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# Aerenchyma development and oxygen transport in the estuarine cordgrasses *Spartina alterniflora* and *S. anglica*

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## Abstract

*Spartina alterniflora* and *Spartina anglica* are intertidal cordgrasses that have the capacity to develop extensive aerenchyma systems. Aerenchyma may supply submerged portions of the plant with atmospheric oxygen as well as lower metabolic demands of the plant. These physiological benefits help to make *Spartina* grasses formidable invasive species in areas where they have been introduced. Aerenchyma development was investigated in *S. alterniflora* and *S. anglica* maintained in greenhouse experiments under flooded and drained soil conditions. Amounts of aerenchyma along the lengths of roots were calculated from digital images of serial root cross-sections using image analysis software. Maximal aerenchyma formation occurred in *S. alterniflora* following exposure to flooded conditions, while aerenchyma in *S. anglica* did not increase under the same conditions. Aerenchyma function was investigated by testing individual *Spartina* plants for their ability to transport oxygen from leaves to roots. Oxygen transport capacities provided information about the plants' oxygen demands and the overall effectiveness of their aerenchyma systems. *S. anglica* plants were able to transport substantial oxygen to their roots, but no oxygen transport was detected in *S. alterniflora* plants under the same conditions. Increased aerenchyma formation in flooded *S. alterniflora* did not enhance oxygen transport and may function primarily in reducing metabolic oxygen demands.

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## 1. Introduction

Plants growing under submerged conditions often experience oxygen deficiencies at root tissues (Jackson and Armstrong, 1999). Regularly flooded soils remain highly reduced

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because the diffusion rate of oxygen through water is about 10,000 times slower than through air (Armstrong et al., 1994), and most available oxygen is utilized by microorganisms within the top few millimeters of soil (Teal and Kanwisher, 1966).

Many flooding-tolerant and wetland plants counter oxygen deficiency by forming aerenchyma that enhances metabolic efficiency and facilitates internal oxygen transport (Armstrong, 1979; Armstrong et al., 1991; Jackson and Armstrong, 1999). The benefits of aerenchyma are two-fold. First, aerenchyma reduces the volume of respiring tissue, lowering the metabolic demands of the plant. Secondly, aerenchyma lacunae can form an unobstructed pathway for atmospheric oxygen transport from the leaves to the tips of the roots (Teal and Kanwisher, 1966; Arenovski and Howes, 1992), supporting respiration by submerged tissues (Williams and Barber, 1961). Some wetland plants can have as high as 60% air space in their root, shoot, and leaf cortex (Armstrong, 1979), allowing transport at rates up to two times higher than required for root metabolism (Teal and Kanwisher, 1966). Excess oxygen can also be released to the rhizosphere to oxidize phytotoxins (e.g.  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{S}^{2-}$ ) to less harmful chemical species (Mendelsohn and Postek, 1982; Lee et al., 1999).

Aerenchyma lacunae have been found to form a contiguous pipeline through plants (Howes and Teal, 1994). This can be verified by blowing into a cut-off wetland plant stub and watching air bubbles emerge from roots suspended in water (Teal and Kanwisher, 1966). The presence of lacunae greatly lowers resistance to gas movement within the plant, representing an unobstructed passageway for internal oxygen transport. Numerous means of internal oxygen transport have been elucidated in wetland plants, including simple diffusion (Armstrong, 1979), pressurized throughflow (Dacey, 1980), venturi-induced convection (Armstrong et al., 1992), and external transport through hydrophobic air layers along leaves (Raskin and Kende, 1983). Formation of aerenchyma can be induced by environmental conditions, such as hypoxia resulting from the onset of flooding. Hypoxic conditions promote the synthesis of the plant hormone ethylene from its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) (Kende, 1993). Ethylene/ACC exposure promotes the synthesis of lysigenous aerenchyma in many wetland plants (Kawase, 1976; Kawase, 1978).

The ability to transport oxygen is paramount to survival in waterlogged regions. In the present study, aerenchyma formation and oxygen transport were investigated in cordgrasses of the genus *Spartina*. *Spartina alterniflora* Loisel. is native to the East and Gulf Coasts of North America (Mobberley, 1956). In its native range, *S. alterniflora* is known as a prominent component of estuarine communities, regularly dominating intertidal habitats as far as 50°N latitude (Long et al., 1975). *Spartina anglica* C.E. Hubbard is a new species of cordgrass that first appeared in the British Isles in the late 1800s (Gray et al., 1991). This species is a fertile hybrid resulting from chromosome doubling (allopolyploidy) in sterile hybrids of accidentally introduced *S. alterniflora* and native *S. maritima* (Curtis) Fernald (Thompson, 1991).

*Spartina* grasses flourish under saline and anoxic estuarine conditions that are often uninhabitable by other plants (Teal and Teal, 1969). Due to their vigorous growth in stressful conditions, *S. alterniflora* and *S. anglica* have been introduced throughout Europe, North America, Australia, and Asia in efforts to prevent shoreline erosion (Spicher and Josselyn, 1985; Gray et al., 1991; Thompson, 1991; Gray and Raybould, 1997; Hedge et al., 1997). Founder populations have since grown and spread vigorously to overtake many estuarine

habitats, converting them into monospecific stands of *Spartina*. *S. alterniflora* was introduced into Willapa Bay, Washington in 1894 (Mobberley, 1956; Dumbauld et al., 1997), where it has currently colonized 4000–10,000 hectares (Kim Patten, pers. comm.). *S. anglica* was introduced into Puget Sound, Washington in 1961, where it has affected 3,311 hectares (Hacker et al., 2001).

Introductions of non-native species of *Spartina* into estuaries of the West Coast of North America have had deleterious ecological and economic impacts. In contrast to North American East Coast estuaries, which are generally densely populated by *S. alterniflora* and *S. patens*, West Coast estuaries often consist of vast mudflats. These mudflats represent uncolonized habitats ideal for proliferation of introduced *Spartina* (Bishop, 1997). Introduced *Spartina* can potentially out-compete native vegetation and alter estuary topography by sediment accretion (Daehler and Strong, 1996; Sanchez et al., 2001). Conversion of estuarine mudflats to *Spartina* stands results in reduced mudflat area available for foraging by shorebirds (Daehler and Strong, 1996), mariculture of oysters and clams (Bishop, 1997), and potentially stimulates production of the toxin hydrogen sulfide (Wang and Chapman, 1999) through decay of organic material that accumulates in *Spartina* stands.

Understanding why *Spartina* is such a successful invasive species and devising effective means of control are key practical issues in North America's Pacific Northwest and elsewhere in the world. To elucidate the mechanisms that enable *Spartina* to invade estuaries, we examined how *S. alterniflora* and *S. anglica* counter effects of flooding stress via aerenchyma formation. Rates of oxygen flux were also investigated from individual *S. alterniflora* and *S. anglica* plants to determine the overall effectiveness of aerenchyma and its role in oxygen transport.

### 1.1. Materials and methods

Sediment cores and associated *Spartina alterniflora* and *Spartina anglica* were collected at sites of invasion in Washington state (WA), USA, and maintained in a greenhouse for several months before initiation of experiments. *S. alterniflora* plants were collected at Willapa Bay, WA and *S. anglica* plants were obtained from Livingston Bay, WA. In the greenhouse, light and dark conditions were maintained at 14L/10D, with temperatures of 26 °C during the day and 18 °C at night. Light intensity averaged around 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during daylight hours in the study, and peaked around 1100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  on sunny days.

For experiments, daughter tillers of greenhouse plants were potted individually in a 50/50 potting soil/sand mixture, and watered twice weekly to saturation. The freshly potted plants were allowed 40 days to acclimate to growing conditions before the onset of experimental flooding treatments. During the acclimation period, the salinity of the solution used to water plants was increased incrementally (2‰ every 4 days) over the last 20 days. Full-strength solution contained 10‰ Instant Ocean salts (Aquarium Systems, Mentor, OH) and Hoagland nutrient solution (Epstein, 1972) at pH 7.5.

*S. alterniflora* and *S. anglica* plants used in experiments were randomly divided into flooded and drained treatments in a 2 × 2 factorial treatment within a randomized block design. Flooded plants were submerged in full-strength solution at a level 1 cm above the soil surface. Drained plants were watered to saturation twice weekly with the same solution. Plants were maintained for at least three weeks in their respective treatments prior to harvest.

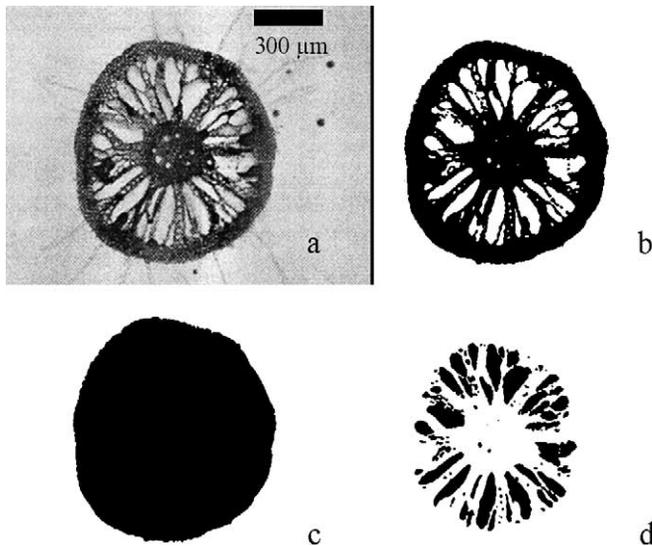


Fig. 1. Representative images used in digital quantification of root porosity. (a) Greyscale digital image of *S. anglica* root cross-section; (b) the same image as (a) converted to black and white; (c) total root cross-sectional area; (d) aerenchyma spaces. Percent aerenchyma is calculated by dividing the number of pixels in (d) by the number of pixels in (c).

At harvest, plants and roots were carefully separated from the soil mixture, and subsequently rinsed several times with 10‰ artificial seawater (ASW) to remove associated soil particles. Senescent roots (flattened, dark coloration) were removed and a subset of the remaining roots were fixed for microscopy in 0.05 M PIPES buffer (pH 7.2) containing 2% glutaraldehyde and 2% paraformaldehyde. Roots of similar size and length were sampled from each of the treatments. Root diameters at 2, 4, 6, 8, and 10 cm from the root tip were not significantly different between species or treatments (ANOVA,  $P = 0.411$ ,  $P = 0.929$ ,  $P = 0.224$ ,  $P = 0.943$ , and  $P = 0.347$ , respectively). Fixed roots were freehand sectioned to a thickness of 0.15–0.20 mm, immersed in distilled water, then viewed with an Olympus BH-2 light microscope. Images were recorded with a Microimage Video Systems digital camera (model A209).

Digital images of root cross-sections were analyzed with Scion Image 1.62a software (Scion Corporation, Frederick, MD) to measure the percentage of root area comprised of aerenchyma. Digital images of root cross-sections (Fig. 1a) were adjusted to maximize contrast by converting the image to black and white only, i.e. “thresholding” (Fig. 1b). To determine total cross-sectional area, aerenchyma spaces were filled, resulting in a solid, black silhouette (Fig. 1c), and the number of pixels was quantified. Lacunal area was determined by thresholding the original image (Fig. 1b), then inverting it to form a negative image (Fig. 1d). Pixels comprised by lacunae were then quantified. The relationship between numbers of pixels and  $\mu\text{m}^3$  was determined using digitized images of a stage micrometer.

Root sections were taken from 1 and 2 cm from the tip, and at 2 cm increments along the rest of their lengths. Two roots were sectioned from each plant, and three sections were

measured and averaged at each representative position on a root. Root porosity results at each position relative to the tip were analyzed using one-way analysis of variance (ANOVA; Minitab release 12;  $\alpha = 0.05$ ).

Representative *S. alterniflora* and *S. anglica* plants were selected from each treatment to test whether aerenchyma facilitates oxygen transport. Oxygen transport was investigated by sealing an intact plant's roots into a flask of nitrogen-flushed 10‰ ASW supplemented with antibiotics. Antibiotics consisted of Penicillin G and streptomycin sulfate at 1 g/l each, and chloramphenicol at 50 mg/l. Initial oxygen concentrations within the flask were 40  $\mu\text{M}$  ( $\pm 5 \mu\text{M}$ ). Oxygen concentrations in the flask were monitored with an oxygen-sensing probe (FOXY-R probe; Ocean Optics Inc., Dunedin, FL). Subsequent increases or decreases in flask oxygen levels were then recorded to assess oxygen release or consumption by the plant. Background rates of oxygen flux were determined in flasks without plants and were negligible and not significantly different than zero.

Additional measurements were conducted in which aerenchyma oxygen transport was blocked (Teal and Kanwisher, 1966; Lee, submitted). The flask and plants were enclosed in a large plastic container (approximately 25 l) that was continually flushed with nitrogen gas (0.55 l/min) to purge the system of oxygen. The plants were maintained in the dark to prevent evolution of photosynthetic oxygen. Oxygen consumption from the flask was then monitored. Consumption under 100% nitrogen gave a measure of total oxygen demand due to respiration of the plant, i.e. consumption not supplemented by aerenchyma supply. The rate of oxygen transport through the plant's aerenchyma system was calculated from the difference between oxygen fluxes under normal and nitrogen-flushed atmospheres, the difference representing the amount of oxygen supplied to the roots through the plant's internal aerenchyma system.

## 2. Results

The percentage of aerenchyma observed in *S. alterniflora* and *S. anglica* ranged from 0 to 35% of total root area. The amount of aerenchyma clearly increased as a function of distance from the root tip (Fig. 2). Lacuna development also appeared to be a function of root age in both *S. alterniflora* and *S. anglica*; cortex tissue near the apical meristem was almost completely devoid of aerenchyma (Fig. 2). Roots from all treatments developed little or no aerenchyma at distances  $\leq 4$  cm from the root tip, and young roots had much less aerenchyma than older counterparts from the same plant (data not shown, not used in comparisons between species/treatments). However, cortex tissue at least 6 cm from the root tip generally contained substantial aerenchyma ( $>10\%$  root area).

Parallel specific gravity measurements were performed on *Spartina* roots in this study to compare with published literature and to verify the validity of our results (Bussiere and Lee, unpublished data). Specific gravity measurements for *S. alterniflora* and *S. anglica* fell within the ranges published by Rozema et al. (1985) and Arenovski and Howes (1992).

In *S. alterniflora* roots, the amount of aerenchyma present changed in response to flooding (Fig. 3a). The percentage of air space in roots of *S. alterniflora* was significantly greater in flooded plants than drained plants at distances of 6, 8, 10, and 12 cm from the root tip (ANOVA,  $P = 0.027$ ,  $P = 0.008$ ,  $P < 0.001$ , and  $P = 0.001$ , respectively). On

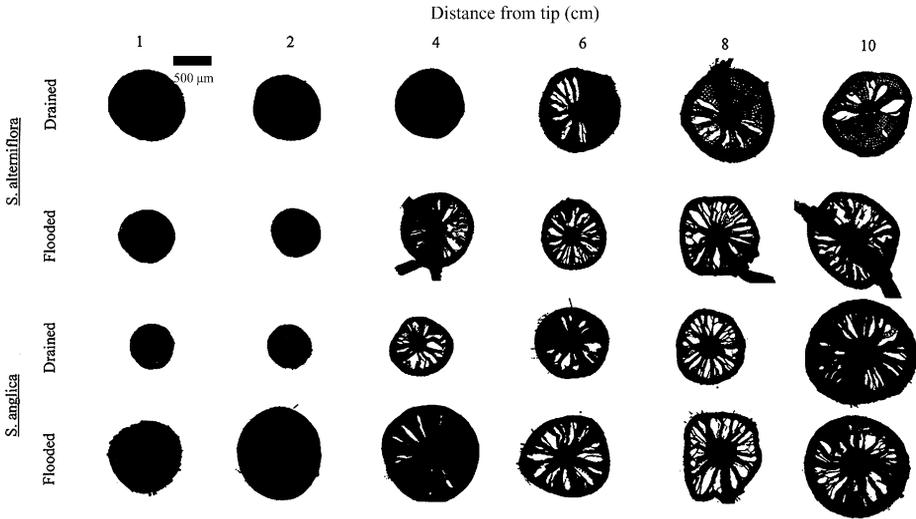


Fig. 2. Threshold images of root cross-sections in *Spartina alterniflora* (first two rows) and *S. anglica* (third and fourth rows). Sections were taken at different distances from the root tip (left to right by column: 1, 2, 4, 6, 8, and 10 cm). The first row is representative of a drained *S. alterniflora* root; the second represents a flooded *S. alterniflora* root. The third row is representative of a drained *S. anglica* root; the fourth represents a flooded *S. anglica* root.

average, *S. alterniflora* flooded roots had 179% more aerenchyma by area than drained roots. This trend was not observed in *S. anglica* (Fig. 3b). There were no significant differences between percent aerenchyma in flooded or drained *S. anglica* roots at any location along the root (ANOVA,  $P > 0.5$ ). Furthermore, percent aerenchyma in flooded and drained roots

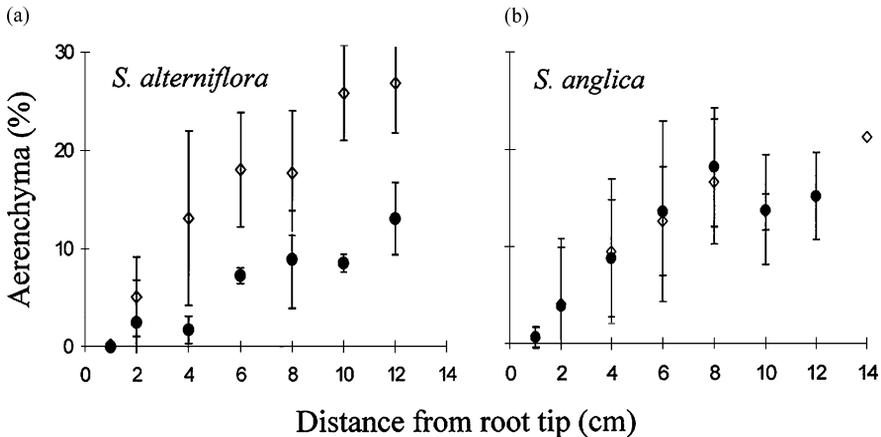


Fig. 3. Amounts of root aerenchyma in *Spartina* plants grown under flooded and drained soil conditions. (a) *S. alterniflora* roots; (b) *S. anglica* roots. Open diamonds represent plants from flooded treatments; filled circles represent plants from drained treatments. Data points are averages of 2–5 plants for each given length from the root tip  $\pm$  1 S.D. Points without error bars are where  $n = 2$ .

Table 1  
Oxygen consumption rates in *Spartina alterniflora* and *S. anglica* roots from flooded and drained soil conditions<sup>a</sup>

		Oxygen flux ( $\mu\text{mols g}^{-1} \text{ root h}^{-1}$ )	
		Normal (air) atmosphere	Under 100% N <sub>2</sub>
<i>S. alterniflora</i>	Drained	$-1.434 \pm 1.065$ (9)	$-1.2648 \pm 0.9495$ (4)
	Flooded	$-1.249 \pm 0.812$ (11)	$-1.2394 \pm 0.3723$ (5)
<i>S. anglica</i>	Drained	$-0.486 \pm 1.347$ (9)	$-1.0150 \pm 0.8775$ (4)
	Flooded	$1.043 \pm 1.815$ (9)	$-0.8826 \pm 0.9105$ (4)

<sup>a</sup> Consumption rates are given for conditions under a normal (air) atmosphere and under 100% nitrogen. Negative oxygen fluxes indicate oxygen consumption from the medium, while positive fluxes indicate oxygen release. Rates shown are mean  $\pm$  1 S.D. (*n*). Units are:  $\mu\text{moles oxygen consumed/released per gram fresh root weight per hour}$ .

of *S. anglica* were not significantly different than *S. alterniflora* from drained treatments (ANOVA,  $P > 0.5$ ).

Oxygen consumption rates between treatments showed that *S. alterniflora* roots consume more oxygen than *S. anglica* roots (Table 1). On average, drained and flooded treatment *S. alterniflora* consumed oxygen at rates of 1.43 and 1.25  $\mu\text{moles g}^{-1} \text{ root h}^{-1}$ , respectively. Drained treatment *S. anglica* oxygen consumption rates were variable and not significantly different from either *S. alterniflora* treatment. On average, drained treatment *S. anglica* oxygen consumption rates were lower than observed in *S. alterniflora* (0.49  $\mu\text{moles g}^{-1} \text{ root h}^{-1}$ ). *S. anglica* plants from flooded treatments released oxygen into the flask at rates of 1.04  $\mu\text{moles g}^{-1} \text{ root h}^{-1}$ . These rates were significantly different from all other treatments (Fisher's pairwise comparison,  $\alpha = 0.05$ ), indicating that flooded *S. anglica* release oxygen into the medium, whereas drained treatment *S. anglica* and drained and flooded treatment *S. alterniflora* do not.

Under a nitrogen atmosphere, flooded and drained treatments of both species had similar oxygen consumption rates (Table 1). Consumption rates averaged from 0.88 to 1.26  $\mu\text{moles g}^{-1} \text{ root h}^{-1}$ , with no significant differences between groups (ANOVA,  $P = 0.88$ ).

The difference between the total consumption rate (measured under a nitrogen atmosphere) and the normal consumption rate (measured under an air atmosphere) represents the amount of oxygen supplied to the roots via the aerenchyma. This value was not significantly different from zero in drained plants of *S. alterniflora* and *S. anglica*, and flooded *S. alterniflora* plants. On the other hand, the aerenchyma in flooded *S. anglica* plants transported an average of 2.10  $\mu\text{moles}$  of oxygen per gram of root weight per hour ( $s = 2.50$ ).

### 3. Discussion

To investigate the dynamics of aerenchyma development in wetland plants such as *Spartina*, a quantitative and rapid method of measuring aerenchyma is needed. A commonly used method involves estimating root porosity via specific gravity measurements. This method has been used in *S. patens* (Burdick and Mendelsohn, 1987; Burdick, 1989;

Naidoo et al., 1992; Pezeshki et al., 1993), *S. alterniflora* (Arenovski and Howes, 1992; Naidoo et al., 1992), and *S. anglica* in one instance (Rozema et al., 1985). However, this method is indirect and does not involve direct measurement of aerenchyma. Fewer studies have involved direct visualization of aerenchyma in root sections, e.g. in *S. patens* (Pezeshki et al., 1991), *S. alterniflora* (Mendelssohn and Postek, 1982; Arenovski and Howes, 1992), and *S. anglica* (Justin and Armstrong, 1987). These studies did not assess variation along the length of the root. As demonstrated in the present study (Fig. 2), the percent aerenchyma is highly dependent on the position from which samples are taken. Better resolution of differences may be possible by examining aerenchyma profiles along the root length, rather than relying on single measurements.

Many studies have been conducted to determine whether aerenchyma formation is linked to flooding or hypoxia. Aerenchyma formation in response to flooding is exhibited in *S. patens* (Burdick and Mendelssohn, 1987; Burdick, 1989; Pezeshki et al., 1991; Naidoo et al., 1992), but this relationship is less clear in *S. alterniflora*. Naidoo et al. (1992) reported that *S. alterniflora* root specific gravity decreased significantly with flooding, yet Arenovski and Howes (1992) found no difference in aerenchyma formation in root cross-sections from flooded and drained treatments. Arenovski and Howes (1992) concluded that *S. alterniflora* attains its maximal lacunal system as a normal part of development regardless of flooding stress. Similarly, specific gravity measurements have shown that aerenchyma formation in *S. anglica* does not appear to be affected by flooding (Rozema et al., 1985). Differences among species are of interest since they provide information with respect to relative tolerance of flooding stress. Our results, examining the aerenchyma profile along the entire root using digital image analysis, clearly show that aerenchyma formation is induced by flooding in *S. alterniflora*, but not in *S. anglica* under the same conditions.

The finding that *S. anglica* aerenchyma formation was not induced by flooded conditions may indicate that *S. anglica* has a different oxygen stress threshold than *S. alterniflora*, and is more tolerant of flooding. Our results show that reduction of oxygen supply necessitates structural changes in *S. alterniflora* but not in *S. anglica*. Oxygen stress brought about by our treatments may not have been sufficient to promote aerenchyma formation in *S. anglica*. Further oxygen reduction could be brought about by bubbling solutions with nitrogen or addition of hydrogen sulfide. Whether such conditions bring about sufficient oxygen limitation to induce *S. anglica* aerenchyma formation is presently being investigated.

The strategy of aerenchyma development in response to flooding may involve a tradeoff between maintaining physiological function and the need to reduce tissue respiration. While aerenchyma systems can provide benefits to the plant in terms of facilitating oxygen transport and increasing metabolic efficiency, the formation of aerenchyma also presents certain costs. Eliminating cortex tissue can impede root functions such as water and mineral uptake and transport (Moog, 1998). Hence aerenchyma in *S. alterniflora* develops maximally only under hypoxic conditions brought about by flooding. Apparently, aerenchyma formation is too costly unless a plant is confronted with hypoxia from flooding stress or a nutrient shortage, necessitating the need for reduced plant tissue and concomitant reduced metabolic demands. We postulate that by not expressing additional aerenchyma, *S. anglica* potentially avoids such associated costs, and utilizes additional adaptations that are not expressed in *S. alterniflora*.

The results of the oxygen transport studies resolved differences in the function of aerenchyma between treatments and species. Based on root structure observations, one might assume that the aerenchyma present in drained *S. alterniflora* is not sufficient to supply oxygen to submerged tissues following flooding. This would account for the greater amounts of aerenchyma in flooded *S. alterniflora* roots. However, the oxygen transport studies showed negligible oxygen transport in either flooded or drained *S. alterniflora* plants. Therefore, the formation of aerenchyma by *S. alterniflora* in response to flooding does not enhance oxygen transport. Rather, it likely acts to reduce metabolic requirements or to transport other gases or waste products. Similarly, Howes and Teal (1994) found that *S. alterniflora* roots always consumed oxygen while only other non-respiratory gases were released from roots suspended in a water phase. These results are consistent with our data. However, we do not rule out the possibility that *S. alterniflora* can release oxygen under some conditions. Rust deposits have been found around roots of field-collected *S. alterniflora* in Louisiana (Mendelssohn and Postek, 1982). This may result from a much larger oxygen gradient between the root cortex and rhizosphere when plants grow in highly reduced mudflats compared to plants suspended in water. Rather than determining absolute rates of oxygen release, this study served as a relative comparison between species.

*S. anglica* clearly transports oxygen more effectively than *S. alterniflora*. Other studies have shown *S. alterniflora* plants kept under flooded conditions for long periods are affected by oxygen deficiencies, despite enhanced aerenchyma systems. Assays of the enzyme alcohol dehydrogenase (ADH) can be used to indicate such oxygen deficiencies; ADH is active during anaerobic metabolism, occurring only during the absence of oxygen (John and Greenway, 1976; Smith et al., 1986; Pezeshki et al., 1993). High ADH levels are usually found in flooded *S. alterniflora* roots and indicate chronic oxygen deficiency even in the presence of enhanced aerenchyma systems (Mendelssohn et al., 1981; Burdick and Mendelssohn, 1987).

The *S. anglica* plants in this study do not appear to be affected by lack of oxygen under flooded conditions. This may suggest that aerenchyma in *S. anglica* plants is more effective at transporting oxygen than *S. alterniflora*, since the constitutive aerenchyma in *S. anglica* is not enhanced under flooded conditions. Our data support this scenario. The abilities of *S. anglica* to transport oxygen are superior to *S. alterniflora* despite having less aerenchyma per root area. All treatments of plants consumed similar amounts of oxygen under a nitrogen atmosphere, so differences in oxygen demand could not be detected.

The fact that *S. anglica* can transport more oxygen with a lower percent aerenchyma is paradoxical. Oxygen transport would seem to be favored by larger lacunae found in *S. alterniflora*. Processes in addition to simple diffusion may account for gas transport in *S. anglica*. *S. anglica* may have an enhanced gas-transport mechanism with a similar basis as pressurized systems proposed for *Nuphar luteum* by Dacey (1980) and *S. alterniflora* by Hwang and Morris (1991). These systems functioned by pressure gradients established by thermal transpiration and hygrometric pressure (Dacey, 1980; Dacey, 1981; Dacey and Klug, 1982; Hwang and Morris, 1991). A similar mechanism may exist in *S. anglica*, allowing substantial oxygen transport without sacrificing the functions of lost cortex tissue. Alternatively, increased oxygen production through photosynthesis may account for enhanced oxygen supply to root tissues. When oxygen partial pressures inside root tissues are elevated above rhizosphere oxygen levels, oxygen can then diffuse outward into the reduced sediments.

Even though *Spartina*'s capacity to transport oxygen has helped make it a formidable invasive species, this ability could possibly be utilized for beneficial purposes. *S. anglica* plants are potentially useful for phytoremediation efforts. For example, breakdown of hydrocarbons from oil spills is accelerated by the presence of oxygen (Lin and Mendelssohn, 1998). Thus, planting *S. anglica* in polluted sediments may accelerate recovery. Other toxins in the soil might also be oxidized to less harmful species by oxygen supplied by *Spartina*. Elucidation of the mechanisms that allow *S. anglica* to cope with oxygen deficiency may also potentially facilitate the development of genetically engineered crop plants with increased resistance to flooding.

#### 4. Conclusions

The enhanced oxygen transport abilities present in *S. anglica* have helped it colonize inhospitable estuarine regions previously devoid of vascular plants. *S. anglica* vigorously grows lower in the intertidal zone than does either parent species (Frenkel, 1987; Sayce and Mumford, 1991), suggesting that the superior aerenchyma efficiency in *S. anglica* is a feature that evolved with the allopolyploidy event that created the species. The *S. anglica* results in this study represent the first documentation of oxygen release by *Spartina* suspended in a liquid phase. The ecological significance of such an ability may be profound, and allow *S. anglica* to thrive in estuaries.

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#### References

- Arenovski, A.L., Howes, B.L., 1992. Lacunal allocation and gas transport capacity in the salt marsh grass *Spartina alterniflora*. *Oecologia* 90, 316–322.
- Armstrong, W., 1979. Aeration in higher plants. *Adv. Bot. Res.* 7, 225–332.
- Armstrong, J., Armstrong, W., Beckett, P.M., 1992. *Phragmites australis*: venturi- and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. *New Phytol.* 120, 197–207.
- Armstrong, W., Brandle, R., Jackson, M.B., 1994. Mechanisms of flood tolerance in plants. *Acta Bot. Neerlandica* 43, 307–358.
- Armstrong, W., Justin, S.H.F.W., Beckett, P.M., Lythe, S., 1991. Root adaptation to soil waterlogging. *Aquat. Bot.* 39, 57–73.

- Bishop, W.S., 1997. Why are there 5,000 acres of *Spartina* in Willapa Bay? In: Patten, K. (Ed.), Proceedings of the Second International *Spartina* Conference, Washington State University-Cooperative Extension, Olympia, WA, pp. 98–99.
- Burdick, D.M., 1989. Root aerenchyma development in *Spartina patens* in response to flooding. *Am. J. Bot.* 76, 777–780.
- Burdick, D.M., Mendelssohn, I.A., 1987. Waterlogging responses in dune, swale, and marsh populations of *Spartina patens* under field conditions. *Oecologia* 74, 321–329.
- Dacey, J.W.H., 1980. Internal winds in water lilies: an adaptation for life in anaerobic sediments. *Science* 210, 1017–1019.
- Dacey, J.W.H., 1981. Pressurized ventilation in the yellow waterlily. *Ecology* 62, 1137–1147.
- Dacey, J.W.H., Klug, M.J., 1982. Tracer studies of gas circulation in *Nuphar*:  $^{18}\text{O}_2$  and  $^{14}\text{CO}_2$  transport. *Physiol. Plant.* 56, 361–366.
- Daehler, C.C., Strong, D.R., 1996. Status, prediction, and prevention of introduced cordgrass *Spartina* spp. invasions in pacific estuaries. *U.S.A. Biol. Conserv.* 78, 51–58.
- Dumbauld, B.R., Peoples, M., Holcomb, L., Ratchford, S., 1997. The potential influence of the aquatic weed *Spartina alterniflora* and control practices on clam resources in Willapa Bay, Washington. In: Patten, K. (Ed.), Proceedings of the Second International *Spartina* Conference, Washington State University-Cooperative Extension, Olympia, WA, pp. 13–16.
- Epstein, E., 1972. *Mineral Nutrition of Plants: Principles and Perspectives*, Wiley, New York.
- Frenkel, R.E., 1987. Introduction and spread of cordgrass (*Spartina*) into the Pacific Northwest. *Northwest Environ. J.* 3, 152–154.
- Gray, A.J., Raybould, A.F., 1997. The history and evolution of *Spartina anglica* in the British Isles. In: Patten, K. (Ed.), Proceedings of the Second International *Spartina* Conference, Washington State University-Cooperative Extension, Olympia, WA, pp. 51–57.
- Gray, A.J., Marshall, D.F., Raybould, A.F., 1991. A century of evolution in *Spartina anglica*. *Adv. Ecol. Res.* 21, 1–62.
- Hacker, S.D., Heimer, D., Hellquist, C.E., Reeder, T.G., Reeves, B., Riordan, T.J., Dethier, M.N., 2001. A marine plant (*Spartina anglica*) invades widely varying habitats: potential mechanisms of invasion and control. *Biol. Invasions* 3, 211–217.
- Hedge, P., Kriwoken, L., Ritar, A., 1997. The distribution of *Spartina* in Victoria and Tasmania, Australia. In: Patten, K. (Ed.), Proceedings of the Second International *Spartina* Conference, Washington State University-Cooperative Extension, Olympia, WA, pp. 11–12.
- Howes, B.L., Teal, J.M., 1994. Oxygen loss from *Spartina alterniflora* and its relationship to salt marsh oxygen balance. *Oecologia* 97, 431–438.
- Hwang, Y.-H., Morris, J.T., 1991. Evidence for hygrometric pressurization in the internal gas space of *Spartina alterniflora*. *Plant Physiol.* 96, 166–171.
- Jackson, M.B., Armstrong, W., 1999. Formation of aerenchyma and the process of plant ventilation in relation to soil flooding and submergence. *Plant Biol.* 1, 274–287.
- John, C.D., Greenway, H., 1976. Alcoholic fermentation and activity of some enzymes in rice roots under anaerobiosis. *Aust. J. Plant Physiol.* 3, 325–336.
- Justin, S.H.F.W., Armstrong, W., 1987. The anatomical characteristics of roots and plant response to soil flooding. *New Phytol.* 106, 465–495.
- Kawase, M., 1976. Ethylene accumulation in flooded plants. *Physiol. Plant.* 36, 236–241.
- Kawase, M., 1978. Anaerobic elevation of ethylene concentration in waterlogged plants. *Am. J. Bot.* 65, 736–740.
- Kende, H., 1993. Ethylene biosynthesis. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 44, 283–307.
- Lee, R.W. Physiological adaptations of the invasive cordgrass *Spartina anglica* to reducing sediments: Rhizome metabolic gas fluxes and enhanced  $\text{O}_2$  and  $\text{H}_2\text{S}$  transport, submitted.
- Lee, R.W., Kraus, D.W., Doeller, J.E., 1999. Oxidation of sulfide by *Spartina alterniflora* roots. *Limnol. Oceanogr.* 44, 1155–1159.
- Lin, Q., Mendelssohn, I.A., 1998. The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands. *Ecol. Eng.* 10, 263–274.
- Long, S.P., Incoll, L.D., Woolhouse, H.W., 1975.  $\text{C}_4$  photosynthesis in plants from cool temperate regions, with particular reference to *Spartina townsendii*. *Nature* 257, 622–624.

- Mendelssohn, I.A., Postek, M.T., 1982. Elemental analysis of deposits on the roots of *Spartina alterniflora* Loisel. *Am. J. Bot.* 69, 904–912.
- Mendelssohn, I.A., McKee, K.L., Patrick Jr., W.H., 1981. Oxygen deficiency in *Spartina alterniflora* roots: metabolic adaptation to anoxia. *Science* 214, 439–441.
- Mobberley, D.G., 1956. Taxonomy and distribution of the genus *Spartina*. *Iowa St. Coll. J. Sci.* 30, 471–574.
- Moog, P.R., 1998. Flooding tolerance of *Carex* species. I. Root structure. *Planta* 207, 189–198.
- Naidoo, G., McKee, K.L., Mendelssohn, I.A., 1992. Anatomical and metabolic responses to waterlogging and salinity in *Spartina alterniflora* and *S. patens*. *Am. J. Bot.* 79, 765–770.
- Pezeshki, S.R., Matthews, S.W., DeLaune, R.D., 1991. Root cortex structure and metabolic responses of *Spartina patens* to soil redox conditions. *Environ. Exp. Bot.* 31, 91–97.
- Pezeshki, S.R., Pardue, J.H., DeLaune, R.D., 1993. The influence of soil oxygen deficiency on alcohol dehydrogenase activity, root porosity, ethylene production, and photosynthesis in *Spartina patens*. *Environ. Exp. Bot.* 33, 565–573.
- Raskin, I., Kende, H., 1983. How does deep water rice solve its aeration problem. *Plant Physiol.* 72, 447–454.
- Rozema, J., Luppens, E., Broekman, R., 1985. Differential response of salt-marsh species to variation of iron and manganese. *Vegetatio* 62, 293–301.
- Sanchez, J.M., SanLeon, D.G., Izco, J., 2001. Primary colonisation of mudflat estuaries by *Spartina maritima* (Curtis) fernald in northwest Spain: vegetation structure and sediment accretion. *Aquat. Bot.* 69, 15–25.
- Sayce, K., Mumford, T.F., 1991. Identifying the *Spartina* species. In: Mumford, T.F. et al. (Eds.), *Proceedings of the The Spartina Workshop Record on Washington State Sea Grant Program*, University of Washington, Seattle, WA, pp. 9–14.
- Smith, A.M., Hylton, C.M., Koch, L., Woolhouse, H.W., 1986. Alcohol dehydrogenase activity in the roots of marsh plants in naturally waterlogged soils. *Planta* 168, 130–138.
- Spicher, D., Josselyn, M., 1985. *Spartina* (Gramineae) in northern California: distribution and taxonomic notes. *Madrono* 32, 158–167.
- Teal, J.M., Kanwisher, J.W., 1966. Gas transport in the marsh grass, *Spartina alterniflora*. *J. Exp. Bot.* 17, 355–361.
- Teal, J., Teal, M., 1969. The Dominant *Spartinas*. In: *Life and Death of the Salt Marsh*, Little, Brown, and Company, Boston, pp. 84–101.
- Thompson, J.D., 1991. The biology of an invasive plant: what makes *Spartina anglica* so successful? *BioScience* 41, 393–401.
- Wang, F., Chapman, P.M., 1999. Biological implications of sulfide in sediment—a review focusing on sediment toxicity. *Environ. Toxicol. Chem.* 18, 2526–2532.
- Williams, W.T., Barber, D.A., 1961. The functional significance of aerenchyma in plants. *SEB Symp.* 15, 132–144.