

Root respiration and oxygen flux in salt marsh grasses from different elevational zones

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Received: 5 November 2005 / Accepted: 17 July 2006 / Published online: 21 October 2006
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Abstract Plants growing in waterlogged environments are subjected to low oxygen levels around submerged tissues. While internal oxygen transport has been postulated as an important factor governing flooding tolerance, respiration rates and abilities to take up oxygen under hypoxic conditions have been largely ignored in plant studies. In this study, physiological characteristics related to internal oxygen transport, respiration, and oxygen affinity were studied in low intertidal marsh species (*Spartina alterniflora* and *S. anglica*) and middle to high intertidal species (*S. densiflora*, *S. patens*, *S. foliosa*, a *S. alterniflora* × *S. foliosa* hybrid, *S. spartinae*, and *Distichlis spicata*). These marsh plants were compared to the inland species *S. pectinata* and the crop species rice (*Oryza sativa*), corn (*Zea mays*), and oat (*Avena sativa*). Plants were grown in a greenhouse under simulated estuarine conditions. The low marsh species *S. anglica* was found to transport oxygen internally at rates up to 2.2 $\mu\text{mol O}_2 \text{ g fresh root weight}^{-1} \text{ h}^{-1}$. In contrast, marsh species from higher zones and crop species were found to transport significantly less oxygen internally, although rice plants were able to transport 1.4 $\mu\text{mol g}^{-1} \text{ h}^{-1}$. Under hypoxic conditions, low marsh species were better able to remove dissolved oxygen from the

medium compared to higher marsh species and crops. The oxygen concentration at which respiration rates declined due to limited oxygen (P_{crit}) was significantly lower in low marsh species compared to inland and crop species; P_{crit} ranged from $<4 \mu\text{M O}_2$ in the low marsh species *S. anglica* up to 20 μM in the inland species corn. Flooding-sensitive crop species had significantly higher aerobic respiration rates compared to flooding-tolerant species in this study. Crop species took up 3.6–6.7 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ while all but one marsh species took up $<3.5 \mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$. We conclude that oxygen transport, aerobic demand, and oxygen affinity all play important and interrelated roles in flood tolerance and salt marsh zonation.

Introduction

Many plants are regularly confronted with hypoxic growing conditions, and soil waterlogging is the most common cause of plant oxygen deficiency (Vartapetian and Jackson 1997). Wetlands occupy 6% of the world's land area (Armstrong et al. 1994). Historically, over 15% of U.S. soils are affected by flooding, and 18.5% of all insurance claims for crop loss in the U.S. from 1939 to 1978 resulted from excess water or flooding damage (Boyer 1982). Investigations of plants adapted to chronic flooding can provide insights into physiological characteristics that are related to the ability or lack of ability to tolerate hypoxia and anoxia.

Several physiological processes have been identified that function to supply oxygen to submerged tissues and/or reduce respiratory demands in waterlogged plants (Vartapetian and Jackson 1997). Most notably,

Communicated by P. W. Sammarco, Chauvin.

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many flooding-tolerant and wetland plants counter oxygen deficiency by forming aerenchyma (Jackson and Armstrong 1999). Aerenchyma tissue forms a conduit through plants that allows ventilation of submerged tissues (Armstrong 1979). A supply of oxygen in submerged tissue is necessary to support aerobic respiration (Burdick and Mendelssohn 1987); permanent structural damage and organelle breakdown rapidly occur in anoxic root cells (Linthurst 1980; Vartapetian and Jackson 1997). The fate of oxygen once it reaches submerged tissues may also be important. Waterlogged plants may benefit by forming suberized exodermal layers in roots to prevent loss of oxygen to reduced rhizosphere sediments (Enstone and Peterson 2005). Alternatively, plant roots in some species release oxygen to rhizosphere sediments to oxidize phytotoxins to less harmful chemical species (Mendelssohn and Postek 1982; Vartapetian and Jackson 1997; Lee et al. 1999). In either case, plant growth in reduced sediments would benefit from high rates of oxygen transport.

Soil waterlogging is prominent in estuarine conditions and is a major factor determining zonation of salt marsh plant species (Pennings et al. 2005; Silvestri et al. 2005). One well-known example of plants tolerant of flooded conditions is the genus *Spartina* (Poaceae). *Spartina* flourishes in low intertidal mudflats and tidal channels characterized by highly reducing conditions (Eh is often < -200 mV; Brix and Sorrell 1996). The ability of *Spartina* to colonize these low marsh areas is likely due to physiological adaptations to resist anoxic and sulfidic conditions typical of estuarine mudflats.

Previous studies have suggested that estuarine zonation is influenced by oxygen demand and the ability to transport oxygen (Gleason and Zieman 1981; Bertness 1991; Pennings et al. 2005; Silvestri et al. 2005; Maricle et al. 2006). Oxygen transport has been well documented in *S. alterniflora* Loisel. (Teal and Kanwisher 1966; Mendelssohn and Postek 1982; Hwang and Morris 1991; Arenovski and Howes 1992; Howes and Teal 1994) and *Spartina anglica* C.E. Hubbard (Maricle and Lee 2002; Lee 2003), but no studies have compared oxygen transport rates between high-intertidal and low-intertidal marsh species. Additionally, metabolic characteristics related to respiration and oxygen affinity may also influence zonation in salt marsh plants. In hypoxic sediments, the affinity of plant root tissues for oxygen can potentially be important for survival. Low respiration rates and a high affinity for oxygen within root tissue would be beneficial in hypoxic sediments, but these characteristics have not been previously investigated in wetland plants.

To understand the role of oxygen status in salt marsh zonation, oxygen affinities and rates of respiration and internal oxygen transport were measured and compared between species from different intertidal zones of estuaries. The emergent low marsh species *S. alterniflora* and *S. anglica* were measured and compared to the middle and high marsh species *S. patens* (Ait.) Muhl., *S. densiflora* Brongn., *S. foliosa* Trin., a *S. alterniflora* × *S. foliosa* F1 hybrid, *Spartina spartinae* (Trin.) Merr. ex A.S. Hitchc. and *Distichlis spicata* (L.) Greene. These marsh species were also compared to the freshwater wetland species *S. pectinata* Bosc ex Link as well as three crop grasses, including the wetland species rice (*Oryza sativa* L.) as well as the upland species corn (*Zea mays* L.) and oat (*Avena sativa* L.). The study was designed to address the following questions: what are the rates of oxygen transport and does oxygen transport in excess of aerobic respiration occur? Do low marsh species express adaptations to lower aerobic respiratory demand compared to high marsh species? Do low marsh species have a higher oxygen affinity than high marsh species? It was anticipated that the answers to these questions could provide substantial insight into the mechanisms influencing estuarine zonation, as well as more general mechanisms influencing flooding tolerance across wetland plants.

Materials and methods

Plant material and growth conditions

Experimental plants were collected from the field sites listed in Table 1 and subsequently maintained in a greenhouse. Physiological comparisons were made between these marsh species as well as the upland species oat and corn and the wetland species rice, all grown from commercial seed.

Greenhouse temperatures were maintained at 26°C day/18°C night. Natural lighting provided a photosynthetic photon flux density (PPFD) that averaged 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during daylight hours and peaked around 1,400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on sunny days. Daughter tillers from field-collected plants were potted individually in a 50:50 (vol.:vol.) sand:potting soil mixture and were watered to saturation twice weekly with modified Hoagland nutrient solution (Epstein 1972). Freshly potted plants were placed into large plastic tubs (12 pots per tub in 23 tubs in an unbalanced block design) and were allowed 30 days to recover from potting before flooding. Growth conditions simulated estuarine conditions. All plants were flooded with enough water

Table 1 The plant species used in the study

Species	Ecological functional type	Collected from
<i>Spartina alterniflora</i>	Low marsh ^{B, C, D}	Willapa Bay, WA
<i>Spartina anglica</i>	Low marsh ^{A, C}	Puget Sound, WA
<i>Spartina densiflora</i>	Mid-high marsh ^F	Odeil Salt marshes, SW Spain
<i>Spartina foliosa</i>	Mid-high marsh ^{E, G}	San Francisco Bay, CA
<i>S. alterniflora</i> × <i>S. foliosa</i> F1 hybrid	Mid-high marsh ^{G, I}	San Francisco Bay, CA
<i>Spartina spartinae</i>	Mid-high marsh	Galveston Bay, TX
<i>Spartina patens</i>	Mid-high marsh ^{D, H}	Gulf Coast, NW FL
<i>Distichlis spicata</i>	Mid-high marsh ^{E, H}	Puget Sound, WA
<i>Spartina pectinata</i>	Inland wetland ^H	Butler County, NE
Rice (<i>Oryza sativa</i>)	Inland wetland	Commercial seed
Corn (<i>Zea mays</i>)	Upland	Commercial seed
Oat (<i>Avena sativa</i>)	Upland	Commercial seed

Included is an approximate ecological functional type of each species, based on ecological observations and published tidal ranges [where known: ^AFrenkel (1987); ^BMcKee and Patrick (1988); ^CSayce and Mumford (1990); ^DBertness (1991); ^EZedler et al. (1999); ^FCastillo et al. (2000); ^GAyres et al. (2004); ^HNatural History of Nova Scotia (2004); ^IMaricle (personal observation)], and collection location

to submerge plants to a level 2 cm above the soil surface (about 12 L per tub) and the water was completely replaced weekly. Plants received water containing 0 or 10‰ salt (Instant Ocean salts; Aquarium Systems, Mentor, OH, USA). Plants grew 30–60 days under experimental flooding conditions before testing. The appearance of black metal sulfide precipitation in the tubs was observed during this time, indicating sulfate reduction and redox potentials <−150 mV (Ponnamperuma 1972) and therefore a reasonable representation of field conditions. Corn and oat plants did not survive long-term flooding. These plants were flooded for 6–8 days before testing.

Measures of oxygen consumption and oxygen transport

Rates of oxygen consumption were determined in a closed-chamber respirometer. Plants were carefully removed from the soil and all soil particles were rinsed away from the roots. Plant roots were soaked overnight in a solution containing 1 g L^{−1} of both penicillin G and streptomycin sulfate and 50 mg L^{−1} chloramphenicol to sterilize the roots. The following day, roots of intact plants were suspended in a flask of the same antibiotic solution to prevent bacterial respiration during plant measurements. 125 or 250 mL Erlenmeyer flasks were used, depending on plant size. Stems of plants were wrapped with Parafilm M (American National Can Co., Menasha, WI, USA) and were threaded through a hole in a stopper. The plant roots were suspended in the flask and the stopper was fit snugly into the flask opening. Several drops of paraffin oil were then applied around the Parafilm seal and between plant tillers to ensure an airtight seal, similar to the usage of vacuum grease by

Hwang and Morris (1991). During measurement, these flasks were stirred with a magnetic stir bar at 75 rpm. Dissolved oxygen concentrations within the flask were measured with a fiber optic oxygen-sensing probe (FOXY-R probe; Ocean Optics Inc., Dunedin, FL, USA). The probe was calibrated with air-saturated water [257 μM or 159 torr (mmHg) O₂ at 25°C] and a Na₂S₂O₄ solution (0 μM or 0 torr O₂).

During testing, plants were placed under a 250 W metal halide grow light (Hydrofarm gardening products; Petaluma, CA, USA). At plant level, PPFD was about 150 μmol m^{−2} s^{−1} and air temperature was 28°C. Flask dissolved oxygen levels started at 120 μM (74 torr) oxygen. Plants were then allowed to consume oxygen from the flask water until dissolved oxygen levels were drawn down to 0 μM, typically requiring 1–3 h (Fig. 1a). Flask oxygen consumption was linear until nearing *P*_{crit} (the concentration where oxygen became limiting for respiration). This steady-state decrease of dissolved oxygen allowed oxygen consumption rates (i.e., respiration) to be calculated, standardized to fresh root weight.

Spartina plants have been shown to transport oxygen internally to support respiration in submerged tissues. Therefore, each plant was subjected to three treatments during measurement: (1) shoots of plants were first exposed to air (20.95% or 159 torr O₂). (2) The shoots of plants were then enclosed within a 100% N₂ atmosphere (0% O₂). (3) Finally the shoots of plants were removed with scissors and the stumps were sealed with paraffin oil. Treatments (2) and (3) prevent the entry of atmospheric oxygen into the plants' aerenchyma systems (Armstrong 1964; Teal and Kanwisher 1966), allowing aerenchyma function to be investigated. However, placing the shoot of a plant into an atmosphere of 0% O₂ while the roots remain in a

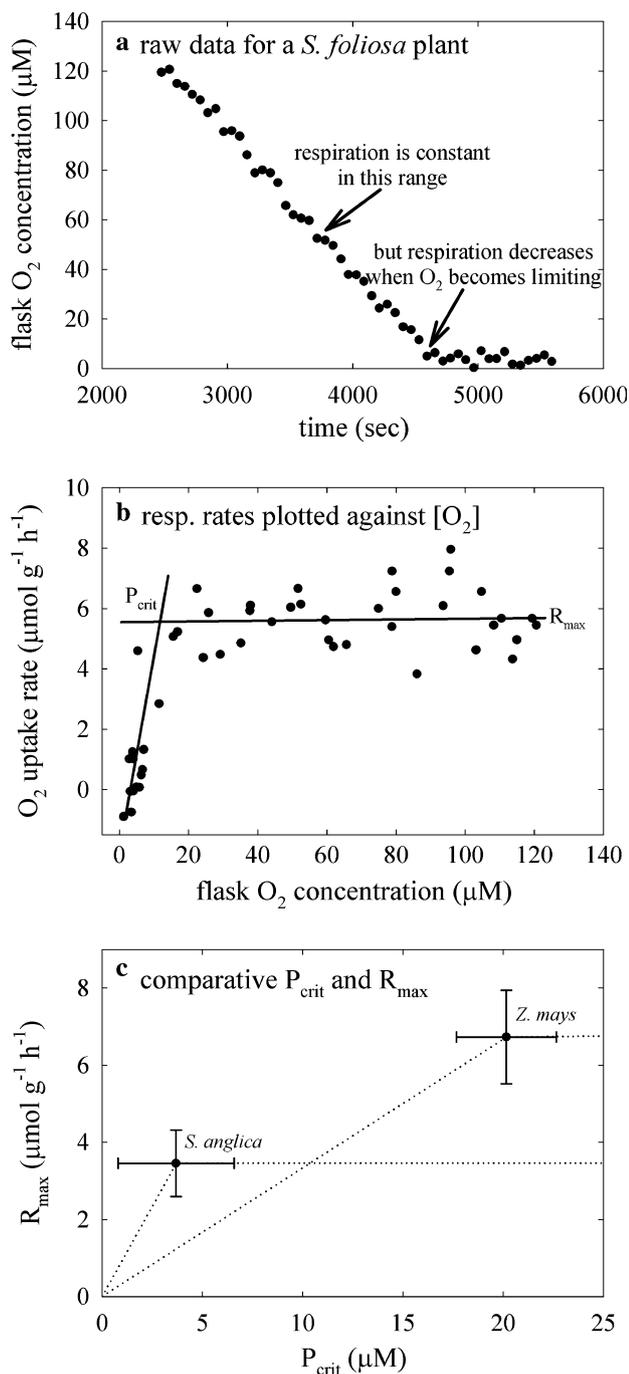


Fig. 1 Methodology for finding respiration rates and P_{crit} values of plants. **a** Data series for oxygen consumption by one *S. foliosa* plant from the experimental flask. Oxygen consumption was linear (constant) until respiration became limited by oxygen shortage. Respiration was found from the slope of the linear portion of this graph, scaled to flask volume and root mass. **b** A biphasic relationship was found when respiration rates were plotted against flask oxygen levels. When oxygen was not limiting, maximum respiration rates were observed (R_{max}). The point where oxygen became limiting for respiration is the critical oxygen pressure (P_{crit}), found by maximizing r^2 values in the separate phases of the graph. **c** The mean P_{crit} and R_{max} are shown for *S. anglica* and *Z. mays*, representing a contrast between a low marsh species and an upland species

- (a) Maximal root respiration rates were calculated by measuring the consumption of oxygen by plants with shoots removed and stumps sealed with paraffin oil. This condition represented maximal oxygen uptake by the roots from the flask medium, since respiration rates were no longer supplemented by internal oxygen transport. Rates of oxygen flux were scaled to flask volume and standardized to g fresh root weight (Fig. 1a).
- (b) Oxygen transport rates under flask conditions were calculated by finding the difference between root oxygen consumption rates under normal (20.95% O_2) conditions and sealed-stump conditions. The sealed stump prevents the entry of oxygen into a plant's aerenchyma system; therefore, the difference between these two flux rates equals the amount of oxygen transported internally through the plant (Lee 2003). Rates of oxygen transport were standardized to g fresh root weight.
- (c) The conductance of oxygen through the plant was calculated according to the general equation: oxygen flux (J_{O_2} ; from previous paragraph) = conductance (g) \times concentration gradient. Therefore $g_{O_2} = J_{O_2}/O_2$ concentration gradient. The inward conductance of oxygen through plants (g_{in}) was found by subtracting the oxygen consumption rate of the plant exposed to air from the oxygen consumption rate of the plant with the sealed stump and dividing this by the average O_2 concentration gradient between the two treatments (122 torr; see Eq. 1 below). This difference represents the maximal inward flux of oxygen since the atmospheric (159 torr) and flask (37 torr) oxygen concentrations are at their greatest difference. g_{out} was found by subtracting the oxygen consumption rate of the plant with the sealed stump from the oxygen consumption rate of the plant under 100% N_2 and dividing

solution of 120 μM O_2 reverses the normal diffusional gradient of oxygen within the plant. This would allow oxygen to diffuse up through the plant and be released into the headspace surrounding the shoots and/or be consumed by respiratory processes in leaf tissue. Cutting off shoot tissue and sealing the stems with paraffin oil stops the flow of oxygen in both directions. Steady-state measurements of flask oxygen consumption under these three conditions allowed calculation of the following:

this by the average O_2 concentration gradient between the two treatments (37 torr; Eq. 2). This difference represents the maximal outward flux of oxygen since the atmospheric (0 torr) and flask (37 torr) oxygen concentrations are at their greatest difference to facilitate an outward flow.

We propose an equation to calculate internal (lacunar) pressurization from conductance measures. The conductances can be expressed as follows:

$$g_{in} = \frac{O_2 \text{ consumption rate under sealed stump} - O_2 \text{ consumption rate under air}}{pO_{2\text{plant}} - pO_{2\text{flask/soil}}} = \frac{J_{O_{2in}}}{pO_{2\text{plant}} - pO_{2\text{flask/soil}}} \quad (1)$$

$$g_{out} = \frac{O_2 \text{ consumption rate under } N_2 - O_2 \text{ consumption rate under sealed stump}}{pO_{2\text{flask/soil}} - pO_{2N_2\text{atm}}} = \frac{J_{O_{2out}}}{pO_{2\text{flask/soil}}} \quad (2)$$

Since the term $pO_{2N_2\text{atm}}$ in equation 2 = 0 torr, and the partial pressure of oxygen in the plant (Eq. 1) can be expressed as:

$$pO_{2\text{plant}} = \frac{\text{internal pressure (torr)}}{760 \text{ torr}} \times pO_{2\text{atm}} \quad (3)$$

We obtain the following equation by substituting the above into equations (1) and (2), then setting $g_{in} = g_{out}$:

$$\frac{J_{O_{2in}}}{\left(\frac{\text{internal pressure}}{760} \times pO_{2\text{atm}}\right) - pO_{2\text{soil}}} = \frac{J_{O_{2out}}}{pO_{2\text{flask/soil}}} \quad (4)$$

One can solve algebraically for plant internal pressure:

$$\text{plant internal pressure (torr)} = \frac{\left(\frac{J_{O_{2in}} \times pO_{2\text{soil}}}{J_{O_{2out}}} + pO_{2\text{flask/soil}}\right) \times 760 \text{ torr}}{pO_{2\text{atm}}} \quad (5)$$

Measures of P_{crit}

Measures of oxygen uptake rates near 0 $\mu\text{M } O_2$ demonstrate the affinity of the plant for oxygen (Saglio et al. 1984). Rates of sealed-stump oxygen consumption were plotted against flask oxygen concentration (Fig. 1b). These plots were biphasic: for the majority of the range of oxygen concentrations in the flask, uptake rates were constant. When flask oxygen levels dropped below a certain point, uptake rates began to drop lin-

early. The critical oxygen pressure (P_{crit}) was taken to be the intersection point of the two phases of the graph, representing the point where respiration rates first became limited by oxygen levels (Mickel and Childress 1982). This intersection point was found by maximizing r^2 values on regression lines plotted through the two phases of the data (Fig. 1b; Yeager and Ultsch 1989).

Using the values for maximum respiration rate and P_{crit} measured in the present study, the slope of the P_{crit} graph between 0 and P_{crit} was calculated for each plant. This slope is the magnitude of the effect of oxygen concentration on respiration under oxygen-limited conditions, a measure of the sensitivity of respiratory processes to oxygen limitation.

Statistical analyses were performed between species with one-way analysis of variance (Proc GLM; SAS

version 8.0, 2001 SAS Institute Inc., Cary, NC, USA; $\alpha = 0.05$). Since there were no effects of salinity on any of the parameters measured (ANOVA, $P \geq 0.459$), 0 and 10‰ salinity treatments were grouped together for comparisons between species. In these models, plants were blocked by tub. Fisher's protected LSD was used for multiple comparisons. Comparisons between ecological functional groups were performed with one-way ANOVA tests comparing species means (low marsh $n = 2$, middle to high marsh $n = 6$, inland wetland $n = 2$, and upland $n = 2$).

Results

Differences between species in oxygen transport rates were apparent in some cases. Despite a fairly high degree of individual variability the data show that *S. anglica* had greater rates of oxygen transport compared to all species except rice (Fig. 2; ANOVA, $P \leq 0.059$). All other species had lower rates of transport, including the low marsh species *S. alterniflora* as well as all higher marsh species, the inland *S. pectinata*, and the crop species oat and corn (Fig. 2). Ecological functional types were not significantly different with respect to one another (ANOVA, $P = 0.178$).

Plant conductance to oxygen (g_{O_2}) paralleled rates of oxygen transport, ranging from slightly negative values (taken to be zero) up to $0.017 \mu\text{mol g}^{-1} \text{h}^{-1} \text{ torr}^{-1}$ in *S. anglica* (Table 2). Even though the flask conditions in this study did not replicate the highly reducing character of estuarine mudflats, these conductance values allow calculations of oxygen transport under field conditions with use of the general formula "oxygen flux (J_{O_2}) = conductance (g) \times concentration gradient". A larger oxygen gradient exists between atmosphere and estuarine sediment under field conditions (159 torr) compared to plants suspended in water under laboratory conditions (122 torr). Rates of oxygen transport in the field may be substantially larger than those measured in the lab, but may be estimable from conductance values such as these.

In contrast to results presented for *S. alterniflora* by Hwang and Morris (1991), there was no evidence for internal pressurization within any species in this study. The ratio internal/ambient pressure in most plants ranged from 0.31 to 0.73 (data not shown). It is possible that the light levels ($150 \mu\text{mol m}^{-2} \text{s}^{-1}$) or temperature (28°C) under lab conditions were not sufficient to allow hygrometric pressurization in these plants, despite modest pressurization in *S. alterniflora* under $165 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 31°C reported by Hwang and

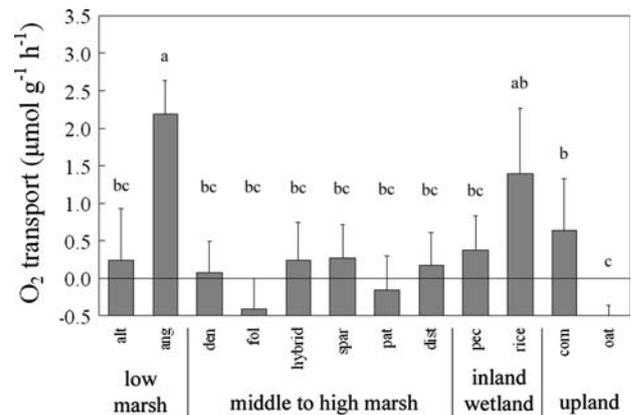


Fig. 2 Rates of internal oxygen transport ($\mu\text{mol g}^{-1} \text{h}^{-1}$) for the species in the study, grouped by ecological functional type. Oxygen transport rates were found by comparing oxygen consumption around root tissue when plants were exposed to air and when shoots were cut off and sealed with paraffin oil, thereby stopping the flow of oxygen. Shown is the least squares mean of 3–15 plants \pm s.e. Letters indicate significant differences between species at $\alpha = 0.05$. Species abbreviations are as follows: *alt* *Spartina alterniflora*, *ang* *S. anglica*, *den* *S. densiflora*, *fol* *S. foliosa*, hybrid *S. alterniflora* \times *S. foliosa* F1 hybrid, *spar* *S. spartinae*, *pat* *S. patens*, *dist* *Distichlis spicata*, *pec* *S. pectinata*, rice *Oryza sativa*, corn *Zea mays*, and oat *Avena sativa*

Morris (1991). Further work may be required to validate Eq. 5 presented above.

Root P_{crit} values closely followed differences in ecological functional type (Fig. 3a). The low marsh species *S. anglica* and *S. alterniflora* showed the highest affinity for oxygen. While some differences were measured between species, comparisons between functional groups illustrate important ecological differences. P_{crit} in low marsh species ranged from 3.7 to $4.5 \mu\text{M}$, significantly lower than the inland and crop species in this study (ANOVA, $P \leq 0.050$), with P_{crit}

Table 2 The inward conductance of each plant species to internal oxygen transport, given in $\mu\text{mol g}^{-1} \text{h}^{-1} \text{ torr}^{-1}$

Species	Conductance to oxygen ($\mu\text{mol g}^{-1} \text{h}^{-1} \text{ torr}^{-1}$) mean \pm std error (n)
<i>S. alterniflora</i>	0.003 ± 0.005 (5)
<i>S. anglica</i>	0.017 ± 0.007 (13)
<i>S. densiflora</i>	0.001 ± 0.002 (14)
<i>S. foliosa</i>	-0.003 ± 0.004 (15)
<i>S. alterniflora</i> \times <i>S. foliosa</i> hybrid	0.002 ± 0.002 (9)
<i>S. spartinae</i>	0.002 ± 0.002 (12)
<i>S. patens</i>	-0.001 ± 0.001 (11)
<i>D. spicata</i>	0.001 ± 0.001 (13)
<i>S. pectinata</i>	0.003 ± 0.002 (11)
Rice	0.011 ± 0.013 (3)
Corn	0.005 ± 0.001 (5)
Oat	-0.004 ± 0.004 (5)

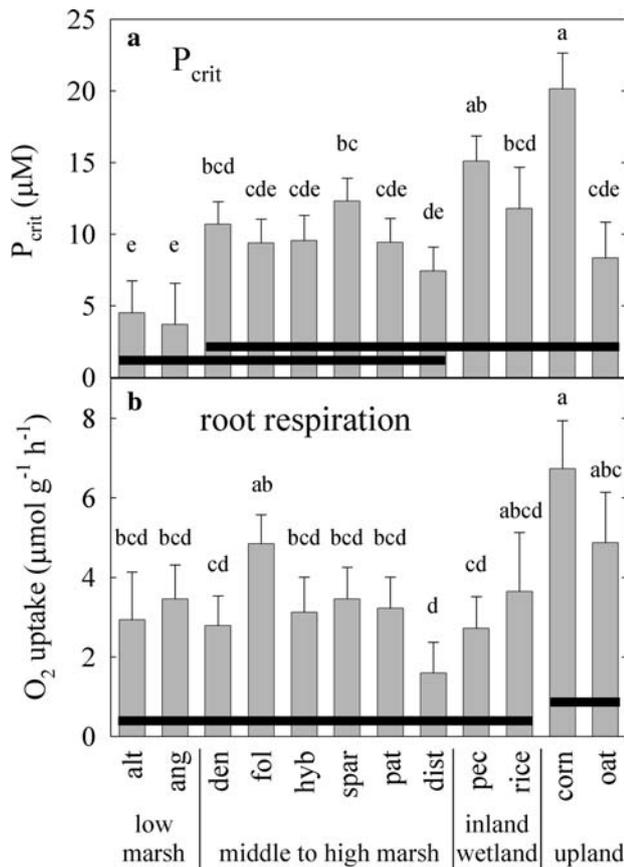


Fig. 3 Oxygen affinity and respiration rates of the plants in this study, grouped by ecological functional type. Measurements were taken after plant shoots had been removed and the stumps were sealed with paraffin oil. **a** The affinity for oxygen is shown as the critical oxygen pressure (P_{crit} ; in μM), the point where dissolved oxygen levels became limiting for respiration. **b** Respiration was measured as the rate of oxygen uptake by root tissues ($\mu\text{mol g}^{-1} \text{h}^{-1}$). Shown is the least squares mean of 3–15 plants \pm s.e. Letters indicate significant differences between species at $\alpha = 0.05$. Black bars connecting the individual species means indicate significant differences between ecological functional groups at $\alpha = 0.05$. Species are labeled as in Fig. 2

values ranging from 11.8 μM in rice to 20.2 μM in corn. Middle and high marsh species had intermediate P_{crit} values ranging from 7.4 to 12.3 μM .

Rates of aerobic respiration in roots ranged from 1.6 $\mu\text{mol O}_2 \text{g}^{-1} \text{h}^{-1}$ in *D. spicata* to 6.7 $\mu\text{mol O}_2 \text{g}^{-1} \text{h}^{-1}$ in corn (Fig. 3b). Flooding-sensitive corn and oats took up 3.6–6.7 $\mu\text{mol O}_2 \text{g}^{-1} \text{h}^{-1}$ while all marsh species except *S. foliosa* took up <3.5 $\mu\text{mol O}_2 \text{g}^{-1} \text{h}^{-1}$. The upland functional group had significantly higher respiration rates compared to all other functional groups (ANOVA, $P = 0.050$). This suggests lower aerobic demand exists in plants adapted to waterlogging compared to flooding-sensitive plants.

The relationship between oxygen concentration and oxygen uptake was determined for all species, and this

relationship was used to calculate the rate of respiration under hypoxic conditions. Oxygen demand and oxygen affinity governed how plants responded to falling oxygen levels. For example, when external oxygen concentrations fall to 1 or 3.5 μM , the low marsh species maintained respiration rates significantly higher than species from other functional types (ANOVA, $P \leq 0.050$; Fig. 4a, b). Therefore, low marsh species are capable of appreciably higher rates of respiration under hypoxic conditions than other species, due to low P_{crit} values. This advantage is lost at higher oxygen levels, when species from higher marsh areas are no longer limited by oxygen (ANOVA, $P = 0.139$; Fig. 4c).

Discussion

Differential abilities to transport oxygen have long been assumed to regulate species zonation across estuaries (Gleason and Zieman 1981; Bertness 1991; Pennings et al. 2005; Silvestri et al. 2005). If this were true, then oxygen transport capabilities would follow a pattern of low marsh > high marsh > inland crop species. Although *S. anglica* exhibited high rates of oxygen transport, there was no apparent correlation between oxygen transport and zonation across the remaining species. The low marsh species *S. alterniflora*, the middle and higher marsh species, and the crop species corn and oat showed little or no ability to transport oxygen under laboratory conditions. The freshwater crop species rice showed a strong ability to transport oxygen. Rice is grown under long-term flooded conditions in paddies, suggesting a need for efficient oxygen transport. The superior ability of *S. anglica* and rice plants to tolerate flooded sediments is at least partly due to high rates of internal oxygen transport. However, aside from *S. anglica* and rice, no other species had a mean oxygen transport rate greater than 0.6 $\mu\text{mol g}^{-1} \text{h}^{-1}$, despite measurements of all ecological functional types. Therefore other physiological factors are also likely involved in flooding tolerance and estuarine zonation.

A low aerobic demand may be beneficial in sediment conditions where oxygen is limiting for respiration. Aerobic demand appeared to differ across estuarine functional types, similar to cytochrome *c* oxidase activities measured for *Spartina* and *Distichlis* (Maricle et al. 2006). Respiration rates in marsh species and flooding-tolerant wetland plants were significantly lower than flooding-sensitive crop species, suggesting a lower oxygen demand in plants adapted to reduced sediments. Additionally, higher respiration rates were

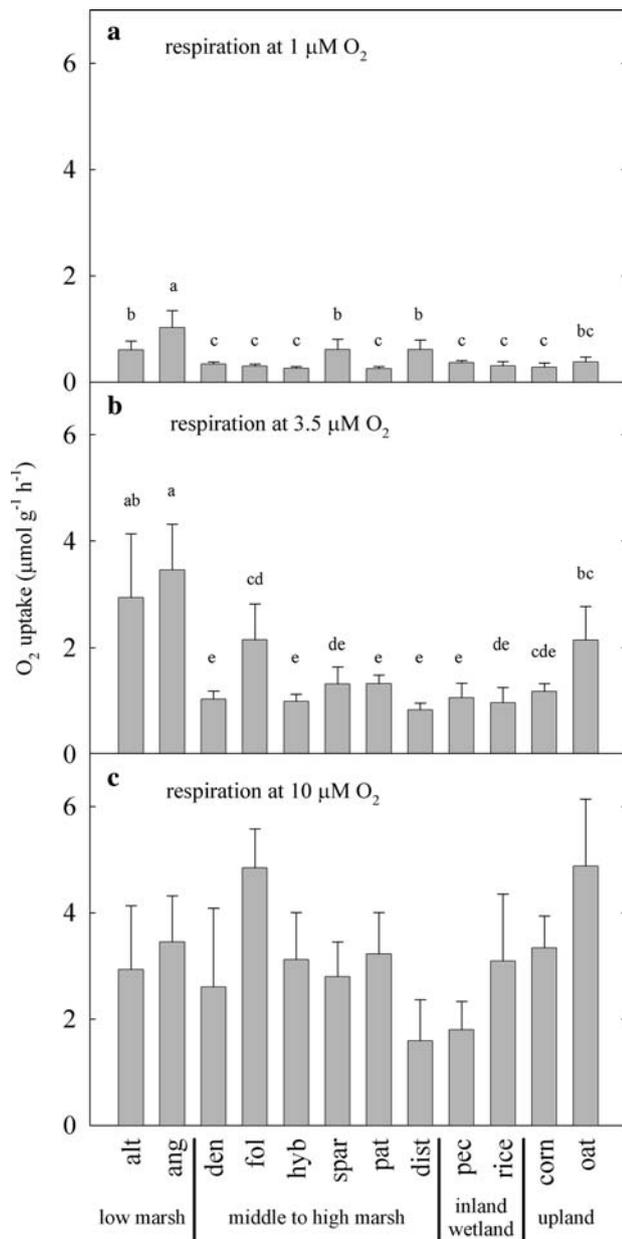


Fig. 4 The ability to take up oxygen from hypoxic substrates was examined based on measures of respiration (Fig. 3b) and P_{crit} (Fig. 3a). Shown is the respiration rate of plants when external oxygen concentration is **a** 1 μM , **b** 3.5 μM , and **c** 10 μM . Shown is the least squares mean of 3–15 plants \pm s.e. Letters indicate significant differences between species at $\alpha = 0.05$, except panel c, where there were no significant differences. Species are grouped by ecological functional type and are labeled as in Fig. 2

related to higher P_{crit} values across species (correlation, $P = 0.003$), suggesting a relationship between high respiratory rates and low oxygen affinity.

Measures of oxygen affinity were more closely related to elevational zonation than oxygen transport or respiration rates. In the present study, P_{crit} values were very low for the low marsh species *S. anglica* as well as

S. alterniflora. These values were similar to critical oxygen pressures for rice root extension reported by Armstrong and Webb (1985). However, these P_{crit} values were lower than previously published P_{crit} values for rice, *Eriophorum angustifolium* (Armstrong and Gaynard 1976), corn (Saglio et al. 1984), onion (Berry and Norris 1949), or *S. alterniflora* (Morris and Dacey 1984). These differences may result from measuring oxygen consumption from a static dissolved medium as opposed to flow-through systems or gaseous media. Nevertheless, a good relationship was observed between increasing P_{crit} and increasing elevational zonation across ecological functional types. P_{crit} values measured in low marsh species were significantly lower than those measured in the inland and crop species. Intermediate P_{crit} values were measured in the middle and high marsh species. This suggests that plants growing in highly reduced low marsh sediments may require higher oxygen affinity compared to plants like corn, onion, or even rice.

Estuarine mudflat conditions are characterized by toxic levels of hydrogen sulfide (Koch and Mendelsohn 1989). The strongly reducing character of sulfidic mudflats introduces an external sink that will compete with root respiratory process for available oxygen (Sorrell 1999). Therefore, much of the oxygen transported to root tissue is lost to rhizosphere sediments and does not support respiration. A high affinity for oxygen within root tissue can help prevent loss of oxygen to the surroundings. Consequently, low P_{crit} values within root tissues are potentially very important for survival in highly reduced sediments. While rice can survive chronic flooding conditions, it does not exhibit the low P_{crit} values observed in low marsh *Spartina*. The fresh water in rice paddies contains little sulfate so there will generally be little sulfide production in contrast to high sulfate levels in seawater inundating estuarine marshes. Consequently, paddy sediments do not have the additional oxygen sink of sulfide and plants may have less of a requirement for high oxygen affinity. Growth in sulfidic estuarine soils may necessitate increased oxygen affinity to resist sediment sulfides, consistent with the low P_{crit} values measured in marsh species.

While oxygen affinity appears to be important in tolerating anoxic sediments, the mechanisms accounting for these differences remain unknown. Oxygen diffusion rates have long been recognized as a factor limiting respiration rates (Berry and Norris 1949). Consequently, differences in P_{crit} may be due to differences in root surface area. While increased root surface area may aid rates of oxygen uptake in roots growing in aerated soils, decreased root surface area

may prevent oxygen loss to anoxic sediments. Under the conditions in this study, *Spartina* species tended to have fewer, larger roots compared to *Distichlis* and crop species (B. R. Maricle and R. W. Lee unpublished observations). Additionally, roots in flooded plants tend to be shorter in length than corresponding non-flooded plants to provide a shorter diffusional path for oxygen to reach root tips (Luxmoore et al. 1970). Flood-tolerant species may be adapted to keep root surface area small. However, very little is known in this area, making it difficult to draw conclusions about differences in P_{crit} . Measurements of root length and surface area and how they relate to P_{crit} and flooding tolerance may be an interesting area for future research.

Measures of oxygen affinity can provide indications of how plants will perform in hypoxic conditions. Respiration rates in plants from higher marsh areas and inland and crop species were quite sensitive to decreasing oxygen concentrations, corresponding with the high P_{crit} values observed in these species. In contrast, the low marsh species *S. anglica* and *S. alterniflora* were able to take up significantly more oxygen under hypoxic concentrations compared to high marsh species. Thus, one can conclude that low respiration rates and a high affinity for oxygen in root tissues are beneficial in hypoxic sediments. Sediment oxygen concentrations must drop below 4 μM before respiration rates are affected in *S. anglica* or *S. alterniflora*. In contrast, the high respiration rates in corn become inhibited when oxygen falls below 20 μM (Fig. 1c). This suggests that while differences among marsh species are correlated with habitat preference, low marsh species are able to maintain normal aerobic respiration despite potential oxygen limitation in other species.

Of all the species investigated in the present study, it appears that only *S. anglica* can maintain an aerobic poise under anoxic conditions. The highly reducing character of estuarine mudflats requires numerous physiological adaptations to maintain an aerobic existence. Soil redox potentials indicate that low marsh sediments are anoxic (Howes et al. 1981; DeLaune et al. 1983). Oxygen to support root respiration cannot be supplied from anoxic rhizosphere sediments, so all oxygen in submerged tissues must come from above ground tissues. Under anoxic conditions (0 torr O_2), *S. anglica* would be expected to transport 2.7 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$, based on conductance values presented in Table 2. If all of this oxygen is kept within roots, this is nearly enough to support the maximal respiration rate of 3.5 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$. Under these conditions, aerobic respiration rates would be greater than anaerobic

respiration. In practice, however, anaerobic respiration capacities are normally much greater than aerobic respiration capacities (Maricle et al. 2006), illustrating the importance of external oxygen sinks under water-logged conditions.

Upland or freshwater plants cannot sustain aerobic processes under anaerobic conditions. Under such conditions (0 torr O_2) corn would be expected to transport 0.8 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ and rice would be expected to transport 1.8 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$, far below their maximal respiration rates of 6.7 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ and 3.8 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$. A plant that cannot maintain adequate oxygen levels in root tissue would be forced to rely on anaerobic metabolism or exhibit reduced ATP demand. Extended fermentation is energetically limiting, and no plant can endure long periods of anoxia in tissues (Crawford 1982). The corn and oat plants could not survive under the long-term flooded conditions in this study, apparently due to oxygen deficiency within tissues and/or a low oxygen affinity in roots. Under field conditions, the highly reducing character of estuarine sediments will cause oxygen shortages even in tolerant low marsh species like *S. alterniflora* (Mendelssohn et al. 1981). Therefore, the capacity to respire anaerobically is important for growth in anoxic sediments. Indeed, one factor suggested to influence flooding tolerance is the ability to express sufficient levels of alcohol dehydrogenase (Crawford 1967).

Wetland plants will often experience oxygen in low amounts due to aeration processes (Sorrell et al. 2000). Under these hypoxic conditions, high oxygen transport and/or high oxygen affinity are advantageous. If oxygen levels are 3.5 μM , the respiratory processes in the low marsh species *S. anglica* would be nearly saturated with oxygen resulting in an uptake rate of 3.5 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$. With the addition of internal oxygen transport rates of 2.6 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$, oxygen needs would be readily fulfilled. Conditions such as this may result in excess oxygen and sediment oxidation, as observed in sediments under tall-form *S. alterniflora* (Howes et al. 1981). In 3.5 μM oxygen, rice plants would only be able to take up 1.0 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ in addition to transporting 1.7 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$. This total is still below rice's maximal respiration rate of 3.6 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$, potentially forcing rice plants to respire anaerobically to make up for the deficit of 0.9 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$. However, it should be noted that plant respiration is likely to deplete oxygen levels in interstitial porewaters. This may increase needs for anaerobic metabolism. Under 3.5 $\mu\text{M O}_2$, corn plants would only be able to take up 1.2 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ and transport 0.8 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$. This total is far below corn's maximal respiration rate of 6.7 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$, creating a large

oxygen deficit in submerged tissues that will likely lead to the death of the plant. Corn is generally regarded as a flooding tolerant plant (e.g., Bray et al. 2000). However, external oxygen lower than about 20 μM corresponds to an oxygen limited condition that may potentially reduce root metabolism and even growth.

Environmental demands change considerably when considering upland soils. Sediment redox potentials will be $> +350$ mV (oxic) in well-drained soils. Oxic soils can supply oxygen for root respiration, lowering the need for high oxygen affinity or high internal oxygen transport. Upland species may then benefit from increased root surface area that allows increased oxygen uptake to support high respiration rates.

In conclusion, a combination of low respiration rates, high oxygen affinity, and high oxygen transport rates may enable *Spartina* to colonize anoxic estuarine areas. While measures of respiration and oxygen affinity under hypoxia have not generally been applied to plants, we feel that they provide insight into physiological capabilities that facilitate utilization of more efficient aerobic respiration in oxygen poor environments. These factors appear to influence flooding tolerance in salt marsh grasses. Therefore, these factors also influence species zonation across estuarine areas. However, species distributions are dependent on a complex mixture of biotic and abiotic components (Pennings and Callaway 1992; Pennings et al. 2005). Other environmental factors may also be important in estuarine species zonation. Highly reduced sediments can lead to the formation of sulfides at toxic levels (Pearson and Havill 1988; Pezeshki et al. 1988; Koch and Mendelssohn 1989; Koch et al. 1990). Marsh plants like *Spartina* are able to resist environmental sulfides (Lee et al. 1999; Lee 2003; Maricle et al. 2006) at levels that may be toxic to other anoxia-tolerant plants like rice (Allam and Hollis 1972; Hollis et al. 1972). Additionally, soil salinity levels are an important factor that can influence species composition within middle and upper intertidal areas of estuaries (Mahall and Park 1976; Pennings and Callaway 1992; Crain et al. 2004).

Spartina anglica grows lower in the intertidal zone than any other emergent vascular species. Its success in these areas appears to be the result of high rates of oxygen transport, low aerobic demand, and a high affinity for oxygen. Similar physiological strategies may influence flooding tolerance across other varieties of wetland plants. Low respiration rates, high oxygen affinity, and high rates of oxygen transport would be universally beneficial in oxygen-deficient sediments.

Acknowledgments The authors thank Paul Rabie for help with statistics; and Kim Patten, Sally Hacker, Eric Hellquist,

M. Enrique Figueroa, Steve Pennings, Chuck Cody, Maurice Ku, Renae Micek, Heather Davis, and Debra Ayres for providing plants. This project was partially funded from the Betty W. Higinbotham Trust and the Biddulph Summer Research Award to BRM. This research was also supported by NSF IBN0076604 and EPA R-82940601. All experiments complied with U.S. laws.

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