PRIMARY RESEARCH PAPER

Can elevated CO$_2$ modify regeneration from seed banks of floating freshwater marshes subjected to rising sea-level?

Beth A. Middleton · Karen L. McKee

Abstract Higher atmospheric concentrations of CO$_2$ can offset the negative effects of flooding or salinity on plant species, but previous studies have focused on mature, rather than regenerating vegetation. This study examined how interacting environments of CO$_2$, water regime, and salinity affect seed germination and seedling biomass of floating freshwater marshes in the Mississippi River Delta, which are dominated by C$_3$ grasses, sedges, and forbs. Germination density and seedling growth of the dominant species depended on multifactor interactions of CO$_2$ (385 and 720 µl l^{-1}) with flooding (drained, +8-cm depth, +8-cm depth-gradual) and salinity (0, 6% seawater) levels. Of the three factors tested, salinity was the most important determinant of seedling response patterns. Species richness (total = 19) was insensitive to CO$_2$. Our findings suggest that for freshwater marsh communities, seedling response to CO$_2$ is species-specific and secondary to salinity and flooding effects. Elevated CO$_2$ did not ameliorate flooding or salinity stress. Consequently, climate-related changes in sea level or human-caused alterations in hydrology may override atmospheric CO$_2$ concentrations in driving shifts in this plant community. The results of this study suggest caution in making extrapolations from species-specific responses to community-level predictions without detailed attention to the nuances of multifactor responses.

Keywords Climate change · Coastal land loss · CO$_2$ enhancement · Floating marsh · Gulf of Mexico · Salinity intrusion · Sea level rise · Seed bank

Introduction

The regeneration of plant species during climate change may hold the key to the future species patterns in ecosystems (Woodward et al., 1991). In coastal ecosystems, regeneration may be dictated by disturbances related to hurricanes, water fluctuation, and/or saltwater intrusion (Platt & Connell, 2003; Middleton, 2009a, b, c, d). These disturbances may increase in the future with climate change, particularly because of increased hurricane activity, sea-level rise (Michener et al., 1997), or declining freshwater input because of inland freshwater diversion or abstraction along rivers (Middleton, 1999). Experimental studies of species in freshwater wetlands indicate that water depth and salinity changes can greatly alter seed germination and seedling growth of species (Middleton, 1999, 2009a, d; Howard & Mendelssohn, 2000; Brock et al., 2005).

B. A. Middleton (✉) · K. L. McKee
U.S. Geological Survey, National Wetlands Research Center, 700 Cajundome Boulevard, Lafayette, LA 70506, USA
e-mail: middletonb@usgs.gov

Especially for freshwater marsh species, the outcomes of post-disturbance regeneration may depend on species-specific responses of seeds and seedlings to future conditions of rainfall, sea level, and associated factors. While "regeneration" refers to the reestablishment of vegetation after disturbance either by seed or vegetative means (Middleton, 1999), in this study, only materials originating from seed banks were considered. Knowledge of the potential role of seed banks in supplying regenerative material to wetlands after disturbance is important; after large-scale disturbance, seed banks can be an important source of regenerative material (Leck, 1989; Middleton et al., 1991), supplying as much as 80% (Jarvis & Moore, 2010). Nevertheless, detailed information regarding species responses to important abiotic and biotic factors is mostly lacking for wetland species (Middleton, 1999). Regeneration studies undertaken in controlled environments could improve our understanding of the potential maintenance of species in future climate settings, and inform modeling efforts.

Future environments for regeneration in freshwater may involve novel combinations of increased atmospheric CO_2 (IPCC, 2007), temperature, sea level, salinity (Michener et al., 1997), precipitation (Easterling et al., 2000), and hurricane activity (Webster et al., 2005). Careful assessment of any interactive effects of these environmental factors is important in projecting the future responses of freshwater wetland species to climate change. Of these factors, CO_2 has received much emphasis in ecosystem modeling (Mayle et al., 2004; Hickler et al., 2009) and empirical field studies in wetlands (Rasse et al., 2005), but only rarely studied in tandem with multifactor interactions (McKee & Rooth, 2008; Cherry et al., 2009; Langley et al., 2009).

While single-factor studies of CO_2 effects may miss critical details of the potential multiple-factor responses of freshwater wetland species to climate change, considerations of the findings of these studies are important. Single-factor studies of CO_2 often report that the carbon assimilation strategies of plant species dominate outcomes, with C_3 species more responsive than C_4 species to elevated atmospheric CO_2 (Osmond et al., 1980; Bazzaz, 1990). For example, C_3 brackish marsh species such as *Schoenoplectus americanus* increase in biomass when subjected to elevated CO_2 levels (Curtis et al., 1989, 1990; Ziska et al., 1990; Rasse et al., 2003). While most studies find that terrestrial C_3 species grow faster under elevated CO_2,

the growth response of C_3 seedlings to elevated CO_2 can be negative (Garbutt et al., 1990), or positive for C_4 species (Wand et al., 1999).

There is a logical expectation that elevated CO_2 could increase the growth of C_3 species under stressful conditions, including anaerobic or saline environments (i.e., an interactive effect). For example, elevated CO_2 offsets the effects of salinity on the biomass of *S. americanus* in brackish marshes (Cherry et al., 2009). C_3 species of salt marshes generally had higher biomass in elevated CO_2, but the response varied for species depending on water regime and/or salinity levels (Cherry et al., 2009). In other studies, C_4 species did not increase growth in enhanced CO_2 in any water regime or salinity level (Rozema et al., 1991; Lenssen et al., 1993). In elevated CO_2, the seedlings of *Taxodium distichum* (C_3), a freshwater swamp species, did not have a higher biomass in flooded conditions, but seedlings of *Orontium aquaticum* (C_3) had higher biomass when exposed to elevated CO_2 in both flooded and unflooded conditions (Megonigal et al., 2005).

As a further complication in understanding the effects of CO_2 on the regeneration of wetland species, single-species experiments could miss important biotic interactions. In one experiment, growth of the mangrove *Avicennia germinans* (C_3) was higher in elevated CO_2 when grown in monoculture, but when grown in mixed cultures with *Spartina alterniflora* (C_4), the growth was suppressed (McKee & Rooth, 2008). While both C_3 and C_4 species may have enhanced growth in elevated CO_2, the specific response of species may depend on a large array of biotic and abiotic factors. Therefore, freshwater wetland species subjected to multiple interacting factors may or may not benefit from CO_2 enrichment. "Benefit" is defined for the purposes of this study as an increase in seed germination density or seedling biomass. Many factors other than CO_2 may affect the outcome of regeneration in freshwater wetlands undergoing climate change. Nevertheless, basic information for species related to either single-factor or interacting multifactor responses to CO_2, water regime and salinity is limited (Middleton, 1999).

Few multifactorial studies have been conducted to explore the responses of wetland species to interactions of CO_2, water regime and salinity (e.g., Cherry et al., 2009; Langley & Megonigal, 2010), even though enhanced CO_2 might have the potential to offset the negative impact of flooding and salinity on

seed germination and seedling growth in wetland species. Controlled greenhouse, laboratory, and field studies could help us better understand species' responses to environmental changes related to climate change factors, to aid modeling, and/or assist efforts to categorize functional types. The species of the floating marshes of Lake Salvador in coastal Louisiana are excellent candidates for studies of potential multifactorial interactions in wetland environments. These wetlands constitute some of the southernmost freshwater wetlands in central North America (Swarzenski et al., 1991), and are thus likely to be directly impacted in the future by shifts in levels of CO_2, water level, and salinity along the Gulf Coast. These wetlands lie within the Barataria Basin of the Mississippi River Deltaic Plain in the most recently abandoned channel of the Mississippi River (Swarzenski et al., 1991).

The overall goal of this study was to examine regeneration from the seed bank of these freshwater floating marshes in response to various combinations of CO_2, water regime, and salinity. Our specific objectives were to determine if (1) elevated CO_2 might ameliorate stress effects of sea-level rise (increased flooding and salinity) on species richness, density, or biomass of seedlings emerging from the seed bank; and (2) species responses to CO_2 and other factors were generally similar or individualistic.

Materials and methods

Study area

Floating freshwater marshes are common in Louisiana (Russell, 1942), India (Middleton et al., 1991; Middleton, 1999), Africa (Migahid, 1947; Gaudet, 1977; Thompson, 1985), and South America (Junk, 1970). In Louisiana, floating marshes often lie on the coastal side of *T. distichum* swamps (Middleton, personal observation). In the Lake Salvador marshes, the water levels are the highest in the winter, and influenced only minimally by tides (Sasser et al., 1996). The vegetation floats on a buoyant mat of peat that is not anchored. The mat may settle on the underlying substrate during low water events. During flooding, water may flow above or below the mat (Swarzenski et al., 1991). Saline water enters Lake Salvador through tidal inlets from the Gulf of Mexico

(maximum salinity = 4; Swarzenski et al., 1991) so that floating marshes near Lake Salvador have a range of salinity from freshwater to brackish (Swarzenski & Swenson, 1994). We studied freshwater marshes in the Lake Salvador Management Area, which had lower levels of salinity than the intermediate type (Swarzenski et al., 1991). The dominant species in the Lake Salvador Management Area are of the C_3 assimilation type including *Cyperus haspan*, *Hydrocotyle verticillata*, *Panicum hemitomum*, *Sagittaria lancifolia*, and *S. americanus* (Swarzenski et al., 1991).

Experimental approach

Seed bank material was collected in the Lake Salvador Management Area (29°48.513N; 90°18.677W) on April 17, 2003. The peat for the seed bank study was lifted from 10 randomly selected points in the marsh. With shears, the shoots were separated from the rhizomes and roots, just below the marsh surface. The entire top 10 cm of peat was removed from each of the sample quadrats in a 1-m^2 area, and placed into a bag. The collected peat was held for 1.5 months in a cold room (at ca. 5°C). On June 7, 2003, rhizomes were removed from the peat, and the peat samples from all collection sites were thoroughly homogenized together by hand. The homogenized peat (1 cm) was placed over a layer of vermiculite and sand in 10 × 15 cm pots, and placed into the Wetland Elevated CO_2 Experimental Facility (WECEF), USGS National Wetlands Research Center, Lafayette, LA (30°22.3N; 92°1.9W).

WECEF consisted of four greenhouses and a control room that housed CO_2 and environmental monitoring equipment. Two greenhouses were randomly assigned to elevated CO_2 (720 µl l^{-1} = elevated) and the other two to ambient CO_2 (385 µl l^{-1}) treatments. CO_2 concentrations were continuously monitored and kept within ±5% of the set-point through automated feedback regulation of flow meters supplying CO_2 gas to the greenhouses (see McKee & Rooth, 2008). One model of the IPCC (2007) projects a doubling of CO_2 by 2050, which was the basis for selecting the elevated level of CO_2 in the experiment. Photosynthetically active radiation, humidity, and temperature were also monitored (data not shown). Greenhouse temperatures were allowed to fluctuate naturally, although evaporative cooling systems prevented higher-than-ambient temperatures during the warmer months. Temperature

control is impossible without shading during hottest months at this latitude in south Louisiana. Shade cloth was present on the roof (not sides) of the greenhouse and only during the hottest months (June–September); light was mainly attenuated from 10 am to 2 pm while the sun was highest (June–September 600 μmol m^{-2} s^{-1} at midday).

Six pots of peat were placed into each of 24 plastic bins, which were assigned to three water and two salinity regimes. Salinity levels were set at 0 and 2 (low and high salinity, respectively), which simulated freshwater and 6% sea-strength conditions using Instant Ocean$^{®}$. The flooding treatments from low to high included freely drained, gradually increasing water levels to 8 cm above soil surface, and initially flooded to 8 cm (low, gradual, and high, respectively). To prevent any floating of materials, all of the seed bank pots in all the treatments were watered to the top of the pot to allow the peat to settle, and then, the water treatments were applied. For the gradually increasing water regime, the water level was increased by 1 cm per week for 8 weeks, starting on June 20, 2003 after seeds had germinated; the flooded treatment was raised to 8 cm slowly on the same day to avoid dislodging peat from the pot. For our purposes, the gradually increasing water regime was an intermediate level treatment between low and high water levels. Each greenhouse (2 ambient, 2 elevated CO_2) contained all combinations of three water regimes and two salinity levels (6 bins).

Each bin was watered daily with distilled water to maintain the water depths and salinity levels in the bins. A pot of vermiculite was placed into each bin to test for seed contamination. To fertilize the plants, a modified Hoagland solution was added to the water weekly (1 mol m^{-3} MgSO$_4$, 2 mol m^{-3} KH$_2$PO$_4$, 2.5 mol m^{-3} CaCl$_2$, 2.0 mol m^{-3} NH$_4$Cl; trace elements as mg dm^3: Fe (2.5), Mn (0.25), Cu (0.01), Zn (0.025), Mo (0.025), B (0.25).

The seedlings were harvested starting on November 18, 2003. Each seedling was separated from the peat, placed into a marked bag, counted, and dried in an oven at 70°C. Roots and shoots were weighed separately. While seed contamination within the study was found to be minimal based on seedling emergence from vermiculate samples, *Utricularia* grew between submerged pots, so that we were unable to determine its origin. Nomenclature follows the U.S. Department of Agriculture (2011a, b).

Statistical analyses

Seed densities were calculated per m^2 based on the pot size (m^2 conversion = 62). This common procedure is used to standardize reported seed germination values, and is based on the idea that the density of seeds is related to the surface volume in the upper 1 cm^2 of the soil sample (Middleton et al., 1991). In this way, the technique of using a thin layer of peat in the greenhouse pots best characterizes the seeds in the upper 10 cm of the peat collected from the field site.

The experimental design was a split-plot with CO_2 as the main plot, and salinity and water level treatments as the subplots. Thus, each pair of elevated-ambient greenhouses constituted a complete block (total of 2 blocks). Three-way ANOVAs were performed on mean seed density, seedling mass, and species richness with main effects of CO_2 level, salinity and water regime, and their interactions examined using a mixed model. CO_2, salinity, and water level were fixed effects in the model; greenhouse (nested within CO_2) was a random effect in the model. The CO_2 effect (main plot) was tested against greenhouse (nested within CO_2), and the subplot effects and interactions were tested with subplot error (Scheiner & Gurevich, 1993). Also, for each dominant species, three way ANOVAs were performed on density, mean total biomass, and the mean biomass per individual. All other species were grouped into a minor category. Using SAS JMP (2007), ANOVA of main effects and their interactions were tested. To examine differences among multiple treatment levels, single degree-freedom contrasts of interest were conducted when there was a significant main effect or interaction. Data were log transformed to meet the normality and homogeneity assumptions of ANOVA.

Results

Seed bank responses

A total of 19 species germinated from the seed banks. Species richness was not affected by the main effects of CO_2, water regime, salinity, or any of their interactions (Table 1A). In contrast, the density of germinating seeds was influenced by the interaction of CO_2 with water regime (Table 1B; Fig. 1a). In elevated (high) CO_2 levels, the gradual water

Table 1 ANOVA analysis of total species responses in freshwater floating marshes in coastal Louisiana for the main effects of CO_2 level (ambient vs. elevated), 385 and 720 $\mu l\ l^{-1}$, respectively, salinity level (salinity = 0 vs. 2, respectively), and water regime (freely drained, 8 cm, and gradually increased by 1 cm a week to 8 cm) and their interactions, for (A) log species richness, (B) log seedling density, and (C) log mean total of individual seedling biomass

Source	F	P
(A) Species richness		
CO_2	0.3	NS
Salinity	0.4	NS
Water regime	0.6	NS
$CO_2 \times$ salinity	0.6	NS
Salinity $\times$ water regime	<0.1	NS
$CO_2 \times$ water regime	2.8	NS
$CO_2 \times$ salinity $\times$ water regime	0.5	NS
(B) Seedling density		
CO_2	<0.1	NS
Salinity	0.3	NS
Water regime	0.5	NS
$CO_2 \times$ salinity	0.6	NS
Salinity $\times$ water regime	3.8	*
$CO_2 \times$ water regime	12.5	***
$CO_2 \times$ salinity $\times$ water regime	0.4	NS
(C) Individual seedling biomass		
CO_2	<0.1	NS
Salinity	28.6	***
Water regime	0.8	NS
$CO_2 \times$ salinity	1.2	NS
$CO_2 \times$ water regime	0.3	NS
Salinity $\times$ water regime	0.9	NS
$CO_2 \times$ salinity $\times$ water regime	3.6	*

Significant relationships are designated as: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$

treatment had higher seedling densities than the low and high water treatments (881.0 ± 48.0 vs. 701.1 ± 40.3 seedlings m^{-2}, respectively; $t = 9.5$, $P = 0.0024$), while in ambient (low) CO_2 levels, seedling densities were higher in high water than in low and gradual waters (975.5 ± 79.8 vs. 729.5 ± 34.5 seedlings m^{-2}, respectively; $t = 13.5$, $P = 0.0003$). Water regime also interacted with salinity level, so that in higher salinity levels (salinity = 2), seedling density was higher in high than in low and gradual water regimes (917.1 ± 78.8 vs. 744.8 ± 58.3, respectively; $t = -2.12$, $P = 0.0357$; Table 1B;
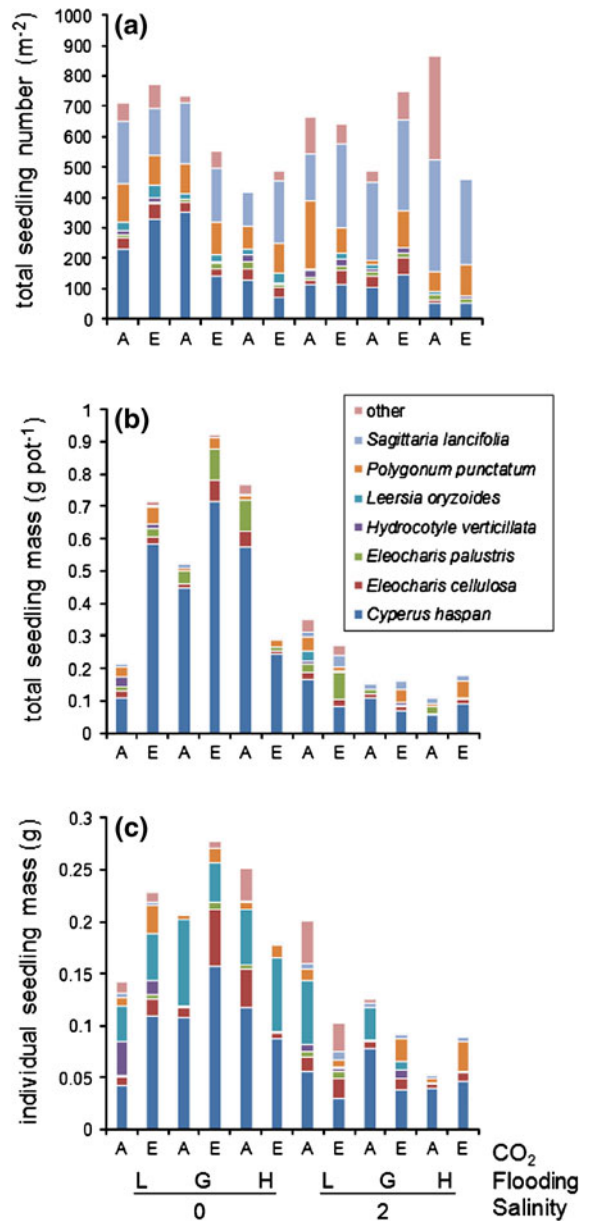

Fig. 1 Bar graphs of seven dominant species and "other" minor species for all treatment combinations showing responses including **a** total seedling number m^{-2} (density: based on pot size), **b** seedling total mass per pot (g), and **c** individual seedling mass (g). Standard errors are omitted for clarity. Treatments included CO_2 levels of ambient and elevated (385 and 720 $\mu l\ l^{-1}$, respectively; *A* and *E*, respectively; water regimes of low, gradual and high (freely drained, gradually increased by 1 cm a week to 8 cm, and 8 cm; *L*, *G*, *H*, respectively), and salinities of 0 and 2

Fig. 1a). *C. haspan* had higher total mass in elevated CO_2 in low flooding and low salinity conditions, and the response of this species dominated the overall

response in the experiment (Fig. 1b). CO_2, water, and salinity interacted with respect to the effects on individual seedling biomass (Table 1C). The nature of this interactive response of individual seedling biomass was that in elevated CO_2, seedling biomass was higher in low water and low salinity (0.105 ± 0.01 per seedling; Table 1C; Fig. 1c), but in gradual water and ambient CO_2 at either low or high salinity, the individual seedling weight did not differ (0.06 ± 0.01 g per seedling; $P > 0.05$). Overall, seedling responses to CO_2 with respect to species richness, density and biomass were not necessarily positive, and depended on interactions with other treatment factors including salinity and water regime (Fig. 1a–c).

Species-specific responses

Seedling density

For certain dominant species, the seedling density response to CO_2 interacted with water regime and salinity level (Table 2A). For *Eleocharis palustris*, the seedling density varied across water regime and CO_2 levels (not salinity) ($CO_2 \times$ water interaction; Table 2A; Fig. 1a). For *C. haspan*, seedling densities were higher in low CO_2 in low salinity and gradual or low water, and in high CO_2 in high or low salinity in low or gradual water, than in other combinations of treatments ($t = 16.9$, $P < 0.00001$; Table 2A; Fig. 1a). For *Polygonum punctatum*, the pattern of response was very similar to *C. haspan* in high CO_2 in all water regimes; however, in low CO_2, the density was lower in gradually increasing water depths in high salinity than in other treatments ($t = 2.95$, $P = 0.0038$; Table 2A; Fig. 1a). For *S. lancifolia*, seedling densities were similar at all water levels, salinities, and CO_2 levels, except that, in low CO_2 in high water and high salinity, the seedling densities were higher than in other treatments ($t = 3.2$, $P = 0.0036$; Table 2A; Fig. 1a). The seedling densities of *E. cellulosa*, *H. verticillata*, and *Leersia oryzoides* did not respond to main effects or combinations of CO_2, salinity, or water regime (Table 2A).

Biomass

The individual seedling biomass of several of the dominant species responded to CO_2, but these responses often interacted with water regime and salinity level (Table 2B). For *E. cellulosa*, higher seedling biomasses were found at low salinities, gradually increasing water regime and low CO_2 levels than in other treatment combinations ($t = -1.98$, $P = 0.05$; Table 2B; Fig. 1c). For *P. punctatum*, biomass increased in the treatments of gradually increasing water regime, low CO_2, and high salinity levels, but not low water in low CO_2 and high salinity ($t = -5.58$, $P < 0.0001$; Table 2B; Fig. 1c). For *C. haspan*, the biomass response generally increased in treatments with low rather than high salinity regardless of CO_2 level or water regime ($t = 7.85$, $P < 0.0001$; Table 2B; Fig. 1c); however, the individual mean weight of seedlings for *C. haspan* was highest in a treatment combination of high CO_2, low salinity and gradual water regime (0.7 g individual^{-1}; $t = 6.46$, $P = 0.0034$; Fig. 1c). For *E. palustris*, mean seedling weight was higher in high CO_2 in low and gradual water, rather than in high water ($t = 2.36$, $P = 0.0199$; Table 2B; Fig. 1c). In high salinity, *E. palustris* had higher mean biomass in low water than in high or gradual water regimes ($t = -2.24$, $P = 0.0269$; Table 2B; Fig. 1c). *S. lancifolia* and *L. oryzoides* did not respond to CO_2 level; instead the weights of seedlings decreased with increasing salinity (Table 2B). For *S. lancifolia*, mean seedling weight was higher in low water, than in gradual or high water regimes (Table 2B). *H. verticillata* did not respond differently to any treatment or treatment combination of salinity, CO_2 or water depth (Table 2B).

Discussion

Climate change may present species with interacting environments, so that the overall effect may be more severe than anticipated based on the analysis of single-environmental factors (Ghosn et al., 2010). This study empirically tested seed germination and seedling responses to interacting environments, which may be associated with changes in sea level and CO_2 in coastal freshwater marshes. We asked the question in the introduction: can the species richness, density, and biomass of freshwater floating marshes be affected by changes in CO_2 concentration and its interactions with water regime and salinity?

Salinity, which reduced total seedling mass by 68% and individual seedling mass by 59%, was the main

Table 2 ANOVA with F ratios of log responses of dominant seed bank species of freshwater floating marshes in coastal Louisiana to CO_2 level (ambient vs. elevated), salinity level (0 vs. 2) and water regime (freely drained, 8 cm, and gradually increased by 1 cm a week to 8 cm) for (A) log seedling density m^{-2} and (B) log individual seedling weight (g); Dominant species exceed a total mean density of 0.1 individuals per pot, or, total mean seedling weight of 0.1 g per pot, respectively[A]

Source of variation	Species						
	C. haspan	E. cellulosa	E. palustris	H. verticillata	L. oryzoides[a]	P. punctatum	S. lancifolia
(A) Seedling density							
CO_2	1.1	0.6	0.6	1.6	0.4	0.4	2.3
Salinity	9.6**	0.8	<0.1	0.5	<0.1	<0.1	8.1**
$CO_2 \times$ salinity	0.6	0.6	0.1	1.2	4.0	3.1	<0.1
H_2O	5.8**	0.8	0.1	0.9	1.2	1.9	<0.1
$CO_2 \times$ water regime	0.2	0.4	5.9**	0.6	0.5	5.4**	<0.1
Salinity $\times$ water regime	0.1	0.3	0.7	2.2	1.1	2.5	3.3*
$CO_2 \times$ salinity $\times$ water regime	7.8***	0.6	0.2	1.3	2.5	3.3*	3.1**
Mean $\pm$ SE density pot^{-1}	178.4 $\pm$ 13.7	39.8 $\pm$ 5.1	138.0 $\pm$ 11.0	12.5 $\pm$ 2.4	18.1 $\pm$ 3.0	101.9 $\pm$ 8.2	224.2 $\pm$ 12.1
(B) Individual seedling weight							
CO_2	<0.1	3.0	1.3	0.1	0.7	7.9	0.2
Salinity	25.3***	3.2	1.6	0.6	5.3*	<0.1	12.1***
$CO_2 \times$ salinity	5.8*	<0.1	0.6	0.5	0.8	0.2	1.5
H_2O	3.4*	0.4	0.8	2.6	<0.1	0.4	4.5**
$CO_2 \times$ water regime	1.1	2.9	3.8*	0.7	0.6	0.5	<0.1
Salinity $\times$ water regime	1.2	1.5	3.2*	1.4	1.0	1.6	0.7
$CO_2 \times$ salinity $\times$ water regime	2.4	3.1*	1.6	0.1	0.7	3.2*	0.7
Mean $\pm$ SE individual weight (mg)	73.8 $\pm$ 6.6	16.2 $\pm$ 3.3	2.2 $\pm$ 0.4	5.6 $\pm$ 2.9	35.5 $\pm$ 8.2	12.0 $\pm$ 1.9	3.4 $\pm$ 0.4

Values per m^2 were based on the pot dimensions. Significant relationships are designated as: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. The carbon assimilation strategy of all species was C_3, with the possible exception of the unknown dicot and monocot. Species that were not dominant included *Alternanthera philoxeroides*, *Ambrosia artemisifolia*[a], *Carex* sp., *Juncus* sp., *P. hemitomon*, *Pluchea odorata*[a], *Ptilimnium capillaceum*[a] and *Vigna luteola* (mean density $\pm$ SE = 0.5 $\pm$ 0.5, 0.9 $\pm$ 0.7, 1.4 $\pm$ 1.4, 0.9 $\pm$ 0.7, 1.9 $\pm$ 1.1, 0.5 $\pm$ 0.5, 0.5 $\pm$ 0.5, 0.5 $\pm$ 0.5 seedlings m^{-2}, respectively). In addition, there were five unknowns that ranged in density from <0.1 to 51.4 seedlings m^{-2}. Species designated with superscript "a", are annuals or short-lived perennials (USDA, 2011a, b). The mean biomass per individual for all of the minor species was <0.01 g per pot. The total species richness was 19, with a total mean density of 786.0 $\pm$ 26.3 seedlings m^{-2} and a mean total biomass of 159.8 $\pm$ 12.5 mg pot^{-1}. Except for *P. hemitomon* and the unknowns, these species are likely to use a C_3 carbon assimilation strategy

treatment driving seedling response patterns in this study, not CO_2 level (minimum and maximum value: total seedling weight per pot = 0.3–733.3 mg). Flooding also had an effect on seedling mass, but less than salinity. Previous studies indicate that some freshwater species are inhibited by flooding and salinity levels above 4 ppt (Baldwin et al., 1996). Experimental studies of species from coastal freshwater wetlands indicate that the seed germination and/or seedling growth of most species decrease with increased salinity (Howard & Mendelssohn, 2000; Brock et al., 2005; Middleton, 2009a). As a rule, salinities above 1,000 mg l^{-1} decrease the species richness of seeds germinating from the seed bank (Nielsen et al., 2003; Brock et al., 2005), and this is true of a wide range of fresh to salt marsh species (Middleton, 2009a). The negative effect of salinity on seed germination for most species may be caused by: (1) reduced seed viability, (2) blocked cues for emergence, or (3) seedling mortality immediately

after emergence (i.e., germination not directly affected) (Nielsen et al., 2003). After storms, coastal freshwater wetlands may increase in salinity and become dominated by different species, even if the species involved do not change (Michener et al., 1997; Middleton, 2009a).

The general characteristics of the seed bank in this floating marsh are roughly similar to those of an oligohaline marsh in Louisiana (Baldwin et al., 1996). While these studies are really not comparable in terms of the study design and treatments, the general results indicate similar seed bank characteristics. The seed banks for the oligohaline marsh had 3–10 species, with a total of 606–2,960 seeds m^{-2}. In comparison, our study had 20 species with a total of 753–828 seedlings m^{-2}, depending on the water regime and salinity treatment. For *S. latifolia*, the seed banks held 202–331 seedlings m^{-2} in the Baldwin et al. study (1996), while our study had 224 seedlings m^{-2}. We know of one other seed bank study of freshwater and freshwater floating marshes in Louisiana, but these marshes had much lower total mean seedling m^{-2} number (106–207 seedlings m^{-2}) in post-hurricane conditions with excessive flooding and/or hurricane sedimentation (Middleton, 2009a).

Our study indicates that CO_2, water regime, and salinity in combination produce seed bank responses not readily predictable from single-factor studies or functional type category species (e.g., graminoid vs. herb). Nevertheless, many climate models use relatively simple assumptions of functional responses, often based on the carbon assimilation strategy of species (Cramer et al., 2001; Krinner et al., 2005), e.g., CO_2 enrichment benefits the growth of C_3 over C_4 species (Bazzaz, 1990; Sage & Kubien, 2003). In contrast, extensive study shows variable responses of species to CO_2 treatment depending on other environmental constraints (Körner, 2006). Similarly in this study, the CO_2 responses of C_3 species in this study followed individualistic patterns.

To further explore potential effects of CO_2 on these species, we examined how CO_2 enrichment might offset the effects of stressful environments. Species richness was unaffected by CO_2, but density and biomass were affected by CO_2, depending on flooding or salinity conditions. The implication is that under a future elevated CO_2 scenario, species dominance may change even if the total number of species does not.

Our findings differ somewhat from germination studies of non-wetland species, which generally conclude that germination is little affected by elevated CO_2 (Garbutt et al., 1990; Huxman et al., 1998; Steinger et al., 2000; Poorter & Navas, 2003). In these terrestrial studies, a lack of germination density response to elevated CO_2 has been attributed partly to the fact that the CO_2 levels of terrestrial soils are higher than in the atmosphere (Bazzaz, 1979). Nevertheless, CO_2 levels higher than those of the soil could promote germination of some species (Baskin & Baskin, 1998). For example, the germination of *Plantago lanceolata* is increased by high CO_2 levels (Wulff & Alexander, 1985). Ethylene synthesis is increased in the seeds of *Helianthus annuus* in higher CO_2 levels (Corbineau et al., 1990). Our findings showed some effect on seed germination, but generally support the broader pattern that CO_2 primarily influences seedling growth, rather than germination, similar to findings of other freshwater wetlands studies (e.g., Megonigal et al., 2005).

Individual species responses to CO_2 were inconsistent, with some species showing an increase in density or biomass under elevated CO_2 (depending on flooding or salinity) and others showing no response or a decrease (Fig. 1; Table 2). CO_2 enrichment might cause seedlings to grow better under stressful edaphic conditions, and any CO_2-related increase in survival could be important to species exposed to rising sea levels. In our study, seedlings did not benefit from higher CO_2 in stressed conditions, although elevated CO_2 promoted higher total biomass for one species under low stress, e.g., *C. haspan* under low flooding and low salinity. Thus, our results do not support the prediction that higher CO_2 concentrations will ameliorate stressful environments related to flooding or salinity levels during early growth of this seed bank community.

Clearly, the responses of these C_3 freshwater wetland species in this study were individualistic and not readily generalized by functional type (graminoid vs. herb) to the entire community. For the purposes of modeling, the regeneration responses of each species should be evaluated separately as a set of potential outcomes related to multifactor interactions. Environmental change in atmospheric CO_2 and sea-level could play a major role in shaping the vegetation of coastal wetlands. Responses of wetland plant species to future environments may be highly individualistic, however, complicating specific predictions of wetland response.

Our findings also showed that early regeneration patterns reflected the interactions of CO_2 with other factors associated with climate change such as sea-level rise; however, not all species in this freshwater community were able to benefit from higher CO_2 levels because of overriding effects of salinity and/or flooding. Of the three environmental factors tested, salinity emerged as the most important determinant of seedling response pattern. This finding suggests that changes in sea-level, leading to salinity increases, may be more important to seedling recruitment in this freshwater community. Flooding can also combine with salinity to further impact regeneration patterns, but this effect may be a minor issue for marshes that float compared with attached freshwater marshes that are more vulnerable to inundation. CO_2 may play a greater role during later growth stages and in relation to biotic interactions of competition, herbivory, or disease. The entire subject is ripe for future study because the individualistic responses of species to abiotic (and biotic) factors can provide the critical framework to inform models predicting impact of climate change on ecosystems.

References

Baldwin, A. H., K. L. McKee & I. A. Mendelssohn, 1996. The influence of vegetation, salinity, and inundation on seed banks of oligohaline coastal marshes. American Journal of Botany 8: 470–479.

Baskin, C. C. & J. M. Baskin, 1998. Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, CA.

Bazzaz, F. A., 1979. The physiological ecology of plant succession. Annual Review of Ecology and Systematics 10: 351–371.

Bazzaz, F. A., 1990. The response of natural ecosystems to the rising global CO_2 levels. Annual Reviews 21: 167–196.

Brock, M. A., D. L. Nielsen & K. Crosslé, 2005. Changes in biotic communities developing from freshwater wetland sediments under experimental salinity and water regimes. Freshwater Biology 50: 1376–1390.

Cherry, J. A., K. L. McKee & J. B. Grace, 2009. Elevated CO_2 enhances biological contributions to elevation change in coastal wetland by offsetting stressors associated with sea-level rise. Journal of Ecology 97: 67–77.

Corbineau, F., S. Bagniol & D. Come, 1990. Sunflower (*Helianthus annuus* L.) seed dormancy and its regulation by ethylene. Israel Journal of Botany 39: 313–325.

Cramer, W., A. Bondeau, F. I. Woodward, I. C. Prentice, R. A. Betts, V. Brovkin, P. M. Cox, V. Fisher, J. A. Foley, A. D. Friend, C. Kucharik, M. R. Lomas, N. Ramankutty, S. Sitch, B. Smith, A. White & C. Young-Molling, 2001. Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. Global Change Biology 7: 357–373.

Curtis, P. S., B. G. Drake, P. W. Leadley, W. J. Arp & D. F. Whigham, 1989. Growth and senescence in plant communities exposed to elevated CO_2 concentrations on an estuarine marsh. Oecologia 78: 20–26.

Curtis, P. S., L. M. Balduman, B. G. Drake & D. F. Whigham, 1990. Elevated atmospheric CO_2 effects on below-ground processes in C_3 and C_4 estuarine marsh communities. Ecology 71: 2001–2006.

Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Chagnon, R. R. Karl & L. O. Mearns, 2000. Climate extremes: observation, modeling and impacts. Science 289: 2068–2074.

Garbutt, K., W. E. Williams & F. A. Bazzaz, 1990. Analysis of the differential response of five annuals to elevated CO_2 during growth. Ecology 71: 1185–1194.

Gaudet, J. J., 1977. Uptake, accumulation and loss of nutrients by papyrus in tropical swamps. Ecology 58: 415–422.

Ghosn, D., I. N. Vogiatzakis, G. Kazakis, E. Dimitriou, E. Moussoulis, V. Maliaka & I. Zacharias, 2010. Ecological changes in the highest temporary pond of western Crete (Greece): past, present and future. Hydrobiologia 648: 3–18.

Hickler, T., S. Fronzek, M. B. Araujo, O. Schweiger, W. Thuiller & M. T. Sykes, 2009. An ecosystem model-based estimate of changes in water availability differs from water proxies that are commonly used in species distribution models. Global Ecology and Biogeography 18: 304–313.

Howard, R. J. & I. A. Mendelssohn, 2000. Structure and composition of oligohaline marsh plant communities exposed to salinity pulses. Aquatic Botany 68: 142–164.

Huxman, T. E., E. P. Hamerlynck, D. N. Jordan, K. J. Salsman & S. D. Smith, 1998. The effects of parental CO_2 environment on seed quality and subsequent seedling performance in *Bromus rubens*. Oecologia 114: 202–208.

IPCC, Intergovernmental Panel on Climate Change, 2007. Climate Change 2007: The Synthesis Report. Cambridge University Press, Cambridge, UK.

Jarvis, J. C. & K. A. Moore, 2010. The role of seedlings and seed bank viability in the recovery of Chesapeake Bay, USA, *Zostera marina* populations following a large-scale decline. Hydrobiologia 649: 55–68.

Junk, W. J., 1970. Investigations on the ecology and production biology of the 'floating meadows' (Paspalo-Echinochloetum)

on the Middle Amazon. I. The floating vegetation and its ecology. Amazonia 2: 449–495.

Körner, C., 2006. Plant CO_2 responses: an issue of definition, time and resource supply. New Phytologist 172: 393–411.

Krinner, G., N. Viovy, N. de Noblet-Ducoudré, J. Ogée, J. Polcher, P. Friedlingstein, P. Philippe Ciais, S. Sitch & I. C. Colin Prentice, 2005. A dynamic global vegetation model for studies of the coupled atmosphere–biosphere system. Global Biogeochemical Cycles 19: GB1015.

Langley, J. A. & J. P. Megonigal, 2010. Ecosystem response to elevated CO_2 levels limited by nitrogen-induced plant species shift. Nature 466: 96–99.

Langley, J. A., K. L. McKee, D. R. Cahoon, J. A. Cherry & J. P. Megonigal, 2009. Elevated CO_2 stimulates marsh elevation gain, counterbalancing sea-level rise. PNAS 106: 6182–6186.

Leck, M. A., 1989. Wetland seed banks. In Leck, M. A., V. T. Parker & R. Simpson (eds), Ecology of Soil Seed Banks. Academic Press, New York: 283–305.

Lenssen, G. M., J. Lamers, M. Stroetenga & J. Rozema, 1993. Interactive effects of atmospheric CO_2 enrichment, salinity and flooding on growth of C_3 (*Elymus athericus*) and C_4 (*Spartina anglica*) salt marsh species. Vegetatio 104(105): 379–388.

Mayle, F. E., D. J. Beerling, W. D. Gosling & M. B. Bush, 2004. Responses of Amazonian ecosystems to climatic and atmospheric carbon dioxide changes since the last glacial maximum. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 359: 499–514.

McKee, K. L. & J. E. Rooth, 2008. Where temperate meets tropical: multifactorial effects of elevated CO_2, nitrogen enrichment, and competition on a mangrove–salt marsh community. Global Change Biology 14: 971–984.

Megonigal, J. P., C. D. Vann & A. A. Wolf, 2005. Flooding constraints on tree (*Taxodium distichum*) and herb growth responses to elevated CO_2. Wetlands 25: 430–438.

Michener, W. K., E. R. Blood, K. L. Bildstein, M. M. Brinson & L. R. Gardner, 1997. Climate change, hurricanes and tropical storms, and rising sea level in coastal wetlands. Ecological Applications 7: 770–801.

Middleton, B. A., 1999. Succession and herbivory in monsoonal wetlands. Wetland Ecology and Management 6: 189–202.

Middleton, B. A., 2009a. Regeneration of coastal marsh vegetation impacted by Hurricanes Katrina and Rita. Wetlands 29: 54–65.

Middleton, B. A., 2009b. Effects of Hurricane Katrina on the forest structure of baldcypress swamps of the Gulf Coast. Wetlands 29: 80–87.

Middleton, B. A., 2009c. Effects of Hurricane Katrina tree regeneration in baldcypress swamps of the Gulf Coast. Wetlands 29: 135–141.

Middleton, B. A., 2009d. Regeneration potential of baldcypress (*Taxodium distichum*) swamps and climate change. Plant Ecology 202: 257–274.

Middleton, B. A., A. G. van der Valk, R. L. Williams, D. J. Mason & C. B. Davis, 1991. Vegetation dynamics and seed banks of a monsoonal wetland overgrown with *Paspalum distichum* in northern India. Aquatic Botany 40: 239–259.

Migahid, A. M., 1947. An ecological survey of the 'Sudd' swamp of the Upper Nile. Proceedings of the Egyptian Academy of Sciences 7: 57–86.

Nielsen, D. L., M. A. Brock, K. Crosslé, K. Harris, M. Healey & I. Jarosinski, 2003. The effects of salinity of aquatic plant germination and zooplankton hatching from two wetland sediments. Freshwater Biology 48: 2214–2223.

Osmond, C. B., O. Björkman & D. J. Anderson, 1980. Physiological Processes in Plant Ecology. Springer-Verlag, Berlin.

Platt, W. J. & J. J. Connell, 2003. Natural disturbances and directional replacement of species. Ecological Monographs 73: 507–522.

Poorter, H. & M.-L. Navas, 2003. Plant growth and competition at elevated CO_2: on winners, losers and functional groups. New Phytologist 157: 175–198.

Rasse, D. P., J.-H. Li & B. G. Drake, 2003. Carbon dioxide assimilation by a wetland sedge canopy exposed to ambient and elevated CO_2: measurements and model analysis. Functional Ecology 17: 222–230.

Rasse, D. P., G. Peresta & B. G. Drake, 2005. Seventeen years of elevated CO_2 exposure in a Chesapeake Bay Wetland: sustained but contrasting responses of plant growth and CO_2 uptake. Global Change Biology 11: 369–377.

Rozema, J., F. Dorel, R. Janissen, G. Lenssen, R. Broekman, W. Arp & B. G. Drake, 1991. Effect of elevated atmospheric CO_2 on growth, photosynthesis and water relations of salt marsh grass species. Aquatic Botany 39: 45–55.

Russell, R. J., 1942. Flotant. Geographical Review 32: 74–98.

Sage, R. F. & D. S. Kubien, 2003. Quo vadis C_4? An ecophysiological perspective on global change and the future of C_4 plants. Photosynthesis Research 77: 209–225.

SAS JMP, 2007. JMP SAS, Version 7.0.1. Statistical Analysis System, Cary, NC.

Sasser, C. E., J. G. Gosselink, E. M. Swenson, C. M. Swarzenski & N. C. Leibowitz, 1996. Vegetation, substrate and hydrology in floating marshes in the Mississippi River delta plain wetlands, USA. Vegetatio 122: 129–142.

Scheiner, S. & J. Gurevich (eds), 1993. Design and Analysis of Ecological Experiments. CRC Press, Boca Raton, FL.

Steinger, T., R. Gall & B. Schmid, 2000. Maternal and direct effects of elevated CO_2 on seed provisioning, germination and seedling growth in *Bromus erectus*. Oecologia 123: 475–480.

Swarzenski, C. M. & E. M. Swenson, 1994. Effect of mat movement on soil salinity fluctuations in some coastal Louisiana marshes. Estuarine, Coastal and Shelf Science 39: 143–156.

Swarzenski, C. M., E. M. Swenson, C. E. Sasser & J. G. Gosselink, 1991. Marsh mat flotation in the Louisiana Delta Plain. Journal of Ecology 79: 999–1011.

Thompson, K., 1985. Emergent plants of permanent and seasonally flooded wetlands. In Denny, P. (ed.), The Ecology and Management of African Wetlands. Dr. W. Junk, The Hague, Netherlands: 43–107.

U.S. Department of Agriculture, 2011a. Integrated Taxonomic Information System. USDA, Washington, DC. www.itis.usda.gov/index.html.

U.S. Department of Agriculture, 2011b. Plants Database. USDA, Washington, DC. http://plants.usda.gov/java/.

Wand, S. J. E., G. F. Midgley, M. H. Jones & P. S. Curtis, 1999. Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. Global Change Biology 5: 723–741.

Webster, P. J., G. J. Holland, J. A. Curry & H.-R. Chang, 2005. Changes in tropical cyclone number, duration, and intensity in a warming environment. Science 309: 1844–1846.

Woodward, F. I., G. B. Thompson & I. F. McKee, 1991. The effects of elevated concentration of carbon dioxide on individual plants, communities and ecosystems. Annals of Botany 67: 23–38.

Wulff, R. D. & H. M. Alexander, 1985. Intraspecific variation in the response to CO_2 enrichment in seeds and seedlings of *Plantago lanceolata* L. Oecologia 66: 458–460.

Ziska, L. H., B. G. Drake & S. Chamberlain, 1990. Long-term photosynthetic response in single leaves of a C_3 and C_4 salt marsh species grown at elevated atmospheric CO_2 in situ. Oecologia 83: 469–473.