and methods

Experimental system

We set up a 'C$_3$ plant–C$_4$ soil' system (Fig. 1) modeled after Cheng (1996). Soils and plants were collected from Kirkpatrick Marsh (38°53'N, 76°33'W), a brackish marsh ecosystem on the Rhode River Estuary, Chesapeake Bay, MD, USA, that has been the site of an elevated-CO$_2$ experiment since 1987 (Drake, 1992; Rasse et al., 2005). The soil is a histosol with a 40.5% C content
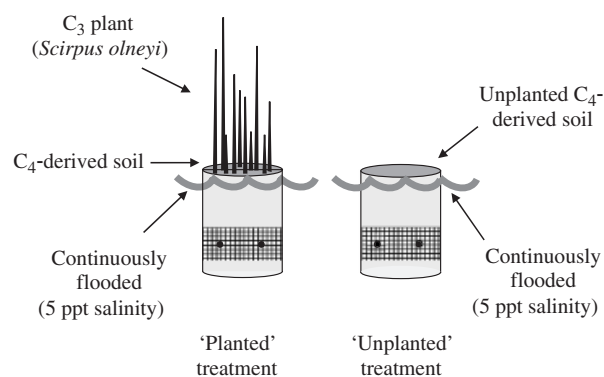

Fig. 1 'C$_3$ plant–C$_4$ soil' system modeled after Cheng (1996). There were a total of 32 pots in the planted treatment and 32 unplanted controls, equally distributed between chambers with ambient ($N = 2$) or elevated ($N = 2$) CO$_2$ concentrations.

and a δ^{13}C of −15.10‰, reflecting herbaceous-cover dominance by the C$_4$ grass *Spartina patens*. Soil was collected from 50 to 100 cm depth but nevertheless contained some roots. The soil was homogenized in a blender and 1215 cm^3 of saturated soil was added to each of 64 PVC pots (10.2 cm diameter × 16 cm height). Pots were sealed on the bottom with a PVC cap and had four 1 cm diameter holes covered with screen at 5 cm above the base to allow for water exchange. Of the 64 pots, 32 were planted with *Scirpus olneyi*, a C$_3$ sedge ('planted' treatment) and 32 were left unplanted ('unplanted' treatment). Plants were harvested as rhizomes from Kirkpatrick Marsh in May 2003. Rhizome nodes were trimmed to the smallest possible sprouting unit and initially sprouted in potting soil at ambient CO$_2$ in a greenhouse. Following sprouting, small shoots (<4 cm) were transplanted to the PVC pots on 7 June 2003. Each planted-treatment pot received eight separate plants corresponding to a realistic field density of 600 shoots m^{-2}. To eliminate the possibility of artificial nutrient limitation induced by confining plants to greenhouse pots, where they did not receive tidal floodwater nutrient inputs, both planted and unplanted pots were fertilized twice during the experiment (26 and 69 days after transplanting) with MiracleGro 15-30-15 NPK fertilizer applied at manufacturer-suggested strength.

Chambers

The experiment was conducted in chambers located in a greenhouse at the Smithsonian Environmental Research Center (SERC) in Edgewater, MD, USA. The chambers were 1.5 m wide × 0.9 m deep × 1.5 m high, covered with clear polyester film, and equipped with a blower for drawing ambient air through the chamber. Two chambers were maintained at ambient atmospheric CO$_2$ concentration and two chambers at an elevated atmospheric CO$_2$ concentration of approximately 735 µL L^{-1} during the day by adding pure CO$_2$ to the air stream; the δ^{13}C of the added CO$_2$ was −11‰, reflecting the isotopic signature of a carbonate CO$_2$ source. CO$_2$ concentrations at night were somewhat higher due to the absence of photosynthetic assimilation. The elevated CO$_2$ treatment began 9 June 2003 and continued until plants were harvested between 14 and 22 October 2003. The CO$_2$ concentration in each chamber was monitored continuously on an Inficon Binos gas analyzer (Oerlikon Corporation, Pfäffikon, Switzerland). Photosynthetically active radiation (PAR) and temperature were monitored in the greenhouse throughout the experiment, and PAR in individual chambers was also measured over a 1-week period.

Each chamber contained two tubs measuring 1 m wide × 0.5 m deep × 0.3 m high. One tub in each chamber contained eight unplanted pots, and the other

contained eight planted pots. Pots were assigned to chambers randomly. Each tub was filled to the soil surface with water from the tidal river adjacent to the Kirkpatrick Marsh that had been diluted by approximately half to a salinity of 5 ppt with tap water. Evaporated water from the tubs was replaced every 2 days with tap water. Water in the tubs was completely changed and pots were rotated within the tubs approximately biweekly.

Sampling

Total (plant + soil) CO_2 and CH_4 flux from individual pots was measured between 13 and 17 September 2003. Before sampling, all algal growth on the soil surface of the pots was removed. An opaque PVC flux chamber consisting of a length of 10.2-cm-diameter PVC pipe with a PVC cap at one end and a PVC coupling at the other was placed on each chamber. All PVC joints were sealed with silicone. The headspace was sampled through a rubber septum located on the flux chamber. During CO_2 and CH_4 efflux measurements, the pots were placed in tubs with 5 ppt salinity river water and temperature was held at 23–25 °C. The bottom of the flux chambers sat below the water surface, ensuring minimal gas exchange with the atmosphere. Samples for CO_2 and CH_4 analysis were taken once per hour over a 5-h period in the middle of the day. CO_2 samples were analyzed on an LI-6251 gas analyzer (LI-COR Biosciences, Lincoln, NE, USA) modified to run in an injection mode. A 4 mL gas sample was loaded into a 500 μL Valco valve sample loop (Valco Instruments Co., Houston, TX, USA), then injected into an N_2 carrier gas flowing at 0.5 L min^{-1}. Peak area was recorded on a Campbell 21X data logger (Campbell Scientific, Logan, UT, USA) and used to calculate CO_2 concentrations based on calibration curves (two replicate injections of four CO_2 standards). Calibration curves were populated before and after each run (spanning about 4 h), and standards were run every 15–20 samples to check for drift. CH_4 samples were analyzed on a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (Shimadzu Corporation, Kyoto, Japan) and a Poropak Q column.

From 5 to 15 October 2003, we sampled each pot for the $\delta^{13}C$ of evolved CO_2 using the above procedure with the following modifications for isotope sampling: Duplicate samples were taken from each flux chamber at 0 and 48 h. One gas sample from each timepoint was analyzed for CO_2 concentration on an LI-6251 gas analyzer in injection mode as described above, while the other was analyzed for $\delta^{13}C$ at the Colorado Plateau Stable Isotope Laboratory (CPSIL) at Northern Arizona University, Flagstaff, AZ, USA with a Thermo Finnigan Delta Plus Advantage gas isotope-ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Between sampling and analysis, CO_2 samples sent to CPSIL were stored in 15 mL Hungate tubes that had been flushed with nitrogen and evacuated before sampling.

Photosynthetic measurements were taken on one healthy stem from each of two planted pots from each chamber. The rate of photosynthetic assimilation at 350 and 700 μL L^{-1} CO_2 was measured using an LI-6400 Photosynthesis System (LI-COR Biosciences).

Planted pots were destructively harvested between 14 and 22 October 2003. All shoots were cut at the soil surface. The number of live and dead shoots per pot was recorded, as well as the number of plants that had produced seeds. Dead material at the tips of live shoots was removed and kept separate. Roots were washed clean of soil. Roots and shoots were dried at 60 °C to constant weight and weights recorded. All plant materials and initial soil samples were ground with a ball grinder and analyzed at CPSIL for $\delta^{13}C$ and C/N with a Carlo Erba NC2100 Elemental Analyzer (CE Instruments, Milan, Italy) in line with a Thermo Finnigan Delta Plus Advantage gas isotope-ratio mass spectrometer (Thermo Fisher Scientific).

Sulfate reduction rate and methanogenesis were measured from 21 October to 13 November 2003. Sulfate reduction was measured by injection of Na^{35}SO$_4$ and anaerobic incubation for 4 h followed by distillation for acid-reducible and chromium-reducible sulfides (Fossing & Jorgensen, 1989). A subsample was also analyzed for organically bound sulfides (Wieder *et al.*, 1985). Methanogenesis was measured on a 4 mL sample of soil in an anaerobic jar over 1 week, after which the samples were dried at 70 °C to constant weight for determination of soil dry weight.

Isotope calculations

The CO_2 emitted from planted-treatment pots was a combination of CO_2 from C_4 SOM mineralization and CO_2 fixed during the experiment by C_3 plants (either in the form of plant respiration or mineralization of C_3 plant-derived material that became incorporated into the soil during the course of the experiment). The relative contributions of CO_2 from C_4 native SOM and C_3 plant assimilation are described by the equation

$$F_t = F_3 + F_4, \tag{1}$$

where F_t is the total CO_2 flux measured from the whole system, F_3 is the amount of CO_2 flux from the C_3 plant and plant-derived materials, and F_4 is the amount of the CO_2 flux from native-SOM mineralization of C_4 soil. The contribution of the respiration of the C_3 plant and plant-derived materials to the total CO_2 flux from each

individual pot was calculated with the following equation (Fu & Cheng, 2002):

$$F_3 = F_t(\delta_t - \delta_4)/(\delta_3 - \delta_4), \qquad (2)$$

where δ_t is the $\delta^{13}C$ of the CO_2 produced by the whole system, δ_4 is the $\delta^{13}C$ of the C_4 soil, and δ_3 is the $\delta^{13}C$ of the C_3 plant. The portion of total flux from SOM mineralization of C_4 soil, the F_4 term, can then be calculated using Eqn (1). We used the average $\delta^{13}C$ of the CO_2 produced by unplanted pots as the δ_4 endmember term ($-17.10‰$), but used a δ_3 endmember specific to each pot. The δ_3 endmember term for each pot was calculated as a mass-weighted average of the $\delta^{13}C$ of root and shoot tissue from that pot, proportional to the dry weight of each tissue type. Average values for this endmember are given in Table 1. F_3 and F_4 values were calculated individually for each pot.

Because the above calculation of the δ_3 endmember is based on the unverified assumption that roots and shoots respire equally per unit mass, a sensitivity analysis was performed in which a range of potential δ_3 terms were used in Eqn (2) to generate the contributions of plant- and soil-derived C to total CO_2 flux for each pot. Three potential δ_3 terms were used: (1) $\delta^{13}C$ of shoots only, (2) $\delta^{13}C$ of roots only, and (3) $\delta^{13}C$ calculated as the mass-weighted contributions of above and belowground biomass $\delta^{13}C$ values, as described above. Average values for each of these δ_3 endmembers are given in Table 1.

Redox measurements

As a follow-up experiment to the one conducted in summer 2003, an experiment nearly identical in design to the one described above was undertaken in the same greenhouse facility at SERC during summer 2005. This study allowed us to gather data on the hypothesis that results from our original experiment were due to increased soil oxygenation via root oxygen loss (ROL). Between July 26 and 29, 2005, soil redox measurements were taken on 16 planted pots and 15 unplanted pots. Four platinum-tipped redox electrode connected to an Orion benchtop conductivity meter (Thermo Electron Corporation) were placed in a pot to a depth of 7 cm below the soil surface and allowed to equilibrate until the digital reading stabilized. Redox potentials reported here were not corrected for the 244 mV difference between our calomel reference electrode and the standard hydrogen electrode. In August 2005, the planted pots were destructively harvested and biomass was dried at 60 °C to constant weight. The four redox readings per pot were averaged and regressed against belowground biomass, with the values for all 15 unplanted pots representing only one point in the regression.

Statistical analysis

CO_2 flux was calculated by regressing CO_2 efflux against time using the SAS regression procedure (SAS Institute Inc., Cary, NC, USA). Data were analyzed for statistical differences between the main effects (planting and CO_2 treatment) by two-way ANOVA using the SAS generalized linear model (GLM) procedure (e.g. Megonigal et al., 2005). Data with heterogeneous variance were log transformed and outliers were removed using Grubbs' outlier test. The type III mean square error of CO_2 treatment nested within chamber was used as the error term in assessing CO_2 effects. An analogous error term was used to assess the effects of the planting treatment and the assigned elevated-CO_2 growth chamber. In cases where the ANOVA analysis showed that chamber was not a significant effect, data from replicate chambers were pooled and reanalyzed without this error term. For biomass analyses, PAR was used as a covariate. To simplify data presentation, the means and standard errors reported in all tables and figures were calculated by pooling data from the two replicate chambers ($n = 16$ for each treatment). However, all of the statistical outcomes reported in the text, figures, and tables are based on the ANOVA tests described above. We set a significance threshold of $P < 0.05$.

Results

Flux measurements

The $\delta^{13}C$ signatures of the CO_2 evolved from the pots varied significantly between the planted and unplanted treatments, but not between the ambient and elevated treatments (Table 1). There was no significant difference in CO_2 flux between the unplanted ambient and unplanted elevated CO_2 treatments (Fig. 2a). Within the planted treatment, total CO_2 flux was significantly higher in the elevated CO_2 treatment ($P < 0.0001$, Fig. 2b). When total CO_2 flux from pots in the planted treatment was partitioned into soil- and plant-derived components as described in Eqn (2), soil-derived CO_2 flux was 157% higher at elevated vs. ambient CO_2 treatment ($P < 0.0001$, Fig. 2b). The percentage of total CO_2 flux from soil-derived sources was also significantly higher ($P < 0.0001$) in planted pots grown at elevated CO_2 compared with those grown at ambient CO_2 (Table 1).

Results from the GLM procedure on log-transformed soil-derived CO_2 flux showed CO_2 treatment, planting treatment, and their interaction to be significant effects ($P < 0.0001$, $P < 0.0001$, and $P = 0.0004$, respectively). Chamber was not a significant effect. This pattern remained the same for each δ_3 endpoint we considered in our sensitivity analysis, though the magnitude of the

Table 1 Endmember values for the δ_3 term used in Eqn (2) and sensitivity analysis, $\delta^{13}C$ of total evolved CO_2 in different treatments, and percentage of flux from soil- and plant-derived sources based on the mass-weighted endmember

Treatment	Shoot $\delta^{13}C$ endmember	Root $\delta^{13}C$ endmember	Mass-weighted $\delta^{13}C$ endmember	$\delta^{13}C$ of evolved CO_2	% flux from plant-derived sources	% flux from soil-derived sources
Planted ambient	-26.47 ± 0.04[a]	-23.96 ± 0.30[a]	-25.12 ± 0.10[a]	-23.70 ± 0.15[a]	82.5 ± 2.07[a]	17.5 ± 2.07[a]
Planted elevated	-27.60 ± 0.22[b]	-25.79 ± 0.33[b]	-26.59 ± 0.22[b]	-23.45 ± 0.24[a]	67 ± 2.13[b]	33 ± 2.13[b]
Unplanted ambient	na	na	na	-17.12 ± 0.27[b]	0	100
Unplanted elevated	na	na	na	-17.09 ± 0.25[b]	0	100

Values for shoot and root $\delta^{13}C$ are measured; the mass-weighted endmember is calculated as a weighted average of the $\delta^{13}C$ of root and shoot tissue from each pot proportional to the dry weight of each tissue type. Units for $\delta^{13}C$ are ‰. Values are means pooled across replicate chambers $\pm$SE. Within a column, values followed by different letters are significantly different. In this table, significant differences are all $P < 0.001$.
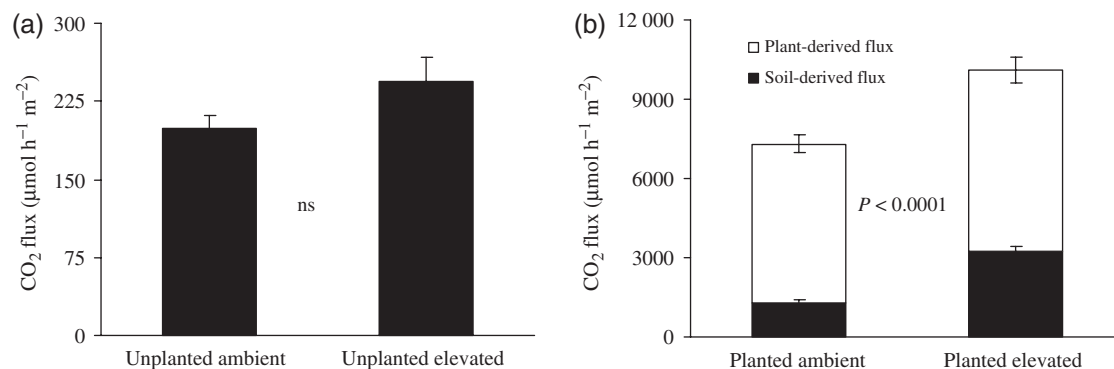

Fig. 2 CO_2 flux from plant- and soil-derived C in (a) the unplanted treatment and (b) the planted treatment. Values are means pooled across replicate chambers $\pm$ standard error. P-values represent the comparison of total CO_2 flux between ambient and elevated CO_2 treatments; ns, not significant.

CO_2 effect (elevated over ambient) ranged from 83% to 218% (Fig. 3). Redox potential was positively and significantly correlated with belowground biomass ($r^2 = 0.56$ and $P = 0.0006$) (Fig. 4).

Methane flux was negligible from pots in all treatments. When flux regressions with $r^2 < 0.5$ are excluded (excludes 24 of 64 pots), methane fluxes were -0.028 ± 0.02, -0.0032 ± 0.03, 0.022 ± 0.02, and $0.091 \pm 0.03 \, \mu g \, CH_4 \, m^{-2} \, day^{-1}$ for the unplanted ambient, unplanted elevated, planted ambient, and planted elevated treatments, respectively. There was no significant difference between any of the treatments.

Sulfate reduction and methanogenesis

Sulfate reduction rates were not significantly different between the ambient and elevated CO_2 treatments (Fig. 5). However, sulfate reduction rates in planted soils were nearly threefold higher ($P < 0.0001$) than in unplanted soils (Fig. 5).

Methanogenesis was negligible during the 1-week soil incubations. The average methane flux of soil from all pots was $1.16 \times 10^{-4} \, \mu mol \, h^{-1} \, g \, dry \, weight^{-1}$, with no significant differences between any of the treatments.

Plant attributes

Elevated CO_2 significantly increased belowground plant biomass. After one growing season, total belowground biomass (including fine roots and rhizomes) was 49% greater at elevated CO_2 than at ambient CO_2 ($P < 0.0001$, Table 2). A marginally significant 17% increase in aboveground biomass was also seen under elevated CO_2 ($P = 0.06$, Table 2). The number of shoots per pot increased by 17% and the shoot C/N ratio was 22% higher in plants grown under elevated CO_2 (Table 2). The shoot $\delta^{13}C$ signature was 4.3‰ more depleted in
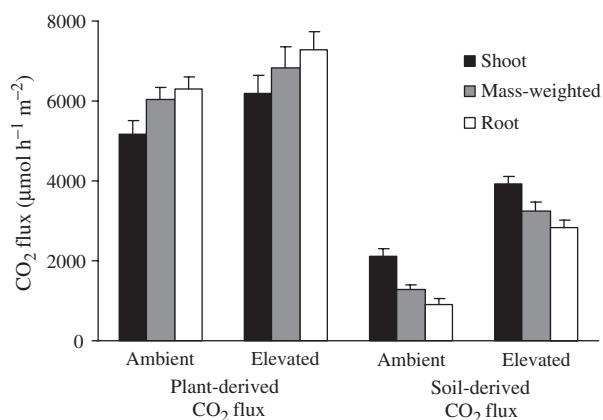
Fig. 3 Sensitivity analysis of the δ_3 term used to generate the contributions of plant- and soil-derived C to total CO$_2$ flux. Presented here is the outcome of Eqn (2) using three potential δ_3 endmember terms: (1) δ^{13}C of shoots only ('shoot'), (2) δ^{13}C calculated as the mass-weighted contributions of above and belowground biomass δ^{13}C values ('mass-weighted'), and (3) δ^{13}C of roots only ('root').
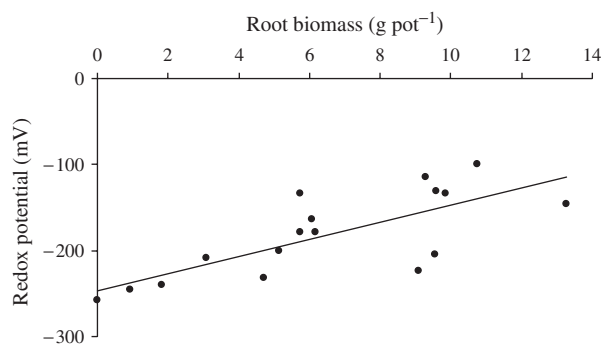

Fig. 4 Soil redox potential vs. root biomass in individual pots. The solid line is a linear regression with $r^2 = 0.56$ ($P = 0.0006$). Increasingly positive redox potentials indicate increasingly oxidized soil environments.

plants from the elevated treatment (Table 1), reflecting the slightly depleted δ^{13}C (−11‰) of the CO$_2$ added to increase CO$_2$ concentration in the elevated chambers as compared with the δ^{13}C of ambient CO$_2$ (−8‰). In the roots, C/N ratio was 16% higher in plants grown under elevated CO$_2$ and the δ^{13}C was 7.6% more depleted (Table 1). Plants grown under elevated CO$_2$ showed photosynthetic acclimation to higher CO$_2$ concentrations; Photosynthetic assimilation at 350 μL L^{-1} CO$_2$ was 36.5% lower in plants grown in elevated CO$_2$ chambers compared with those grown in ambient chambers ($P = 0.045$, Table 1). Despite acclimation, elevated CO$_2$ plants had higher instantaneous photosynthetic rates than ambient CO$_2$ plants at their respective growth-CO$_2$ concentrations (means = 13.9 μmol m^{-2} s^{-1}
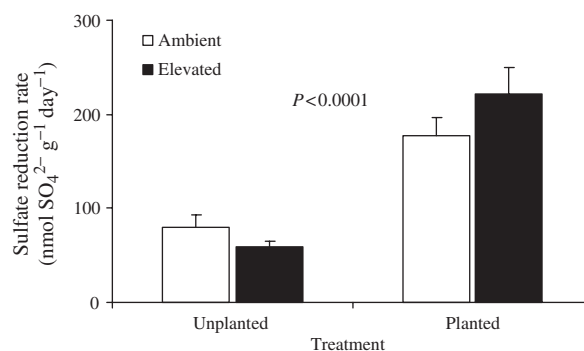

Fig. 5 Sulfate reduction rates across treatments. Values are means pooled across replicate chambers ±SE. The P-value is a comparison between planted and unplanted treatments.

for elevated CO$_2$ plants at 700 μL L^{-1} vs. 8.64 μmol m^{-2} s^{-1} for ambient CO$_2$ plants at 350 μL L^{-1} CO$_2$). Relative levels of PAR varied by ±9% between the chambers. This variation in light levels was positively correlated with differences between chambers in belowground biomass.

Discussion

Elevated CO$_2$ significantly increased soil organic matter decomposition by 83–218% in a simulated wetland. Based on natural-abundance stable C isotope tracing, we show that this increase in decomposition was from native soil organic matter, not recently fixed C compounds. Furthermore, increased belowground biomass raised the redox potential of wetland soils, providing evidence that an elevated-CO$_2$-induced increase in ROL from wetland plant roots may explain the observed increase in SOM decomposition at elevated CO$_2$.

Previous studies have proposed that global climatic changes such as warming and changing precipitation patterns may 'unlock' C stored in peat soils (Gorham, 1991; Hogg et al., 1992; Oechel & Vourlitis, 1994; Bridgham et al., 1995). Here, we show that elevated CO$_2$, the most ubiquitous global change, can independently induce an increase in native SOM decomposition in a highly organic anaerobic soil. Two non-exclusive mechanisms may explain this observed pattern: (1) elevated CO$_2$ led to an increase in root exudates, leading to increased decomposition via a 'priming effect' (Kuzyakov, 2002), and (2) elevated CO$_2$ caused an increase in belowground biomass, leading to increased ROL and an increase in aerobic SOM decomposition.

In support of the first possibility, Freeman et al. (2004a) demonstrated that elevated CO$_2$ increased the release of recently photosynthesized C compounds into peatland soils. In addition, an increase in the release of labile C compounds from wetland plants has been

Table 2 Plant attributes by CO_2 treatment for planted pots

CO_2 treatment	Shoots per pot	Shoot biomass	Root biomass	Shoot C/N	Root C/N	Photosynthetic assimilation at ambient [CO_2]
Ambient	26.00 ± 0.80	4.92 ± 0.18	5.13 ± 0.26	66.95 ± 1.22	105.72 ± 3.11	8.64 ± 1.42
Elevated	30.42 ± 0.73	5.77 ± 0.36	7.63 ± 0.52	81.91 ± 1.89	122.81 ± 2.83	5.49 ± 1.32
P-value	0.0003	0.06	0.02	<0.0001	0.085	0.045

Data in this table are means pooled across replicate chambers ± SE; *P*-values are based on the two-way ANOVA described in 'Materials and methods' and represent the difference between the ambient and elevated CO_2 treatments. Units for biomass are g dry weight pot^{-1}; units for assimilation are μmol m^{-2} s^{-1}.

invoked to explain increased methane emissions induced by elevated CO_2 (Vann & Megonigal, 2003). An increase in root exudation or root turnover could induce a rhizosphere priming effect (Kuzyakov *et al.*, 2000), whereby an influx of labile C leads to a concomitant increase in native soil organic matter decomposition. Given the increase in leaf-level photosynthesis and root biomass we observed in this study, priming is a possible explanation for an increase in native SOM decomposition. However, we do not have direct evidence to support a positive priming response and cannot separate it from other potential mechanisms. To our knowledge, priming effects have not been studied in depth in wetland soils (but see Li & Yagi, 2004).

Our study does, however, provide evidence supporting the second mechanism. Wetland plants transport O_2 to their roots through aerenchyma tissue, and some of this O_2 leaks out of the roots into the soil, a process termed ROL. Bezbaruah & Zhang (2005) showed that total oxygen released belowground from wetland plants is a function of root length and diameter; it is thus likely that the increase in root biomass at elevated CO_2 shown in our study led to an increase in ROL and total O_2 flux into the soil, and less-negative soil redox potentials (Fig. 4). An increase in the O_2 supply would allow aerobic bacteria to proliferate and facultative anaerobes to switch to an aerobic metabolic pathway, increasing metabolic efficiency and thus rates of decomposition. Oxygen has also been shown to activate the enzyme phenol oxidase; higher rates of ROL could thus allow soil microbes access to previously recalcitrant C compounds via this enzyme (Freeman *et al.*, 2001, 2004b).

The increase in sulfate reduction in the planted vs. unplanted treatments suggests that at least some of the rhizosphere-related increase in decomposition results from an increase in anaerobic decomposition. This could occur via either a priming effect from root exudates, or a secondary increase in labile C compounds released by the hydrolysis of recalcitrant C via aerobic decomposition or phenol oxidase activity.

Some of the conditions of this experiment were necessarily artificial (e.g. the initial homogenization of soil); however, the changes in plant attributes (shoot and root biomass, shoot density, shoot and root C : N, photosynthetic assimilation) we observed with the elevated-CO_2 treatment in the greenhouse very closely mimicked the results of elevated CO_2 enrichment in a natural wetland setting (Drake, 1992; Rasse *et al.*, 2005). Although we would be cautious in extrapolating the absolute magnitude of changes in rates of decomposition to natural systems, the relative responses are likely applicable outside a greenhouse setting.

Further work should focus on identifying *in situ* rates of ROL under elevated CO_2 and discerning the respective contributions to increased decomposition of a priming effect and ROL. In addition, because the increase in SOM decomposition that we found at elevated CO_2 could contribute to an elevated rate of increase in the atmospheric CO_2 concentration, determining the potential extent of this positive feedback in anaerobic systems worldwide and quantifying thresholds for its initiation are important for understanding future carbon-cycling dynamics (DeAngelis *et al.*, 1986).

Conclusions

The results of this study suggest the presence of a positive feedback between rising CO_2 concentrations in the atmosphere and wetland SOM decomposition. In addition to the atmospheric and climatic implications of this feedback, increased rates of decomposition over a sufficiently long period could cause the loss of soil-surface elevation in coastal wetlands and potential inundation of these important coastal ecosystems. Moreover, though this study was conducted in a simulated brackish marsh system, the mechanisms identified are not restricted to a specific type of wetland. Increased ROL at elevated CO_2 is probable in any anaerobic environment that is not subject to severe nutrient limitation and is dominated by emergent vascular plants. Anaerobic systems, including northern peatlands, hold a large portion of the world's soil C, and substantial losses of soil C have already been observed in some of these systems (Bellamy *et al.*, 2005). Taken together,

these results suggest a limitation on the size of the carbon sink presented by anaerobic wetland soils in future atmospheric CO$_2$ conditions and should be considered by both modelers and policy makers concerned with quantifying the future carbon-sequestration potential of these systems.

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