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Does sulphate enrichment promote the expansion of *Typha domingensis* (cattail) in the Florida Everglades?[1]

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SUMMARY

1. The expansion of *Typha domingensis* into areas once dominated by *Cladium jamaicense* in the Florida Everglades has been attributed to altered hydrology and phosphorus enrichment, although increased concentrations of sulphate and phosphorus often coincide. The potential importance of hydrogen sulphide produced from sulphate in the expansion of *Typha* has received little attention. The present study aimed to quantify the comparative growth and photosynthetic responses of *Cladium* and *Typha* to sulphate/sulphide.
2. Laboratory experiments showed that *Cladium* is less tolerant of sulphide than *Typha*. *Cladium* was adversely affected at sulphide concentrations of approximately 0.22 mM, while *Typha* continued to grow well and appeared healthy up to 0.69 mM sulphide.
3. Experiments in field mesocosms provided strong support for species-specific differences in physiology and growth. Regardless of interstitial sulphide concentrations attained, *Typha* grew faster and had a higher photosynthetic capacity than *Cladium*. However, sulphide concentrations in the mesocosms reached only 0.18 mM which, based on the hydroponic study, was insufficient to affect the growth or photosynthetic responses of either species. Nevertheless, the upper range of sulphide (0.25–0.375 mM) in Everglades' soil is high enough, based on our results, to impact *Cladium* but not *Typha*.
4. This research supports the hypothesis that sulphide accumulation could affect plant species differentially and modify species composition. Consequently, the role of sulphate loading should be considered, in conjunction with hydroperiod, phosphorus availability and disturbances, in developing future management plans for the Everglades.

Keywords: *Cladium*, Everglades, growth, sulphide toxicity, *Typha*

Introduction

The Florida Everglades in the United States, among the world's best-known oligotrophic wetland systems, has undergone dramatic changes since the early 1900s (Maltby & Dugan, 1994). The ''River of Grass'' once extending from Lake Okeechobee to Florida Bay has been hydrologically fragmented, resulting in an alteration of the natural hydroperiod of the Everglades (Worth, 1988; Myers & Ewel, 1991; Light & Dineen, 1994). Further, the runoff of nutrients, particularly phosphorus (P), from fertilised agricultural fields adjacent to the Everglades has caused site-specific eutrophication (Belanger, Scheidt & Platko, 1989; Davis, 1989, 1994; Nearhoof, 1992). Concurrent with hydroperiod and nutrient alterations, the Everglades system has been influenced by introduced exotic plants (Bodle, Ferriter & Thayer, 1994), increased frequency of fire (Bancroft, 1976; Steward & Ornes, 1983; Gunderson & Snyder, 1994), drought and other disturbances (Hofstetter, 1976; Swift & Nicholas, 1987; Davis & Ogden, 1994).

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In particular, two impacts to the Everglades, (1) the expansion of *Typha domingensis* Pers. (cattail) into areas previously occupied by the historically dominant *Cladium jamaicense* Crantz (sawgrass) and (2) mercury contamination of the Everglades biota, are of primary concern and have received considerable attention. The *Typha* expansion has been attributed to P enrichment from agricultural runoff and to altered hydroperiod resulting from water management (Davis & Ogden, 1994; Busch, Loftus & Bass, 1998; Miao & DeBusk, 1999). The mercury problem (Arfstrom, MacFarlane & Jones, 2000) is exacerbated by increased sulphate concentrations and by microbial reduction of sulphate (Gilmour *et al*., 2007) that results in the methylation of natural and anthropogenic inputs of mercury, making it more bioavailable and toxic. Sulphate concentrations, which are highest in canals that intersect the Everglades Agricultural Area (Bates *et al*., 2002), are above background in 60% of the freshwater Everglades (Axelrad *et al*., 2007). Sulphate concentrations tend to show a north–south gradient in surface water, ranging from 60 mg L^{-1} in the northern Everglades to <1 mg L^{-1} in the south, far from canal discharge points (Orem, 2005). Interestingly, these two apparently independent perturbations, *Typha* expansion and mercury contamination, may actually be closely associated.

Sulphate enrichment often leads to eutrophication and has caused serious problems in freshwater wetlands (Lamers, Tomassen & Roelofs, 1998; Lamers *et al*., 2002). A major end product of microbial sulphate reduction is hydrogen sulphide (H_2S), and concentrations of H_2S range up to 0.25–0.375 mM in areas of the Everglades with high surface water sulphate concentrations resulting from canal discharge (W. Orem, unpubl. data). Sulphide is a known plant toxin (Mendelssohn & McKee, 1988; Koch & Mendelssohn, 1989), which can inhibit oxygen uptake by macrophyte roots, react with metals to make them unavailable for plant uptake, inhibit root energy production and the active uptake of plant nutrients, and negatively affect other aspects of plant metabolism (Mendelssohn & Morris, 2000).

Although most wetland plants can detoxify H_2S in the oxidised rhizosphere surrounding the roots (Mendelssohn & Postek, 1982; McKee, Mendelssohn & Hester, 1988), the extent of detoxification varies among species with the ability to create an oxidised rhizosphere (McKee *et al*., 1988; Chabbi, McKee & Mendelssohn, 2000). It has been clearly demonstrated that *Typha* has a significantly greater ability to transport oxygen to its roots and to produce a more extensive oxidised rhizosphere than *Cladium* (Chabbi *et al*., 2000; Sorrell *et al*., 2000). Hence, *Typha* may be more tolerant to H_2S production than *Cladium* because of the formers greater ability to generate an oxidised rhizosphere and, thereby, to oxidise toxic H_2S to non-toxic sulphate. As a result, *Typha* dominance in areas of the Everglades receiving nutrient input may be due, at least in part, to the greater tolerance of sulphide in *Typha*. In fact, increased sulphate has been observed in the northern Everglades where *Cladium* has been replaced by the invasion of *Typha* (Orem, 2007).

Therefore, both mercury accumulation and *Typha* expansion into native *Cladium* marshes may be a response to sulphate enrichment and its subsequent reduction to H_2S. However, we have no direct evidence that *Typha* is indeed more tolerant to sulphide than *Cladium* and that sulphate loading to the Everglades will generate concentrations sufficient to limit the growth of either species. The present paper addresses these information gaps. Specifically, we asked two questions: (1) Is *Cladium* more sensitive to sulphide than *Typha*? and (2) Can species-specific differences in sulphide tolerance help to explain the expansion of *Typha* into areas previously dominated by *Cladium?*

Methods

Two approaches were taken to investigate the effect of sulphate/sulphide on *Typha domingensis* and *Cladium jamaicence*. A hydroponic experiment in an environmentally controlled growth chamber tested the growth responses of *Typha* and *Cladium* to different concentrations of sulphide compared with aerated and hypoxic controls. A 3-year field mesocosm experiment was also conducted to evaluate the effect of sulphate loading on the growth responses of *Typha* and *Cladium* in the Everglades.

Experiment 1 – effect of sulphide on plant growth responses in hydroponic culture

Plant materials and pre-treatment. Seeds of *Cladium jamaicence* and *Typha domingensis* were collected from Water Conservation Area (WCA)-3A in the Florida

Everglades, U.S.A. in 2005. They were germinated on 16 November 2005 and 9 January 2006, respectively, with commercial potting mixture (Jiffy-Mix Plain; Jiffy Products of America, Chicago, IL, U.S.A.) as the substratum in a growth chamber (Environmental Growth Chambers, Model M-75 equipped with Sunbrella fixtures, Chagrin Falls, OH, U.S.A.). Environmental conditions were 14 : 10 h, 25 : 10 °C day : night photo- and thermo-period with photosynthetically active radiation (PAR) of 300 μmol m^{-2} s^{-1} on the soil surface. These conditions have previously been found to stimulate their germination (Lorenzen *et al.*, 2000). Seedlings about 15-cm tall were transferred to plastic cups (one seeding per cup) filled with the same potting soil.

Two weeks before the experiment began, seedlings were transferred to a hydroponic nursery system within the environmental growth chamber. The chamber was operated with a 14 : 10 h photoperiod and a 28 : 20 °C thermoperiod. The light intensity was *c.* 1000 μmol m^{-2} s^{-1} PAR at shoot base level. The nursery unit consisted of an 11-L hydroponic container (Rubbermaid Roughtote; Rubbermaid Home Products, Wooster, OH, U.S.A.) with six holes on the lid to pre-condition the plants to hydroponic conditions. The growth medium was a modified 1/2-strength Hoagland's solution, with iron in the form of Fe-ethylenediaminetetraacetic acid (EDTA) to prevent iron sulphide precipitation. The chemical components and their concentrations were $MgSO_4 \cdot 7H_2O$ (1 mM), $CaCl_2 \cdot 2H_2O$ (2.5 mM), KCl (2.5 mM), NH_4Cl (0.4 mM), KH_2PO_4 (0.3 mM), H_3BO_3 (22.5 μM), $MnCl \cdot 4H_2O$ (4.5 μM), $ZnSO_4 \cdot 7H_2O$ (0.5 μM), $CuSO_4 \cdot 5H_2O$ (0.15 μM), MoO_3 (0.07 μM) and Fe-EDTA (45 μM). The solution was buffered with $CaCO_3$ and pH was adjusted to 6.5. The nutrient solution was renewed once in the middle of the pre-treatment.

Experimental procedure. Plants uniform in size (approximately 55-cm tall) and appearance were chosen for the experiments. Experiments on *Typha* and *Cladium* were conducted sequentially because of the time-consuming process of culture medium replenishment. Study plants were exposed to six treatments, with six replicates per treatment per species in April 2006. Treatments included: (1) aeration, medium was bubbled with air continuously, (2) hypoxia, medium was bubbled with nitrogen gas for 45 min before introducing the plants and each day when nutrient solutions were renewed, (3) hypoxia with 0.25 mM sulphide, (4) hypoxia with 0.5 mM sulphide, (5) hypoxia with 0.75 mM sulphide and (6) hypoxia with 1 mM sulphide. Actual concentrations of sulphide achieved in the four hypoxic sulphide treatments (treatments 3–6) were 0.22, 0.46, 0.69 and 0.92 mM, respectively, as determined by standardisation with lead perchlorate titration.

The experiments were conducted in sealed Erlenmeyer flasks (250 mL). Plants were fixed into position using a non-toxic silicone caulk (GE translucent RTV 128 Silicone Rubber Adhesive Sealant; GE Silicones, Waterford, NY, U.S.A.) through holes in the rubber stoppers. Study plants were exposed to the sulphide treatments for 7 days. The culture medium was changed daily for all groups because concentrations of sulphide decreased as sulphide escaped from the media as H_2S gas and could be re-oxidised to sulphate because of radial oxygen loss from the plants. Armstrong, Afreen-Zobayed & Armstrong (1996), Smolders & Roelofs (1996) and Seliskar *et al.* (2004) also found frequent medium changes to be best for maintaining sulphide concentrations. When sulphide and nutrient solutions were renewed, the roots were kept under a nitrogen environment by allowing nitrogen gas to displace the nutrient solution after which new nutrient solution was added while nitrogen flowed through the rooting environment. Each targeted sulphide concentration was achieved by adding 50 mM $Na_2S \cdot 9H_2O$ to the solution via syringe; the total volume of the rooting solution was 250 mL.

A 3 mL sample of the medium was extracted daily by syringe from each flask just before medium replenishment and immediately injected into an equal volume of antioxidant buffer (Sulphide Anti-oxidant Buffer II; Thermo, Beverly, MA, U.S.A.). Sulphide concentrations were measured using an Orion 3 Star portable meter and an Orion Sure-Flow Combination Silver/Sulphide electrode (Thermo). A standard curve was generated from a series of dilutions of $Na_2S \cdot 9H_2O$ prepared with antioxidant buffer.

Redox potential (Eh) was measured daily for the solutions just after they were renewed. Platinum-tipped electrodes were inserted into the medium. Eh was recorded using an OAKTON pH/mV/°C 10 series meter and a calomel reference electrode (Corning, NY, U.S.A.). Eh values were corrected by adding the potential of the calomel reference electrode (244 mV) to the millivolt readings.

Experiment 2 – effect of sulphate dosing on plant growth responses in the Everglades

The mesocosm experiment was conducted in the central Everglades near the middle of WCA-3A (25°58.026′N, 80°39.989′W). High rates of methylmercury production have been measured previously in this region, but have declined substantially in recent years along with a reduction in sulphate concentration in surface waters (Axelrad *et al.*, 2005).

The mesocosms, which consisted of 1.0 m diameter plastic rings that were inserted approximately 30 cm into the soil, were installed in adjacent *Typha* and *Cladium* dominated sites and allowed to equilibrate for a period of 2 months. Holes in the sides of the mesocosms allowed exchange of water with the outside during this equilibration period. The experimental design was a randomised complete block with repeated measures analysis over time. Within each block, five treatments were randomly assigned: (1) ambient, 0 mg L^{-1} added sulphate and no mesocosms. PVC pipes were used to delineate a 2 m × 2 m plot; (2) control, 0 mg L^{-1} added sulphate in the mesocosms; (3) low, 20–40 mg L^{-1} added sulphate in the mesocosms; (4) medium, 50–100 mg L^{-1} added sulphate in the mesocosms and (5) high, 100–200 mg L^{-1} added sulphate in the mesocosms. These treatment levels were replicated in three blocks for a total of 30 experimental units (two species × five sulphate loadings × three blocks). Sulphate was applied 16 times, on 18 November 2003 (day 1), 19 April (day 154), 26 July (day 252), 16 August (day 273), 28 September (day 316) and 27 December (day 406) 2004, 29 March (day 498), 24 May (day 554), 1 July (day 592), 14 September (day 667) and 7 December (day 751) 2005, 24 January (day 799), 8 March (day 842), 14 April (day 879), 24 August (day 1011) and 4 November (day 1083) 2006. From 14 September 2005, the sulphate doses were doubled (40, 100 and 200 mg L^{-1} for low, medium and high sulphate treatment-levels respectively) to approach growth-limiting concentrations more closely.

Plant measurements

Net photosynthesis rate. Measurements of net photosynthesis rate (P_n, μmol CO_2 m^{-2} s^{-1}) were taken on the second or third fully developed and healthy leaf from the centre for all study plants using a portable infrared gas analysis system (LI-6400; LiCor, Lincoln, NE, U.S.A.) between 08 : 00 and 12 : 00 h before the medium changes on days 4 and 7 in experiment 1. P_n was also recorded on three to five leaves per experimental unit (mesocosm or ambient plot) in experiment 2. One measurement was taken per leaf, at each sampling time including November 2003, March and November 2004, March and August 2005, and March, August and December 2006.

Leaf elongation rate. In experiment 1, leaf elongation rate (cm day^{-1}) was determined as the change in the distance between the tip of the second youngest leaf to a fixed point over a period of 3 days. In experiment 2, it was determined on three leaves that were approximately one third expanded in each experimental unit, at the same sampling times as for P_n.

Light response and CO_2 response curves. In experiment 2, assimilation versus light intensity curves, as well as assimilation versus CO_2 curves, were determined with the photosynthetic system described previously. One light response curve and one CO_2 response curve were generated on the third fully developed and healthy leaf from the centre for each experimental unit for both species in March and November 2004 and March and August 2005. However, not all response curves were generated in March, August and December 2006. Measurements were taken at nine light intensities: 2000, 1500, 1000, 500, 200, 100, 50, 20 and 0 μmol m^{-2} s^{-1} and 10 CO_2 concentrations: 400, 300, 200, 100, 50, 400, 400, 600, 800 and 1000 μmol mol^{-1}. Photosynthetic parameters were calculated by fitting the equations of Farquhar, von Caemmerer & Berry (1980), using the software Photosyn Assistant (version 1.1; Dundee Scientific, Scotland, U.K.). Based on light response curves, respiration (RESP), apparent quantum efficiency (AQE), maximum photosynthesis (A_{max}), light compensation point (LCP) and light saturation estimate (LSE) were determined. Based on CO_2 response curves, maximum rate of caboxylation by Rubisco (Vc_{max}) and PAR-saturated rate of electron transport (J_{max}) were calculated.

Chlorophyll fluorescence. In experiment 2, chlorophyll fluorescence was determined on intact leaves with the previously described LI-6400 and a chlorophyll fluorescence leaf chamber in March 2004 and March and August 2005. Prior to measurement, one fully developed and healthy leaf in each experimental unit

(mesocosm or ambient plot) was chosen and dark adapted for at least 30 min using a dark-adapting clip especially designed for the LI-6400. Maximum quantum efficiency of Photosystem II (PSII) reaction centres (F_v/F_m) was recorded: $F_v/F_m = (F_m - F_o)/F_m$, where F_m is the maximal fluorescence achieved with a saturating flash and F_o is the dark fluorescence yield.

Turnover rate. In experiment 2, four to five individual leaves were tagged in each experimental unit on 17 November 2004, and then checked for mortality on 6 February and 9 March 2005. Mortality was again recorded on 7 August 2005 for leaves tagged on 10 March 2005. Similarly, leaves tagged on 25 March 2006 were checked on 15 August 2006. Leaves marked on 15 August 2006 were used to determine the turnover rate on 7 December 2006. Turnover rate (% day^{-1}) = number of dead leaves (completely brown)/number of tagged leaves × 100% day^{-1}.

Plant cover. In experiment 2, plant cover for live and dead *Typha* in *Typha* sites and *Cladium* in *Cladium* sites (Cov_{live} and Cov_{dead} respectively) in each experimental unit was recorded, at the same sampling times as for P_n. Cover was placed in one of seven categories by eye. Cover categories were converted into percentage data using class midpoints (Table 1).

Biomass. Plants were harvested at the end of the experiment 1 (day 7). Each plant was then sectioned into live and dead leaves and roots, shoot base and rhizomes, and dried in an oven at 65 °C to constant mass (g). Total biomass (live and dead) and total live biomass were then calculated. The ratio of live and dead root-supported biomass (leaves, shoot base and rhizomes) to live and dead root biomass (total RSB/RB ratio) as well as live RSB/RB ratio [(live leaf, shoot base and rhizome biomass)/live root biomass ratio] were also calculated. All biomass was measured and calculated on a dry mass basis.

At the end of the experiment 2 (December 2006), soil samples were taken with a Model-A Russian Peat Borer (50.8 cm in length and 5.08 cm in diameter; Aquatic Research Instruments, Lemhi, ID, U.S.A.) for all experimental plots (one sample per plot). Collected plant materials were separated into floating plant materials (primarily living roots), plant debris and rhizomes. Their biomasses were then determined based on the dry mass (g) per metresquare of marsh surface area after they were dried at 65 °C.

Tissue nutrients. In experiment 2, leaf tissue of both species was collected in all experimental units in March and December 2006, immediately frozen with dry ice, and kept frozen until dried in the laboratory at 65 °C. The tissue was then ground with a ball mill (MM200; Retsch, Newtown, PA, U.S.A.), and sulphur (S), nitrogen (N) and carbon (C) concentrations (%) were determined using an Elemental Combustion System (ECS 4010; Costech Analytical Technologies, Valencia, CA, U.S.A.).

Table 1 Cover classes used to estimate species dominance

Cover class	Range of cover (%)	Class midpoints (%)
6	95–100	97.5
5	75–95	85.0
4	50–75	62.5
3	25–50	37.5
2	5–25	15.0
1	1–5	3.0
0	0.01–1	0.5
–	0	0

Data analyses

Experiment 1 followed a completely randomised design for both species. Data for P_n and leaf elongation rate from days 4 and 7 were pooled. One-way ANOVA (SAS 9.1) was used to test the differences in means of plant response variables between six treatments for *Cladium* and *Typha* separately. Fisher's protected least significant difference (LSD) test was used to examine all pair-wise group differences. Principal component analysis was performed to combine net photosynthetic and leaf elongation responses into one growth variable (i.e., component 1) for each individual plant.

Species, sulphate loading, time and their interactions were fixed effects within a randomised complete block design in experiment 2. Time was a repeated measure and block was a random effect. Data for all photosynthetic parameters generated by light and CO_2 response curves were pooled across sampling time. The effects of sulphate dosing and species on those parameters were tested by one-way ANOVA and *t*-test separately. Tukey's test was used for pair-wise comparisons.

Data were tested for normality and homogeneity of variances to meet the ANOVA assumptions and transformed if necessary. Differences were considered significant at $P < 0.05$.

Results

Experiment 1 – effect of sulphide on plant growth responses in hydroponic culture

Sulphide concentrations. Sulphide concentrations in aerated and 0 mM flasks were below detection limits (<1 p.p.b.). Average sulphide concentrations measured in the culture solution for *Typha* were lower than for *Cladium* under all hypoxic sulphide treatments (Table 2). Sulphide concentrations in the culture solution decreased from target values to those presented in Table 2 during each 24-h exposure period of the 7-day experiment.

Solution Eh. Average Eh in the aerated treatment remained >400 mV for both species, indicating oxic conditions. Eh was moderately reduced in media bubbled with nitrogen gas (Table 2). As sulphide concentration increased in the hypoxic culture solution, Eh decreased further. Overall, the Eh of the *Typha* nutrient solution was more positive (less reduced) than that of *Cladium* (Table 2).

Plant measurements. The P_n and growth were comparable between the aerated and anoxic treatments (0 mM sulphide) for both *Typha* and *Cladium* (Table 3; Figures 1–3). However, plants were adversely affected when sulphide was added to the culture media. As compared with the hypoxic control (0 mM sulphide), P_n for *Typha* was adversely impacted at a sulphide concentration of 0.92 mM, while it took only half of that sulphide concentration to affect *Cladium* significantly (Fig. 1a,c). Leaf elongation rate for *Typha* was significantly affected at a sulphide concentration of 0.69 mM, which was three times higher than for *Cladium* (Fig. 1b,d). Principal component analysis was performed to combine net photosynthetic and leaf elongation responses into one growth variable, which explained 88.5% of the variance. This growth variable for *Cladium* was negatively affected at a sulphide concentration of 0.22 mM, 67% lower than for *Typha*.

Table 3 ANOVA results (*F*-value, d.f. and *P*-value) for the effect of sulphide on photosynthetic and growth parameters of *Cladium* and *Typha*

		Cladium		*Typha*	
Variables	d.f.	*F*-value	*P*-value	*F*-value	*P*-value
P_n	5	6.51	**<0.0001**	2.40	**0.0468**
Leaf elongation rate	5	7.14	**<0.0001**	4.66	**0.0011**
Leaf biomass					
Live	5	3.66	**0.0105**	1.19	0.3398
Dead	5	9.66	**<0.0001**	3.94	**0.0076**
Total	5	0.83	0.5386	1.10	0.3802
Root biomass					
Live	5	3.00	**0.0260**	0.30	0.9063
Dead	5	5.00	**0.0012**	3.77	**0.0094**
Total	5	0.67	0.6508	0.27	0.9279
Shoot base biomass	5	0.96	0.4548	0.87	0.5146
Rhizome biomass	5	2.86	**0.0313**	2.32	0.0868
Total biomass					
Live + dead	5	1.08	0.3891	0.75	0.5922
Total live biomass					
Live	5	4.45	**0.0038**	0.82	0.5445
Total RSB/RB ratio					
Live + dead	5	0.81	0.5519	1.26	0.3085
Live RSB/RB ratio					
Live	5	0.36	0.8727	1.26	0.3091

Those parameters included net photosynthesis rate (P_n), leaf elongation rate, shoot base and rhizome biomass, live, dead and total leaf and root biomass, total biomass, total live biomass, total root supported biomass to root biomass ratio (total RSB/RB ratio), and live RSB/RB ratio.
Values presented bold are significant effects at $P < 0.05$.

Table 2 Average sulphide concentration (mM) and redox potential (Eh) in six treatments (aerated, hypoxic with 0 mM sulphide, and hypoxic with sulphide concentrations of 0.22, 0.46, 0.69 and 0.92 mM) measured after each 24-hour exposure during the 7-day experiment for *Typha* and *Cladium*

Measurement	Species	Aerated	0	0.22	0.46	0.69	0.92
Sulphide	*Typha*	*	*	0.02 ± 0.03	0.07 ± 0.06	0.16 ± 0.03	0.28 ± 0.08
	Cladium	*	*	0.15 ± 0.04	0.27 ± 0.07	0.36 ± 0.09	0.45 ± 0.07
Eh	*Typha*	434 ± 12	210 ± 7	184 ± 4	88 ± 9	23 ± 6	−6 ± 3
	Cladium	445 ± 10	183 ± 9	101 ± 12	61 ± 9	−5 ± 7	−21 ± 4

Each value represents the mean of concentration and Eh for 36 replications (six flasks × six times) ± SE.
*Sulphide concentrations in aerated and 0 mM flasks were below detection limits (<1 p.p.b.).

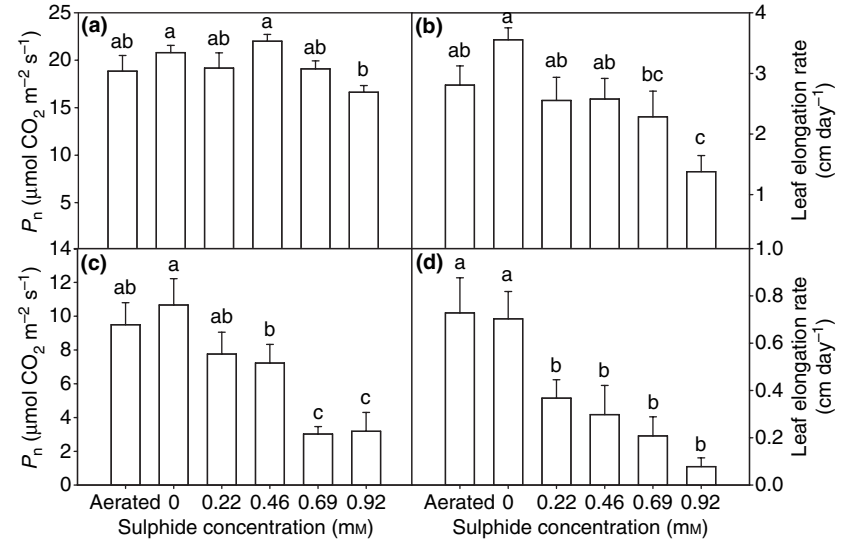

Fig. 1 Net photosynthesis rate (P_n) and leaf elongation rate for *Typha* (a, b) and *Cladium* (c, d) in response to six treatment-levels (aerated, hypoxic with 0 mM sulphide, and hypoxic with sulphide concentrations of 0.22, 0.46, 0.69 and 0.92 mM). Each value is the mean ± SE. Different letters indicate significant differences across treatments for each parameter within each species identified with Fisher's protested least significant difference test.

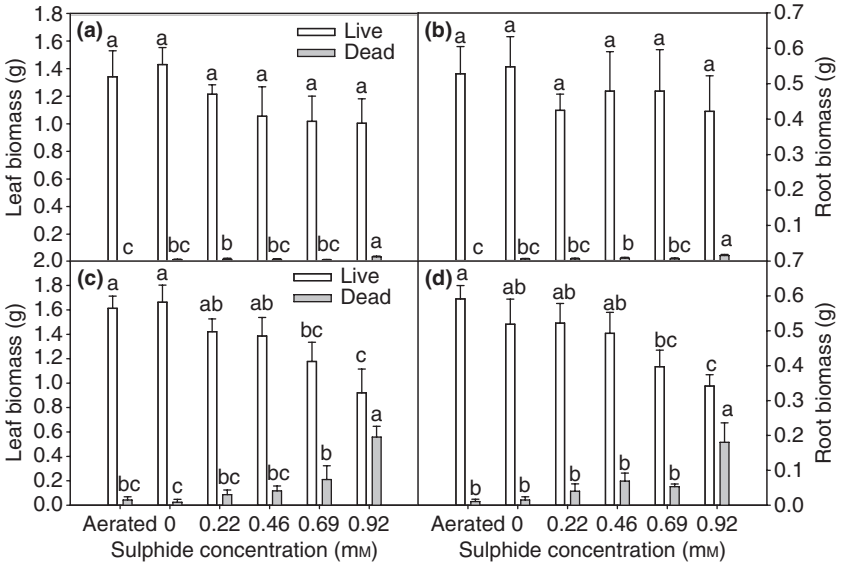

Fig. 2 Biomass of live and dead leaves and roots for *Typha* (a, b) and *Cladium* (c, d) in response to six treatment-levels (aerated, hypoxic with 0 mM sulphide, and hypoxic with sulphide concentrations of 0.22, 0.46, 0.69 and 0.92 mM). Each value is the mean ± SE. Different letters indicate significant differences across treatments for each parameter within each species identified with Fisher's protested least significant difference test.

Live leaf biomass for *Typha* did not significantly differ among the treatments (Table 3; Fig. 2a). Dead leaf biomass for *Typha* increased at 0.92 mM sulphide. In contrast, 0.69 mM sulphide significantly reduced live leaf biomass and increased dead leaf biomass in *Cladium* (Table 3; Fig. 2a,c). Root biomass demonstrated a similar pattern as for leaf biomass for the two species under all sulphide concentrations, except that both live and dead root biomass were significantly affected at 0.92 mM sulphide for *Cladium* (Table 3; Fig. 2b,d).

Differences between *Typha* and *Cladium* among the six treatments were also found in final rhizome biomass and total live biomass. Both parameters were reduced at 0.69 mM sulphide for *Cladium*, while they were unaffected for *Typha* (Table 3; Fig. 3). No effects of sulphide on total leaf and root biomass, shoot base biomass, total plant biomass, total RSB/RB ratio and live RSB/RB ratio were detected for either species (Table 3).

Experiment 2 – effect of sulphate dosing on plant growth responses in the Everglades

Net photosynthesis rate and leaf elongation rate. The effect of plant species on P_n was dependent on sampling time (significant species × time interaction; Table 4). P_n for *Typha* was higher than that for *Cladium* on all sampling dates except August 2006, when they did not differ significantly. In addition, P_n for both *Typha* and *Cladium* changed significantly over time.

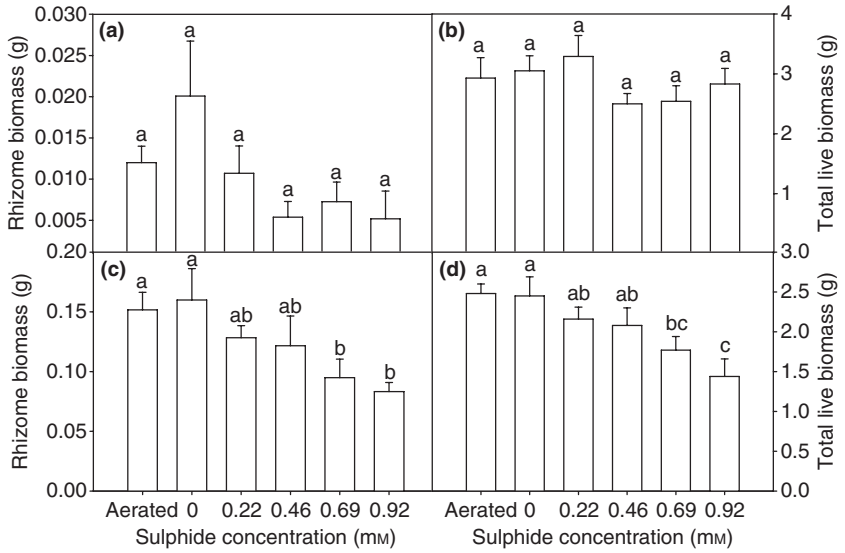

Fig. 3 Rhizome biomass and total live biomass for *Typha* (a, b) and *Cladium* (c, d) in response to six treatment-levels (aerated, hypoxic with 0 mM sulphide, and hypoxic with sulphide concentrations of 0.22, 0.46, 0.69 and 0.92 mM). Each value is the mean ± SE. Different letters indicate significant differences across treatments for each parameter within each species identified with Fisher's protested least significant difference test.

Table 4 Analysis of variance for the main effects and their interactions for responses of *Typha* and *Cladium* to species, sulphate dosing and sampling time

Response	Species	Dosing	Time	Species* dosing	Species* time	Dosing* time	Species* dosing*time
P_n	0.0005	0.0510	<0.0001	0.1522	**<0.0001**	0.4957	0.5627
Elongation	<0.0001	0.0352	<0.0001	**0.0104**	**0.0004**	**<0.0001**	0.2020
F_v/F_m	**<0.0001**	**0.0058**	**<0.0001**	0.0779	0.2852	0.3515	0.2975
Turnover	<0.0001	0.3593	<0.0001	**0.0256**	**<0.0001**	0.5279	0.3466
Cov_{live}	0.0012	0.0982	<0.0001	0.1396	**<0.0001**	0.3464	0.6237
Cov_{dead}	0.0027	0.4862	<0.0001	0.9491	**<0.0001**	0.0699	0.4003
Live root	0.0031	0.0290		**0.0453**			
Debris	0.3750	**0.0440**		0.0963			
Rhizome	0.3259	0.1775		0.2013			
S	<0.0001	<0.0001	0.0035	0.4300	**0.0247**	**0.0187**	0.1760
N	**0.0024**	0.3566	0.3799	0.4289	0.1411	0.2176	0.5096
C	0.0015	0.2470	<0.0001	0.5147	**0.0057**	0.1571	0.6194

The responses included net photosynthesis rate (P_n), leaf elongation rate, maximum quantum efficiency of Photosystem II reaction centres (F_v/F_m), turnover rate, live plant cover (Cov_{live}), dead plant cover (Cov_{dead}), live root biomass, plant debris biomass, rhizome biomass, leaf tissue nitrogen (N), carbon (C) and sulphur (S) concentrations.
Values presented bold are significant effects at $P < 0.05$.

Highest P_n in both species was recorded in March 2005 (Fig. 4a). Sulphate loading did not significantly affect P_n (Table 4).

The effect of time on leaf elongation rate was dependent on species (significant species × time interaction; Table 4). Leaf elongation rate for *Typha* was about three times higher than for *Cladium* on average. Significantly higher leaf elongation rate for *Typha* compared with *Cladium* occurred at all sampling times except for March 2005. In addition, *Typha* in November 2003, November 2004, August 2005, and March, August and December 2006 grew much faster than in March 2005. On the other hand, *Cladium* maintained a relatively constant, albeit low, elongation rate over time (Fig. 4b). Elongation rate also depended on the interactive effects of species and sulphate loading (significant species × dosing interaction; Table 4). *Typha* responded to low and high sulphate dosings with higher elongation (3.71 ± 0.39 cm day^{-1} and 3.48 ± 0.32 cm day^{-1} respectively) than those under mesocosm control (2.39 ± 0.27 cm day^{-1}) and medium sulphate (2.35 ± 0.36 cm day^{-1}) conditions, while sulphate did not have any significant effect on elongation for *Cladium*, ranging from 1.17 ± 0.11 to 0.90 ± 0.06 cm day^{-1}. In addition, the effect of dosing on elongation rate depended on time (significant

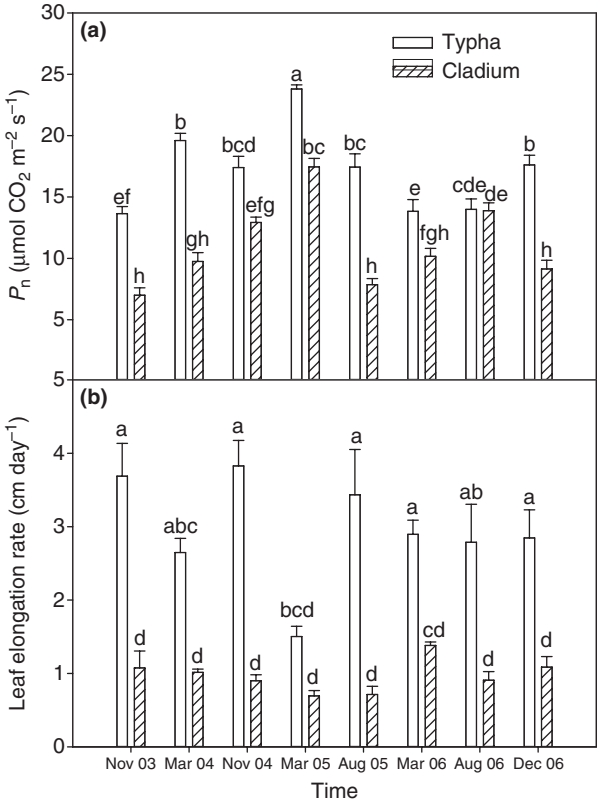

Fig. 4 Net photosynthesis rate (P_n, a) and leaf elongation rate (b) for *Typha* and *Cladium* in November 2003, March and November 2004, March and August 2005, and March, August and December 2006 across five sulphate levels. Each value is the mean ± SE. Different letters indicate significant differences across species and sampling times for each parameter identified with Tukey's procedure.

dosing × time interaction; Table 4). However, no significant differences between treatments levels across time were detected by Tukey's procedure.

Light response and CO_2 response curves. As average light intensity and CO_2 concentration rose, P_n for both species significantly increased. *Typha* had higher CO_2 fixation than *Cladium* in response to the same levels of light intensity and CO_2 (data not shown). Overall, *Typha* had significantly higher RESP, AQE, A_{max}, LCP and LSE, generated from light response curves, as well as higher Vc_{max} and J_{max}, produced from CO_2 response curves, than *Cladium* (Table 5). Sulphate dosing effect was not statistically significant (data not shown).

Chlorophyll fluorescence. Significant differences were found in F_v/F_m between the two species, the five

Table 5 *t*-Test for the effect of species on all photosynthetic parameters

Parameter	*P*-value	*Typha*	*Cladium*
RESP	**0.0326**	1.18 ± 0.30	0.75 ± 0.25
AQE	**0.0461**	0.0563 ± 0.0023	0.0418 ± 0.0033
A_{max}	**0.0399**	23.79 ± 0.96	11.07 ± 0.78
LCP	**<0.0001**	42.19 ± 1.75	34.36 ± 4.73
LSE	**0.0397**	495.24 ± 22.59	351.52 ± 33.70
Vc_{max}	**<0.0001**	51.32 ± 3.82	28.72 ± 1.83
J_{max}	**<0.0001**	141.06 ± 10.18	73.68 ± 4.77

Photosynthetic parameters: respiration (RESP), apparent quantum efficiency (AQE), maximum photosynthesis (A_{max}), light compensation point (LCP), light saturation estimate (LSE), maximum rate of caboxylation by Rubisco (Vc_{max}), PAR-saturated rate of electron transport (J_{max}) for *Typha* and *Cladium* generated from light and CO_2 response curves, and mean ± SE across sulphate dosing and sampling time
Values presented bold are significant effects at $P < 0.05$.

sulphate dosing concentrations and the three sampling periods (Table 4). Mean F_v/F_m for *Typha* was 0.776 ± 0.004, 5.4% higher than that for *Cladium* across all dosing treatments and all sampling times. Plants under low sulphate treatments tended to have higher F_v/F_m (0.768 ± 0.006) than those under mesocosm control conditions (0.737 ± 0.010). Also, F_v/F_m in August 2005 was 0.741 ± 0.007, 33.9% lower than that in both March 2004 and March 2005.

Turnover rate. Differences in turnover rate between the two species depended on sampling time and dosing (significant species × time and species × dosing interactions; Table 4). *Cladium* had a much smaller turnover rate than *Typha* at all sampling times except for August 2005, when there was no difference. In addition, *Typ*ha turned over more slowly in August 2005 than at any other time, while the highest mortality was observed in December 2006 and lowest in February 2005 for *Cladium* (Fig. 5). Higher mortality for *Typha* than *Cladium* was also found under all sulphate dosing treatments and the turnover rates were comparable among all dosing levels for both species (from 0.735 ± 0.0005 to 0.592 ± 0.0008% day^{-1} for *Typha*, and from 0.222 ± 0.0007 to 0.110 ± 0.0005% day^{-1} for *Cladium* across dosing treatments).

Plant cover. Cov_{live} for *Cladium* was overall higher than for *Typha* at all sampling times. However the difference in Cov_{live} between these two species varied with the sampling period (significant species × time interaction; Table 4). Cov_{live} for *Typha* remained

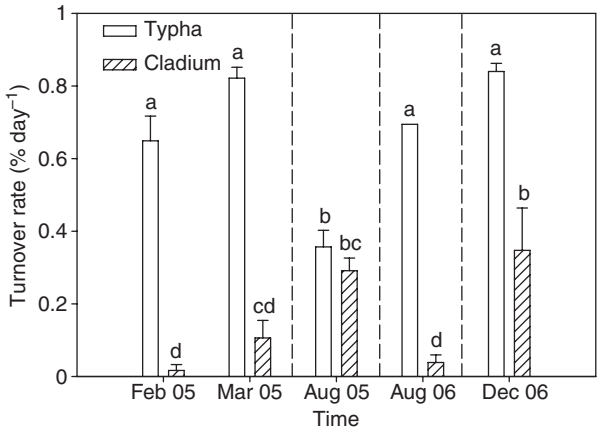

Fig. 5 Turnover rate for *Typha* and *Cladium* in February, March and August 2005, and August and December 2006 averaged over five sulphate levels. Leaves tagged on 17 November 2004 were checked on 6 February and 9 March 2005. Mortality was again recorded on 7 August 2005 for leaves tagged on 10 March 2005. Similarly, leaves tagged on 25 March 2006 were checked on 15 August 2006. Leaves marked on 15 August 2006 were used to determine the turnover rate on 7 December 2006. Each value is the mean ± SE. Different letters indicate significant differences across species and sampling times identified with Tukey's procedure.

constant over time (ranging from 11.7 ± 2.4% to 7.33 ± 2.7%), while for *Cladium* it was greatest in December 2006 (45.8 ± 3.2%) and lowest in November 2003 (19.5 ± 2.4%). Cov_{dead} for *Typha* and *Cladium* showed similar trends as Cov_{live}.

Biomass. Differences in live root biomass, determined by the floating method, between the two species was dependent on dosing treatments (significant species × dosing interaction; Table 4). *Cladium* root biomass was 1.3–10.1 times higher than for *Typha* across all dosing treatments, and significant differences between species were found for both the mesocosm control and medium sulphate conditions. Moreover, there was significantly more (2.3 times) live root biomass in control mesocosms than in the ambient plots (116.2 ± 47.3 g m^{-2}) for *Cladium*, while it was relatively constant across sulphate treatments for *Typha* (21.3 ± 1.1–67.8 ± 5.2 g m^{-2}). Sulphate also had a significant influence on plant debris biomass (Table 4), with that in the low sulphate plots (655.6 ± 10.9 g m^{-2}) being significantly lower than in the control mesocosms (1468.2 ± 17.1 g m^{-2}). No effect of dosing or species on rhizome biomass was found (Table 4).

Tissue nutrients. The effect of plant species on sulphur concentration was dependent on sampling time (significant species × time interaction; Table 4). Sulphur concentrations for *Cladium* were 0.274 ± 0.025% and 0.210 ± 0.018% in March and December 2006. Significantly higher (26.3% higher) S content for *Typh*a than for *Cladium* was detected in March 2006, while S concentration was not significantly different between the two species in December 2006. Tissue S concentration was also strongly affected by the interaction of sulphate dosing and time (Table 4) and was positively linked to sulphate dosing treatment at both sampling times. Sulphur concentration was significantly higher in high sulphate plots (0.532 ± 0.079%) than in the other treatment levels (ranging from 0.195 ± 0.025% to 0.324 ± 0.033%) in March 2006. However, no significant differences between the five sulphate treatments were found in December 2006, although leaf S levels tended to increase linearly with higher sulphate dosing (ranging from 0.177 ± 0.022% under ambient to 0.343 ± 0.033% under highly dosed conditions).

Difference in N concentration between the two species was significant (Table 4), with *Typha* being 12.8% higher than *Cladium* (1.070 ± 0.028%). In addition, the difference in C concentration between the two species depended on sampling time (significant species × time interaction; Table 4). Carbon content in *Typha* tissue was 52.790 ± 0.500% in March 2006, significantly lower than that in *Cladium* (56.612 ± 0.593%). In contrast, there was no significant difference between the two species in December 2006 (58.176 ± 0.275% for *Typha* and 59.400 ± 0.434% for *Cladium*). For both species, C concentrations were significantly higher in December than in March 2006.

Discussion

In the hydroponic study (experiment 1), we found a negative relationship between sulphide treatment and solution Eh, as carried out by Connell & Patrick (1968), Mendelssohn & McKee (1988) and Koch, Mendelssohn & McKee (1990). This result demonstrates the role of sulphide as an oxygen scavenger and in increasing electron availability and implies that plants exposed to sulphide were simultaneously subjected to a second stress, reducing conditions. It was also noted that Eh was higher for *Typha* than for *Cladium* under all hypoxic and hypoxic sulphide treatments, while sulphide concentrations showed

an opposite trend. Such species differences in Eh and sulphide concentration can be attributed to their differences in root porosity, radial oxygen loss, pressurised gas flow and extent of oxidised rhizosphere (Chabbi *et al*., 2000; Sorrell *et al*., 2000). The higher root aeration capacity in *Typha* enables it to both increase the Eh of the rooting medium and to detoxify biochemically reduced phytotoxins, such as sulphide, to a greater extent than *Cladium*, which has a lower root aeration capacity (Chabbi *et al*., 2000).

Both *Typha* and *Cladium* displayed their tolerance to anoxic root environments (0 mM sulphide) by maintaining photosynthesis and growth compared with the aerated treatment. Wetland plants including *Typha* and *Cladium* adapted to anoxic soil conditions possess a number of morphological, anatomical and physiological features including an ability to produce adventitious roots (Davis, 1982) with an aerenchymatous cortex, which facilitates internal gas transport in tissues growing in an anoxic environment (Armstrong, Brändle & Jackson, 1994).

Adding sulphide to the culture media in the hydroponic dose–response study resulted in reduced P_n and leaf elongation, along with greater dead leaf and dead root biomass, in both species. Elevated sulphide concentration was also linked to decreased live leaf biomass, live root biomass and rhizome biomass for *Cladium*. Hydrogen sulphide, resulting from sulphate reduction, may be highly toxic to plants at high concentrations (Tanaka *et al*., 1968; Havill, Ingold & Pearson, 1985; Koch & Mendelssohn, 1989; Armstrong *et al*., 1996; Van der Welle *et al*., 2006). Sulphate contamination is also an important factor in causing increased mercury methylation in the Everglades through microbial sulphate reduction (Gilmour *et al*., 2004). Sulphide has been found to adversely affect photosynthesis, aerobic and anaerobic metabolism, nutrient uptake, growth and survival of wetland plants (Koch & Mendelssohn, 1989; Pezeshki, DeLaune & Pan, 1991; Armstrong *et al*., 1996; Seliskar *et al*., 2004). Additionally, sulphate loading can lead to the increased mobilisation of phosphate from the sediment to the porewater and thus cause eutrophication (Lamers *et al*., 1998, 2002). Sulphide also binds to iron, which may cause iron deficiency in wetland plants (Smolders, Nijboer & Roelofs, 1995). As a result of its adverse effect on plant function, free sulphide has been documented experimentally as an important mediator in dieback events of seagrass, such as *Thalassia testudinum* Banks ex König (Borum *et al*., 2005) and *Zostera marina* L. (Pedersen, Binzer & Borum, 2004), and wetland plants including *Phragmites australis* (Cav.) Trin. ex Steud. (Armstrong *et al*., 1996), *Spartina alterniflora* Loisel. (Mendelssohn & McKee, 1988) and *Stratiotes aloides* L. (Smolders *et al*., 2003). Therefore, excess sulphate input can severely influence plant species composition, toxicity and mobilisation of heavy metals, and thus pose a major threat to biogeochemical function and biodiversity of freshwater wetlands (Lamers *et al*., 2002; Van der Welle *et al*., 2007).

Even though *Typha* and *Cladium* were both negatively affected by sulphide, species-specific differences in sensitivity were noted in experiment 1. In *Cladium*, P_n, leaf elongation rate, dead leaf biomass and dead root biomass were all negatively affected at a lower sulphide concentration than in *Typha*. The principal component analysis, which combined leaf photosynthesis and elongation rates, showed that *Cladium* was negatively affected at a sulphide concentration of 0.22 mM, as opposed to 0.69 mM for *Typha*. The species difference was further demonstrated by the fact that live leaf biomass, live root biomass, rhizome biomass and total live biomass of *Typha* were not affected by sulphide, while they were adversely affected in *Cladium*. Overall, these results demonstrated that *Typha* is more tolerant of sulphide than *Cladium*. This advantage of *Typha* over *Cladium* can be attributed the greater root aeration capacity of *Typha*, which alleviates the adverse effects of both low Eh and high sulphide conditions. We did not find any significant differences in total leaf biomass, total root biomass, shoot base biomass, total biomass, total RSB/RB ratio and live RSB/RB ratio among treatments for either species for the time period tested. A longer experimental period might have allowed for significant effects on biomass-related parameters. It is expected that those parameters in *Typha* would be less impacted by sulphide than in *Cladium*. However, note that our results are for relatively immature plants (55-cm plant height), grown for a relatively short period (7 days) in a high nutrient, hydroponic culture. Additionally, both *Cladium* and *Typha* are large clonal perennials with a large interconnecting rhizome system and will presumably be more effective at detoxifying sulphide by radial oxygen loss than individual plants, even those of the same size, under hydroponic conditions.

In the field mesocosm experiment (experiment 2), we found large and significant differences in growth and photosynthetic responses between the two species. *Typha* had a higher CO_2 assimilation rate, a faster growth rate, and a higher turnover rate than *Cladium*, which agrees with previous findings (Davis, 1990, 1994). Leaf fluorescence (F_v/F_m) and the light and CO_2 response curves for the two species provided additional support for this finding. F_v/F_m is highly correlated to the quantum yield of PSII and is typically used to estimate quantum efficiency (Maxwell & Johnson, 2000). Furthermore, both light and CO_2 response curves are widely used to compare the photosynthetic capacities among species or varying environmental conditions such as temperature, light, salinity, nutrients, etc. (Plus *et al*., 2005). As expected, *Typha* reached higher photosynthesis rates (A_{max}), needed more light to saturate photosynthesis (LSE), possessed a higher dark respiration rate (RESP) and a higher AQE, and required more light to balance leaf respiration and achieve zero P_n (i.e. *Typha* had a higher instantaneous LCP) than did *Cladium*. In addition, the maximum rate of carboxylation or Rubisco activity (Vc_{max}) and rates of electron transport used to regenerate RuBP (J_{max}) were also greater for *Typha*. These results further support the theory that *Typha* is inherently a better competitor than *Cladium*.

Previous studies reported many other significant species-specific differences for *Cladium* and *Typha*. For instance, Miao & Sklar (1998) found that *Typha* had higher nutrient concentrations than *Cladium* in tissues primarily associated with growth functions, such as leaves, roots and rhizomes. In addition, *Cladium* exhibits characteristics of species adapted to low nutrient environments while *Typha* is considered as an opportunistic species from nutrient enriched habitats (Davis, 1989; Lorenzen *et al*., 2001). Miao (2004) also found a contrasting growth pattern between the two species and concluded that *Typha* has a greater capacity to pre-empt space. Together with our findings, these differences in growth and life history characteristics between the two species help to explain the ability of *Typha* to dominate over *Cladium* in areas of the Florida Everglades influenced by canal water discharge (once the former has become established).

However, sulphate loading did not generally have any significant effect on growth of either species in experiment 2, even though leaf S concentrations in both species were much higher in high sulphate plots than the others. Therefore, the field experiment did not provide evidence, *per se*, that *Typha* expansion in the Everglades is because of its greater ability to tolerate sulphate-induced H_2S as compared with *Cladium*. This may be because sulphate loading was interrupted by drought events over several months each year (preventing dosing). Also, buildup of soil H_2S levels lagged behind what was expected based on sulphate dosing levels, possibly because of the reaction of H_2S with organic matter in these soils. As a result, H_2S concentrations never reached target values and the highest concentration of H_2S was just 0.18 mM in March 2006 (W. Orem, unpubl. data), which was below the sublethal level detected in experiment 1.

Ecological implications

Evidence has been accumulating that altered hydrology and increasing P loading are both key factors responsible for the expansion of *Typha* into areas previously dominated by *Cladium* in the Everglades. *Typha* possesses both morphological and physiological characteristics that give it a competitive advantage over *Cladium* under low Eh conditions. Experimental work also indicates that *Typha* is favoured by the presence of P enriched conditions in the Everglades. However, surface water sulphate is also enriched in areas of the Everglades where *Typha* has displaced *Cladium*, and where elevated soil and surface water P occurs. Sulphate can be reduced to toxic sulphide by sulphate reducing bacteria under anoxic conditions. Our study clearly indicated that *Cladium* was impacted by sulphide before *Typha*. *Cladium* was adversely affected when sulphide concentrations reached approximately 0.22 mM, while *Typha* continued to do well and appeared healthy until 0.69 mM. Table 6 presents growth-limiting sulphide concentrations for a diverse range of plant species, including *Cladium* and *Typha* from this study. It is clear that porewater sulphide concentrations in the field mesocosm study never reached a growth limiting level for either *Cladium* or *Typha*. However, the highest sulphide concentrations recorded in the Everglades (0.25–0.375 mM) is greater than the minimum level required to impact young *Cladium* plants, while not affecting similarly aged *Typha*. These areas of very high soil sulphide most often occur in *Typha*-dominated areas of the ecosystem. Therefore, we conclude that, in combination with altered hydrology and

Table 6 Comparison of species-specific tolerance to sulphide

Species	Marsh type	Growth limiting (S^{-2}), mM	Reference
Spartina alterniflora	Salt	1.13	Koch & Mendelssohn (1989)
Thallasia testundinum	Seagrass	2 (acute)	Carlson *et al.* (2002)
Avecinnia marina (L.) L.	Mangrove	0.5–1.0	McKee (1993)
Rhizophora mangle L.	Mangrove	>1.0	McKee (1993)
Oryza sativa L. (rice)	Fresh	0.16–0.31	Tanaka *et al.* (1968)
Panicum hemitomon Schult.	Fresh	0.63	Koch & Mendelssohn (1989)
Carex wetland meadow	Fresh	0.2	Lamers *et al.* (1998)
Cladium jamaicense	Fresh	0.22	This research
Typha domingensis	Fresh	0.69	This research

increased P availability, sulphate contamination and resultant sulphide also has the potential to drive the replacement of the historically dominant *Cladium* by the native-invasive *Typha*. Although further research is required to confirm or reject this potential, the role of sulphate loading should be considered, in conjunction with hydroperiod, P availability and disturbances (e.g. surface fire and herbivory), in developing future management plans for the Everglades.

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