

# Respiratory enzyme activities correlate with anoxia tolerance in salt marsh grasses

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## Abstract

Salt marsh communities are known for well-defined species zonation patterns. Lower limits of plant growth are thought to be set by an ability to tolerate anoxic sediments, but the physiological differences between species have not previously been examined. To investigate responses to anoxic sediments, several estuarine species were grown in greenhouse experiments to compare how respiratory processes were affected by flooding. Metabolic characteristics related to respiration and anoxia tolerance were studied in the emergent estuarine species *Spartina alterniflora*, *S. anglica*, *S. densiflora*, *S. foliosa*, *S. alterniflora* × *S. foliosa* hybrids, *S. patens*, and *Distichlis spicata* and compared to the inland species maize (*Zea mays*). All species showed a strong ability to respire anaerobically, indicating flooding tolerance. High intertidal marsh species had significantly higher root aerobic respiration enzyme activities compared to low intertidal species that may suggest lower aerobic demand in low marsh species. Some higher marsh species showed an apparent high sensitivity to sulfide that may be related to high cytochrome *c* oxidase activities. In contrast, the low marsh species *S. alterniflora* and *S. anglica* had lower aerobic respiration enzyme activities and a lower sensitivity to sulfide. Thus differences in aerobic demand and sulfide sensitivity may influence estuarine species zonation.

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## 1. Introduction

Salt marsh plant communities are characterized by well-defined zonation patterns that are correlated with marsh elevation. Many previous studies have established factors that influence upper and lower limits of plant zonation in salt marshes (e.g., Bertness and Ellison, 1987; Bertness, 1991; Pennings and Callaway, 1992; Crain et al., 2004; Pennings et al., 2005; Richards et al.,

2005). A species' lower elevational limit is determined by abiotic environmental factors and the upper limit is normally determined by biotic factors like competition (but see Pennings and Callaway, 1992).

Changes in marsh elevation correspond with changing environmental conditions. Lower elevational zones are regularly inundated by tides and consequently have low soil oxidation–reduction (redox) potentials. Soil organic matter decomposes anaerobically in low marsh areas, leading to the production of toxic sulfides (Koch and Mendelssohn, 1989). In contrast, higher elevational areas are only intermittently flooded, leading to higher soil redox potentials (Bertness and Ellison, 1987). Extended periods between tides in the high marsh lead to

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high evaporative conditions and can elevate soil salinity to levels higher than those found in the low marsh (Pennings and Callaway, 1992). However, salinity can be variable between sites. In some cases soil salinities have been found to simply decrease with increasing distance from the shore (Bertness and Ellison, 1987).

Despite the variability in salinity measures, the aforementioned studies agree that high marsh species are excluded from lower marsh areas because of sediment conditions like redox potential, sulfide concentration, or salinity. However, the relative importance of each is not known and is extremely difficult to determine by field manipulations or transplant experiments (e.g., Pennings et al., 2005).

Superimposed on the dynamics of estuarine zonation is the success of invasive *Spartina* species (Poaceae) in areas of introduction. Exotic low marsh *Spartina* species are successful in North American West Coast estuaries (and others throughout the world) because they can occupy an empty niche; conditions in low-elevation mudflats and tidal channels exclude native species. Given the importance of abiotic factors in structuring normal marsh communities, it is important to compare the relevant physiological responses between species in order to explain the success of invaders in the low marsh.

Investigation of physiological characteristics may provide information that is synergistic with information gained from experimental ecology. Physiology can, in part, structure ecological systems (Brown et al., 2004). Biochemical characteristics of species are potentially well suited for studies on the scales relevant to address ecological questions. For example, measurements of enzymatic activity can be conducted without highly specialized equipment and in a relatively short period of time. Enzymatic activity is the maximal rate of an enzyme-catalyzed reaction for a given plant tissue. Thus activity measurements represent potential flux, and can correlate with flux exhibited *in vivo*. Enzymatic activity is due to inherent characteristics of a species and environmental influences. Differences between plants grown under common conditions reflect species characteristics. Differences in activity in response to flooding reflects the degree of environmental regulation. Both parameters are important when considering responses to potentially deleterious environmental conditions.

Metabolic respiratory pathways can be important when considering plant life in anaerobic sediments. Survival of plants in these areas depends on efficient oxygen supply and usage in submerged tissues. Aerobic respiration occurs via glycolysis, the TCA cycle, and mitochondrial oxidative phosphorylation. Additionally, the ability to respire anaerobically is likely to be important to survive in

these sediments. The enzyme alcohol dehydrogenase (ADH) catalyzes the final reaction in fermentative ethanol synthesis. ADH activity in plants subject to anoxia stress appears to be an adaptation for anoxia tolerance (Crawford, 1967). Consequently, TCA cycle and mitochondrial enzyme activities are reflective of the potential for aerobic respiratory flux and ADH is a measure of the potential for anaerobic respiratory flux.

In the present study we investigated metabolic respiratory characteristics of several common marsh species, including invasive *Spartina* species and two vigorous *Spartina* hybrids. Measures of metabolic characteristics allowed separation of phenomena associated with flooding from salinity effects in order to isolate flooding adaptations. Such experiments may elucidate the physiological underpinnings of zonation patterns observed by field ecologists. In this study, the following general questions were addressed: Can biochemical indices provide insight into estuarine zonation? Is there evidence that metabolic characteristics may prevent high marsh species from establishing in low marsh environments? Do invasive hybrids differ from progenitor species in metabolic characteristics and could these characteristics contribute to invasiveness?

These questions were addressed in the emergent estuarine species *Spartina alterniflora* Loisel. and *S. anglica* C.E. Hubbard (low marsh species), as well as *S. densiflora* Brongn., *S. foliosa* Trin., a *S. alterniflora* × *S. foliosa* hybrid (middle marsh species), *S. patens* (Ait.) Muhl., *Distichlis spicata* (L.) Greene (high marsh species), and the flood-tolerant inland species maize (*Zea mays* L.). Plants were grown under drained and flooded soil conditions in greenhouse experiments, allowing physiological responses to be compared between species. Several metabolic parameters were measured, including aerobic vs. anaerobic respiration enzyme expression and root sulfide oxidation capacity.

## 2. Materials and methods

### 2.1. Plant material and growing conditions

Marsh plants were collected from field sites and were subsequently maintained under greenhouse conditions on the Washington State University campus in Pullman, WA. *Spartina alterniflora* plants were collected in Willapa Bay, WA and *S. anglica* was collected from northern Puget Sound, WA. Additionally, *S. patens* plants were obtained from the Gulf Coast of NW Florida, *S. densiflora* plants were obtained from the Odiel Salt marshes, SW Spain, and *S. foliosa* and *S. alterniflora* × *S. foliosa* hybrids were obtained from San Francisco Bay, California.

The estuarine grass *Distichlis spicata* was collected in northern Puget Sound, WA. Although phenotypic variations exist across plants collected from different geographic regions, the relative intertidal growth zones (i.e., high marsh vs. low marsh) do not change between species (e.g., Bertness, 1991; Pennings and Callaway, 1992; Pennings et al., 2005). Therefore, physiological comparisons between species should be valid despite representation from a single collection. Metabolic comparisons were made between these marsh species as well as the flood-tolerant inland species maize (*Zea mays*), grown from commercial seed.

Greenhouse conditions were 26 °C day/18 °C night with natural lighting. Photosynthetic photon flux density averaged 400  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  during daylight hours and peaked around 1500  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  on sunny days. Field-collected plants were maintained several weeks before initiation of experiments. Daughter tillers from these plants were potted individually in a 50/50 (vol./vol.) sand/potting soil mixture in 11 cm  $\times$  11 cm pots. Plants were watered to saturation twice weekly with modified Hoagland nutrient solution (Epstein, 1972). Freshly potted plants were selected for uniformity in size, randomized between flooded and drained treatments, and allowed 30 d to recover from potting before flooding. Following recovery, plants were placed into 50 cm  $\times$  36 cm plastic tubs (12 pots per tub across 45 tubs in an unbalanced block design). Flooded treatment plants were submerged in water to a level 2 cm above the soil surface (about 12 L) and the water was completely replaced weekly. Drained plants were watered to saturation with nutrient solution twice weekly. While greenhouse conditions may not replicate the highly reducing character found in some estuarine mudflats, sulfate reduction was observed in most of the flooded treatment tubs, indicating redox potentials  $< -150$  mV (Ponnamperuma, 1972) and therefore a reasonable representation of field conditions.

Plants were allowed 60–80 d in their respective treatment (drained or flooded) before harvest. Maize plants died under long-term flooding. As a result, data for flooded treatment maize plants represent flooding for 6–8 d. All statistical analyses were performed between species and treatments with a two-factor (species and treatment) analysis of variance (Proc mixed; SAS version 8.0, 2001 SAS Institute Inc., Cary, NC;  $\alpha=0.05$ ). In this model, species were blocked by tub. Comparisons between ecological functional groups were performed with two-way ANOVA tests comparing species means between low marsh ( $n=2$ ) and middle to high marsh ( $n=5$ ) groups. Significance is reported for highest-level interactions in all cases.

## 2.2. Preparation of enzyme extracts

At harvest, root samples were obtained from each plant, flash-frozen in liquid nitrogen, and stored at  $-80$  °C. Cytochrome *c* oxidase (CytOx) and sulfide oxidase (SOx) activities were measured in extracts from root tissue samples. Roots were ground in liquid nitrogen and cold extraction buffer was added at 2 mL  $\text{g}^{-1}$ . The extraction buffer contained 0.1 M  $\text{Na}_2\text{HPO}_4$  at pH 7.1 with 0.1% v/v Triton X-100 (Maxwell and Bateman, 1967) (All reagents were purchased from Sigma Chemical Company, St. Louis, MO). This mixture was homogenized with a mortar and pestle, filtered through Miracloth (Calbiochem; San Diego, CA), and centrifuged at 1000 *g* for 20 min at 4 °C. The supernatant was used in CytOx and SOx assays. Alcohol dehydrogenase (ADH) was extracted from root tissue samples after John and Greenway (1976). Roots were ground in liquid nitrogen and cold extraction buffer was added at 5 mL  $\text{g}^{-1}$ . ADH extraction buffer was 50 mM HEPES (4-2-hydroxyethylpiperazine-1-ethanesulfonic acid) (pH 8.0), containing 5 mM  $\text{MgCl}_2$ , 2 mM cysteine hydrochloride, and 2% w/v PVP-40 (polyvinylpyrrolidone, MW  $\approx 40,000$ ) (John and Greenway, 1976). The resulting mixture was homogenized with a mortar and pestle, filtered through Miracloth, and centrifuged at 10,000 *g* for 10 min at 4 °C. All enzyme assays were performed spectrophotometrically at 25 °C.

## 2.3. Aerobic and anaerobic respiration enzyme activities

CytOx assays were performed in a procedure after Smith (1955). Enzyme extract was assayed in the presence of 15  $\mu\text{M}$  reduced cytochrome *c* (from horse heart) in 50 mM  $\text{Na}_2\text{HPO}_4$  (pH 7.0). Cytochrome *c* was reduced with 4 mM  $\text{Na}_2\text{S}_2\text{O}_4$ ; a  $A_{550}/A_{565}$  ratio  $> 6.0$  indicates reduced cytochrome *c* (Smith, 1955). Enzyme activity was determined as the rate of cytochrome *c* oxidation, measured as a decrease in absorbance at 550 nm, using an extinction coefficient of 19.6  $\text{mol m}^{-3} \text{cm}^{-1}$  (Pearson and Havill, 1988). Rates of CytOx activity were corrected for background rates of cytochrome *c* oxidation, then standardized to *g* fresh root weight.

For the ADH assays, the supernatant was assayed spectrophotometrically in the ethanol-forming direction. Enzyme extract was assayed in the presence of 80  $\mu\text{M}$  NADH and 10 mM acetaldehyde in a buffer solution of 40 mM bicine and 5 mM  $\text{MgCl}_2$  (pH 8.0) (John and Greenway, 1976). Enzyme activity was determined as the rate of NADH oxidation, measured as a decrease in absorbance at 340 nm, using an extinction coefficient of 6.22  $\text{mol m}^{-3} \text{cm}^{-1}$  (Pearson and Havill, 1988). Rates

of NADH oxidation in the presence of acetaldehyde were corrected for background rates, then standardized to g fresh root weight.

#### 2.4. Sulfide oxidase assays

A colorimetric method was developed to estimate the activity of root sulfide oxidation processes. 50  $\mu\text{L}$  aliquots of root extract were added to each of three vials of 0.95 mL de-oxygenated 50 mM  $\text{Na}_2\text{HPO}_4$  buffer solution (pH 7.0) containing 100  $\mu\text{M}$   $\text{Na}_2\text{S}$  that were then allowed to oxidize. 40  $\mu\text{L}$  of Cline reagent (Cline, 1969) was added to vials after 0, 10, and 20 min to determine residual sulfide concentrations. Background rates of sulfide oxidation were measured by adding 50  $\mu\text{L}$  buffer instead of enzyme extract to a series of vials containing 100  $\mu\text{M}$   $\text{Na}_2\text{S}$ . Sulfide oxidation was measured as a decrease in sulfide concentration over time. Sulfide concentrations were determined from a standard curve made with 0–100  $\mu\text{M}$   $\text{Na}_2\text{S}$ . Rates of SOx activity were corrected for background rates of spontaneous sulfide oxidation, then standardized to g fresh root weight. Rates of nonenzymatic sulfide oxidation were determined using 50  $\mu\text{L}$  aliquots of boiled enzyme extract. Enzymatic rates of sulfide oxidation were calculated from the difference between the rates of total sulfide oxidation and the nonenzymatic sulfide oxidation.

### 3. Results and discussion

#### 3.1. Aerobic respiration

Mean cytochrome *c* oxidase (CytOx) activities for all species ranged from 0.023 to 0.328  $\mu\text{mol g}^{-1} \text{min}^{-1}$  across species and waterlogging treatments (Fig. 1). When expressed by root protein levels, these activities are equivalent to protein-specific activities measured for wetland and nonwetland plants by Pearson and Havill (1988), but slightly lower than tissue-level values presented for *Echinochloa crus-galli* measured by Wang (1980). CytOx is a critical step in aerobic respiration, as it catalyzes the terminal electron transport to oxygen in aerobic respiration. Enzyme activities represent metabolic potential ( $V_{\text{max}}$ ; i.e., with unlimited substrate concentrations). Drained treatment root CytOx activities in the present study corresponded well with previously measured root oxygen uptake rates in all species (Maricle and Lee, in press), so drained soil treatments appear to allow cytochrome *c* oxidase to operate near  $V_{\text{max}}$ .

CytOx activities differed significantly among species, with the low marsh species *S. alterniflora* exhib-

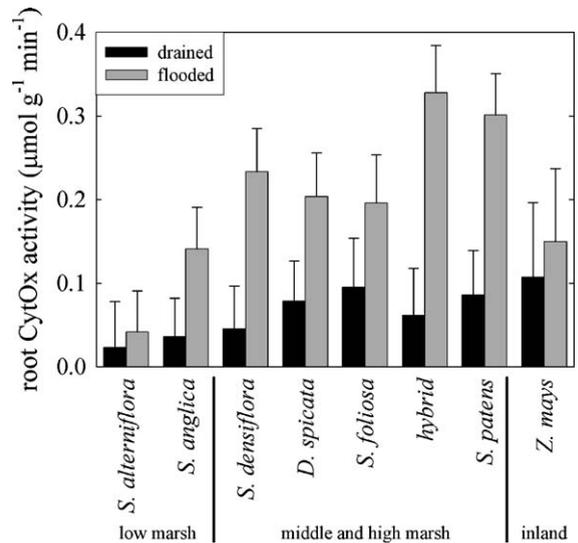


Fig. 1. Cytochrome *c* oxidase (CytOx) activities ( $\mu\text{mol g}^{-1} \text{min}^{-1}$ ) of *Spartina*, *Distichlis*, and *Zea* plants grown under drained and flooded soil treatments. The least squares mean of 4–15 plants  $\pm$  SE is shown. Species are grouped by elevational zonation patterns.

iting activities significantly lower than the middle and high marsh species *S. foliosa*, the hybrid, *S. patens*, and *D. spicata* (ANOVA,  $p \leq 0.02$ ). Additionally, the low marsh species *S. anglica* had CytOx activities significantly lower than the middle marsh species *S. foliosa* and the hybrid (ANOVA,  $p \leq 0.02$ ). When grouped by ecological functional type, CytOx activities in the low marsh species were significantly lower than in the middle and high marsh species (ANOVA,  $p < 0.01$ ). Although drained CytOx activities in maize are consistent with previously measured high aerobic demand (Maricle and Lee, in press), flooded-treatment CytOx activities in maize were found to be within the range of activities measured in marsh species. These results suggested that high marsh species may have an aerobic oxygen demand that is too high to allow survival in highly anoxic low marsh conditions. In contrast, the low marsh species had low aerobic demand, which may be advantageous for survival under anoxia.

Low aerobic respiration capacity appeared to be related to ability to tolerate anoxic conditions across species, similar to results in wheat strains differing in flood tolerance (Huang and Johnson, 1995). In the present study, CytOx activities significantly increased across species with the onset of flooding (ANOVA,  $p < 0.01$ ). Waterlogged conditions will result in decreased oxygen available for respiration (Vartapetian and Jackson, 1997), so it is unlikely that oxygen uptake rates can increase under flooded conditions. Instead, increased CytOx enzymatic activities under flooded conditions may serve as an increased oxygen

scavenging system in oxygen-deficient tissues (Pearson and Havill, 1988).

### 3.2. Anaerobic respiration

The ADH activities observed in these species suggested a well-developed capacity for anaerobic fermentation, an important factor in flooding tolerance (Crawford, 1967). Mean ADH activities ranged from 0.65 to 5.33  $\mu\text{mol g}^{-1} \text{min}^{-1}$  across species and treatments (Fig. 2), similar to results presented by Mendelssohn et al. (1981) for *S. alterniflora* and Smith et al. (1986) for other marsh plants. Flooded soil conditions resulted in significantly higher ADH activities across species (ANOVA,  $p < 0.01$ ). There were no significant differences between ADH activities of low marsh and high marsh species groupings (ANOVA,  $p = 0.24$ ). Although there was no difference between individual species (ANOVA,  $p = 0.39$ ) and no significant interaction between species and treatment ( $p = 0.45$ ), ADH activities in *S. anglica* did not show the characteristic increase with flooding. This may suggest *S. anglica* is more resistant to flooding compared to the other species in this study. Further work may be required to investigate the respiratory capabilities of *S. anglica*. Additionally, a supply of oxygen from internal aeration may allow marsh plants like *Spartina* to respire aerobically despite growing in waterlogged substrates. The superior oxygen transport abilities of *S. anglica* (Maricle

and Lee, 2002) may have accounted for the apparent lack of increase in root ADH activities observed in this study.

### 3.3. Sulfide oxidase

The plants in the present study showed varying degrees of sulfide oxidation capacity. Total sulfide oxidation ranged from 37.3 to 162.2  $\text{nmol g}^{-1} \text{min}^{-1}$  across species and treatments (Fig. 3a). Rates of total sulfide oxidation were significantly greater in the high marsh species *D. spicata* and the middle marsh species *S. foliosa* compared to all other species (ANOVA,  $p \leq 0.05$ ). Total sulfide oxidation rates did not differ between drained and flooded treatments (ANOVA,  $p = 0.61$ ) or between low marsh and middle to high marsh species groupings (ANOVA,  $p = 0.19$ ).

Sulfide oxidation processes can result from uncharacterized enzymatic processes or from nonenzymatic catalysts like metals (Lee et al., 1999). Thus, gross sulfide oxidation was partitioned into enzymatic and nonenzymatic processes. Rates of enzymatic sulfide oxidation ranged from 14.2 to 96.8  $\text{nmol g}^{-1} \text{min}^{-1}$  across species and treatments (Fig. 3b). Enzymatic SOx activities were highest in the high marsh species *D. spicata* and middle marsh species *S. foliosa* and were significantly lower in all other species (ANOVA,  $p \leq 0.01$ ). Enzymatic SOx activities did not change in response to flooding (ANOVA,  $p = 0.36$ ). A strong trend was observed where the middle to high marsh species grouping appeared to have higher enzymatic SOx activities compared to the low marsh species grouping (ANOVA,  $p = 0.07$ ).

Nonenzymatic processes were also found to be important in sulfide oxidation. Mean nonenzymatic sulfide oxidation rates ranged from 6.5 to 64.8  $\text{nmol g}^{-1} \text{min}^{-1}$  across species and treatments (Fig. 3c). There were no significant differences in rates of nonenzymatic sulfide oxidation between species (ANOVA,  $p = 0.08$ ), or treatments (ANOVA,  $p = 0.75$ ), or species groupings (ANOVA,  $p = 0.73$ ), suggesting similar nonenzymatic sulfide oxidation mechanisms across all species.

The anaerobic decay of material in estuarine sediments can lead to the production of hydrogen sulfide at toxic levels (Koch and Mendelssohn, 1989). The toxicity of sulfide results from its inhibition of cytochrome *c* oxidase, thereby stopping aerobic respiration (Bagarinao, 1992). Plants growing lower in the intertidal zone would encounter lower soil redox potentials, and thus encounter higher levels of sulfides. These plants showed low CytOx activities, so they may require less protection against sulfide toxicity. Plants

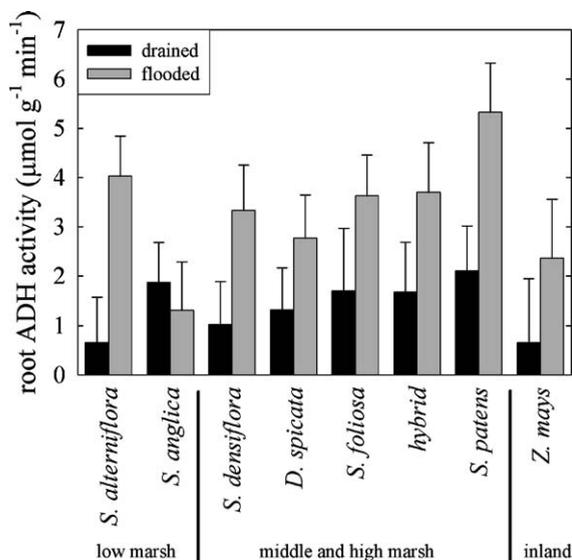


Fig. 2. Alcohol dehydrogenase (ADH) activities ( $\mu\text{mol g}^{-1} \text{min}^{-1}$ ) of *Spartina*, *Distichlis*, and *Zea* plants grown under drained and flooded soil treatments. The least squares mean of 5–14 plants  $\pm$  SE is shown. Species are grouped by elevational zonation patterns.

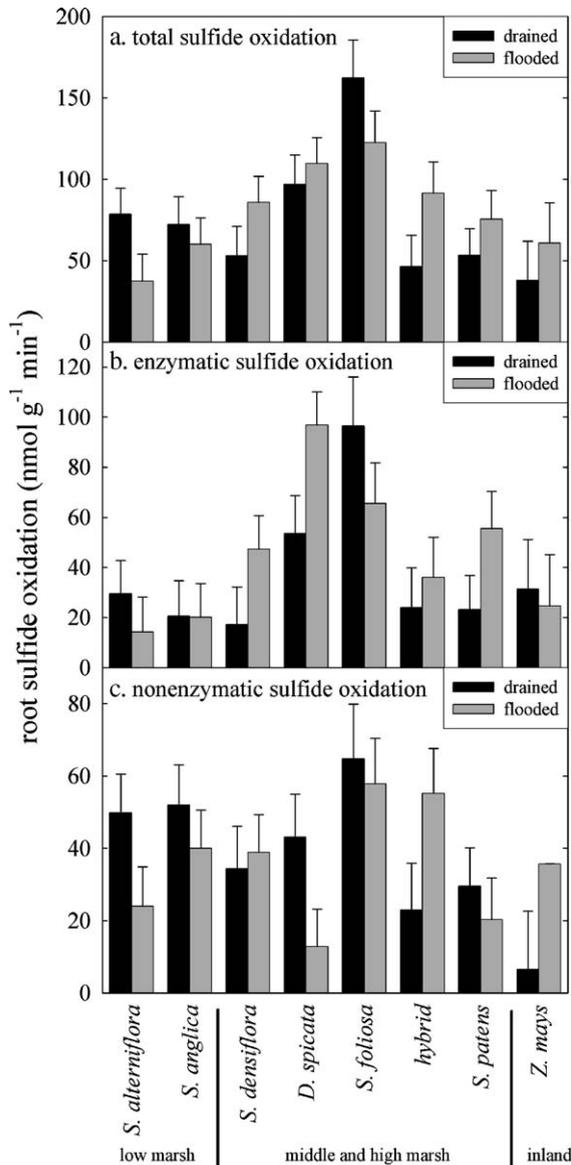


Fig. 3. Sulfide oxidase (SOx) activities ( $\text{nmol g}^{-1} \text{min}^{-1}$ ) of *Spartina*, *Distichlis*, and *Zea* plants grown under drained and flooded soil treatments. Shown are (a) total sulfide oxidation rates, (b) enzymatic, and (c) nonenzymatic rates of sulfide oxidation. The least squares mean of 4–10 plants  $\pm$  SE is shown. Species are grouped by elevational zonation patterns.

growing higher in the intertidal zone experience higher soil redox potentials and lower sulfide levels. These species showed higher CytOx activities, which may require greater protection against sulfides, even in low concentration. In contrast, inland species like maize will encounter little sulfide even under waterlogged conditions. Unlike seawater, there is generally less sulfate in freshwater that can be reduced to sulfide.

Therefore, inland species may not express adaptations to detoxify sulfides.

#### 4. Features of middle and high marsh species (*Spartina densiflora*, *S. foliosa*, *S. alterniflora* $\times$ *S. foliosa* hybrid, *S. patens*, and *Distichlis spicata*)

The upper regions of salt marshes are characterized by oxidized soils since tidal flooding is rare. However, episodic flooding at the highest tides can result in occasional anoxic and sulfidic conditions. Therefore, plants from the high marsh are not forced to withstand chronic anoxia. The middle and the high marsh species in this study had high aerobic enzyme activity. This aerobic oxygen demand may be too high to allow survival in anoxic low marsh conditions, where plants had lower aerobic demand. Anaerobic pathways (root ADH activities) increased dramatically after flooding in all middle and high marsh species suggesting a high sensitivity to soil waterlogging. Differences in aerobic demand may therefore help to influence zonation within estuaries. The high aerobic demand of high marsh species, suggested by high CytOx activities, were shared by inland maize plants. High oxygen demand is clearly a disadvantage for life in anaerobic sediments.

Higher SOx activities were found in high marsh species compared to low marsh species. These trends suggested that high marsh species were more sensitive to sulfide and required greater protection of aerobic respiration. This idea is consistent with the finding that many of these species exhibited higher activities of CytOx, which is the site of sulfide inhibition of aerobic respiration (Bagarinao, 1992). High rates of aerobic respiration and apparent sulfide sensitivity may be reasons why these species are excluded from low marsh zones.

#### 5. Features of low marsh species (*Spartina alterniflora* and *S. anglica*)

Plants inhabiting low marsh regions must be able to tolerate highly reducing and sulfidic sediment conditions. The low zones of salt marshes are characterized by frequent tidal flooding. This leads to highly reduced sediments, often containing high levels of sulfides. *Spartina alterniflora* is the dominant low marsh species in many North American East- and Gulf-Coast estuaries (Bertness, 1991). *Spartina anglica* can grow lower in the intertidal range than *S. alterniflora* (Raybould et al., 1991), and therefore any other species in this study. Low marsh species appeared to have a low aerobic demand as suggested by low root CytOx activities and previously measured root oxygen uptake rates (Maricle and Lee, in

press). This may pose a significant advantage for survival under anoxia. The ability to supply oxygen to submerged tissue is crucial to survival in the low marsh, as no plant tissue can endure anoxia indefinitely (Crawford, 1982). Low marsh species must also possess an enhanced ability to respire anaerobically since demand for oxygen from highly reduced sediments may overwhelm transport processes. Root ADH activities measured in all species indicated a well-developed capacity for fermentation. However, increases in root ADH in response to flooding were not observed in *S. anglica*. High rates of oxygen transport in *S. anglica* measured by Maricle and Lee (2002) and Lee (2003) might be adequate to supply the needed oxygen, as suggested by low root ADH activities. Low marsh species may also be more resistant to sulfide compared to high marsh species. Lower aerobic respiration rates and lower CytOx activities can relax needs for intense sulfide oxidation requirements. Alternatively, higher rates of oxygen transport in low marsh species may help to oxidize rhizosphere sulfides, resulting in reduced SO<sub>x</sub> activity. However, the acute toxicity of dissolved sulfides around roots still necessitates moderate SO<sub>x</sub> activities in low marsh species.

The physiological mechanisms that allow plant growth in low marsh habitats also contribute to the invasiveness of *Spartina* species in areas of introduction. The low marsh species *S. alterniflora* has colonized many hectares of low-elevation intertidal mudflat in Willapa Bay, WA (Hedge et al., 2003) and San Francisco Bay, CA (Daehler and Strong, 1994). The hybrid origin *S. anglica* has shown extreme vigor during its establishment in low intertidal zones of Puget Sound, WA (Hacker et al., 2001). Similarly, the *S. alterniflora* × *S. anglica* hybrid shows a greater ecological amplitude than its parent species (Ayres et al., 2004), giving it a wider growth range and helping to confer competitive success. The results of this study help to explain the mechanisms of estuarine zonation and consequently the success of invasive *Spartina* in low marsh zones. Sediment oxygen dynamics govern plant distributions in intertidal areas. Lower aerobic demands, high anaerobic capacities, and low sulfide sensitivity are all advantageous in anaerobic sediments. These three invasive species all appeared to be largely insensitive to sulfide and/or had low CytOx activities, consistent with the conclusions on middle and low marsh zonation.

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