Oxygen Deficiency in *Spartina alterniflora* Roots: Metabolic Adaptation to Anoxia

Abstract. *The aerenchyma (air-space) tissue in the wetland macrophyte* Spartina alterniflora *conveys sufficient oxygen to roots for predominately aerobic respiration in moderately, but not highly, reduced substrates. Continuously flooded plants survive by respiring anaerobically, although growth is decreased. Two metabolic adaptations to flooding are displayed in this species, depending on the degree of soil reduction.*

Root oxygen deficiencies resulting from substrate waterlogging in salt marshes have generally been discounted in influencing the growth of *Spartina alterniflora* Loisel (*1*) because root oxygen concentrations, renewed by a well-developed aerenchyma (air-space tissue) system, have been indicated in excess of that needed for aerobic root respiration (*2*). We now present data demonstrating that root oxygen deficiencies do occur in *S. alterniflora* and may produce variations in its height and productivity. The induced alcoholic fermentation caused by root oxygen deficiencies is significant in increasing root adenosine triphosphate (ATP) levels and root adenylate energy charge ratios in mildly stressed inland *Spartina* to levels comparable to that in those plants respiring aerobically; severely stressed plants of the dieback sites (*1*) have a lower energy status. We also show that *Spartina* has the capability of responding metabolically to soil waterlogging in two ways, depending on the degree of soil reduction.

The study area was an *S. alterniflora* salt marsh located near Barataria Bay, Louisiana (29°15′N, 90°0′W). Sampling sites were located at 2-m intervals along two parallel transects oriented perpendicular to a tidal stream and 5 m apart (Fig. 1a). Each transect intersected the gradient in *Spartina* height forms over a distance of 24 m from the tidal stream to the marsh interior. On 12 June 1979, turgid and structurally intact living roots were collected at the sampling sites and stored on dry ice for the determination of alcohol dehydrogenase (ADH) activity (*3*) and ethanol and malate concentrations (*4*). Since ADH, the adaptive enzyme catalyzing the reduction of acetaldehyde to ethanol during alcoholic fermentation, is inducible during anaerobic respiration (*3, 5*), its activity is an index of root oxygen deficiency. On 19 June 1979, root samples were collected at the sampling sites and analyzed for adenylate energy charge ratios (*6*). The adenylate energy charge ratio (*7*), ([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP]), a measure of the energy-rich adenosine phosphate compounds in a cell, was calculated from measured adenine nucleotide levels (*6*) to assess the energy status of the root tissue as a function of anaerobic root respiration and soil redox potentials. On 8 November 1979, the aboveground standing crop, culm height, and density (number of culms per unit area) were determined in 0.25-m^2 plots located at the sampling sites; these measurements serve as indices of plant vigor at the end of the growing season. Soil redox potentials (E_h) were measured at this time with platinum electrodes (*8*).

Significant changes in various abiotic and biotic measurements were evident along the environmental gradient between streamside and inland sites (Fig. 1, b–d). Redox potentials were relatively high throughout the more productive streamside zone, but decreased significantly within the inland *Spartina* zone and remained at this low level through the dieback area (Fig. 1c). The lower elevation and reduced interstitial water movement of inland sites result in a relatively greater confinement of water and consequently lower substrate E_h, a situation also found along the Atlantic Coast (*9*). Root ADH activity, a measure of root alcoholic fermentation, was low in the more oxidized substrate of the streamside zone (Fig. 1c) where aboveground biomass and plant density were maximal (Fig. 1b). The ADH activity increased significantly as soil E_h decreased within the inland *Spartina* zone and then declined in the dieback site to a lower, but still significantly greater, level than that of the streamside plants (Fig. 1c). A shift in respiratory metabolism from predominately aerobic to predominately anaerobic at low substrate oxygen concentrations is well documented (*3, 10*). Aboveground standing crop and culm density decreased significantly as ADH activity increased along these transects (Fig. 1, b and c). Culm height also decreased along these transects, with inland plant height reduced to 55 percent of that of streamside plants (Fig. 1b). Significantly higher ADH activities demonstrated that the inland *Spartina* roots were respiring anaerobically and thus were oxygen-deficient. The oxygen deficiency in inland *Spartina* roots was apparently a result of both the greater oxygen demand in these soils (Fig. 1c) and the reduced oxygen diffusion from aerial parts to the roots (*11*). In rice *Oryza sativa* L., a plant that also possesses well-developed aerenchyma tissue (*12*), oxygen movement to the roots from the leaves was found to be insufficient during flooding to sustain the full potential of at least some metabolic processes including ion uptake and aerobic respiration (*3, 13*).

The adenylate energy charge ratio is a good indicator of whether or not a plant tissue is maintaining an adequate supply of ATP for optimum metabolic activity (*12*). As substrate E_h levels decreased in association with increased soil waterlogging within the streamside zone, the energy charge ratio, the ATP concentration, and the total adenine nucleotide levels decreased significantly (Fig. 1, c and d). Since ADH activity was relatively constant within this zone (Fig. 1c), this decrease in energy status suggested a decrease in aerobic respiration. At a soil E_h of approximately −200 mV, the oxygen in the root tissue was depleted enough to induce anaerobic respiration as evidenced by high ADH activity (Fig. 1c), synthesis of ATP (Fig. 1d), and an increased energy charge ratio (Fig. 1d). The higher rates of alcoholic fermentation in inland *Spartina* roots were great enough to generate energy charge ratios equal to that of streamside roots, which primarily synthesize ATP aerobically. The high energy status of the inland roots was probably dependent on an operative Pasteur effect, which increases ATP yield at the expense of considerable glucose consumption (*14, 15*). The potential carbon deficit resulting from stimulated glucose consumption during alcoholic fermentation in the inland roots may contribute to the reduced growth in this area of the transect. The higher respiration rates measured for leaves of short (inland) *S. alterniflora* compared to that for the tall (streamside) plants in a Georgia salt marsh were attributed in part to CO_2 released from belowground tissues (*16*), which may have been a response to a greater oxygen deficit in the short plant zone. The relation between the energy charge ratio and alcoholic fermentation was further demonstrated within the dieback zone (Fig. 1a) where the energy charge ratio was significantly lower in response to a decreased rate of alcoholic fermentation (Fig. 1, c and d). Thus, in the extremely waterlogged dieback sites where energy status is low and where potentially toxic sub-

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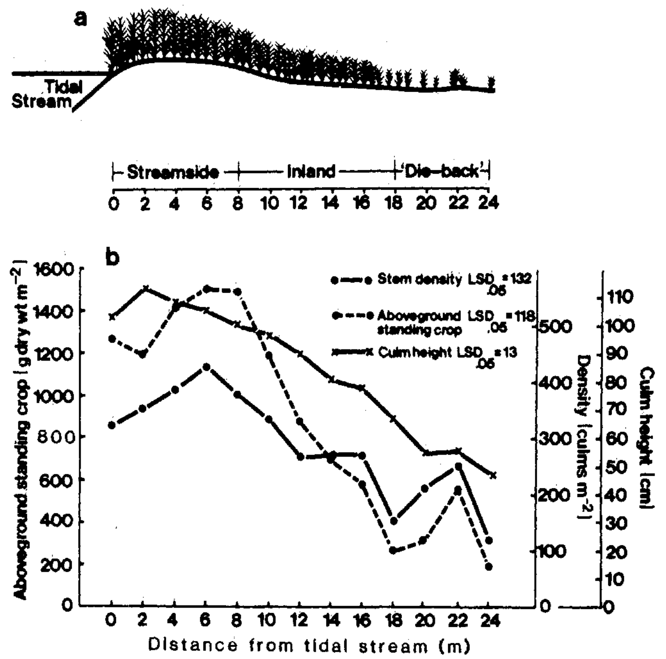
stances—for example, H_2S—may accumulate, growth is severely reduced.

Root ethanol concentrations were relatively constant throughout the transect (Fig. 1c); in view of the high ADH activity found in inland roots, this suggests that the ethanol produced during anaerobic respiration is easily diffused from *Spartina* roots, as is the case for rice (*17*). Therefore, ethanol toxicity is not a likely cause of the reduced growth along the streamside-to-inland transects. However, the loss of carbon in the form of ethanol from the plant would augment the potential carbon deficit resulting from increased glucose consumption during glycolysis and may contribute indirectly to controlling the height of *Spartina*.

Malate, which accumulates in some flood-tolerant plants, may be a nontoxic end product of anaerobic respiration (*12*). Malate concentrations in the roots of streamside *Spartina* were significantly higher than those of inland plants, and the lower concentrations within the inland zone were strongly associated with increased ADH activity and thus with alcoholic fermentation (Fig. 1c). The increase in malate within the streamside zone was closely related to the decreases in ATP and the energy charge ratio within this zone, since this pathway of malate synthesis does not result in net ATP formation (Fig. 1, c and d). The accumulation of malate in response to an ionic disequilibrium in flooded roots (*18*) does not seem to apply in this case because at the point of greatest soil reduction, malate levels were lowest and ADH activity highest, and vice versa (Fig. 1c).

Three zones of *Spartina* vigor can be delineated: (i) a highly productive streamside zone in which the metabolism of *Spartina* roots is primarily aerobic and the production of ATP by oxidative phosphorylation is apparently oxygen-limited; (ii) an inland zone in which anaerobic root respiration compensates for a loss in aerobic ATP production and maintains healthy but less productive plants; and (iii) a continuously flooded inland zone where the accumulation of soil phytotoxins (*19*) and the low energy status of the roots, in combination with a potential carbon deficit, contribute to a decline in growth and, ultimately, to *Spartina* dieback.

Crawford (*20*) suggested that there are two general types of metabolic adaptations by plants to anoxia, and which type is operative depends on the degree of continuous flooding. (i) Those flood-tolerant plants that are subjected to a limited seasonal flooding adapt physiologically to partial anoxia by simultaneously accumulating nontoxic malate as the major end product of anaerobic root respiration and by limiting any increase in the rates of glycolysis and alcoholic fermentation as a result of this anoxia. This adaptation has the advantage of restricting the generation of toxic ethanol; but it has the disadvantage of preventing net ATP formation and hence is valuable only during relatively short periods of flooding. (ii) Those flood-tolerant plants that endure perpetual flooding and anoxia adapt by stimulating glycolysis, alcoholic fermentation, and ATP synthesis; toxic ethanol is lost by diffusion from the roots, and malate is not accumulated. These metabolic adaptations to anoxia have thus far been thought to be species-



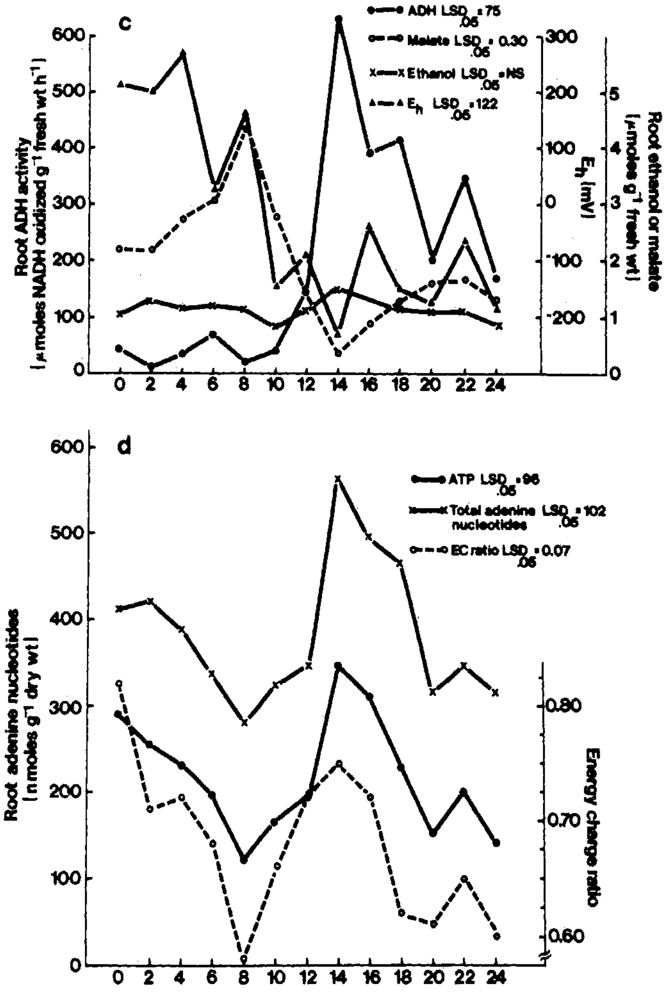


Fig. 1. (a) Sampling transect through streamside, inland, and dieback forms of *Spartina alterniflora* in a Louisiana salt marsh. (b) Variation in stem density, aboveground standing crop, and culm height of *S. alterniflora* along the sampling transect. (c) Variation in root alcohol dehydrogenase (*ADH*) activity and malate and ethanol concentrations in *S. alterniflora* and substrate E_h along the sampling transect. (d) Variation in root adenosine triphosphate (*ATP*) and total adenine nucleotide concentrations and adenylate energy charge (*EC*) ratio in *S. alterniflora* along the sampling transect. No significant differences in protein content of root extracts were found along the transects (2.31 ± 0.63 mg of protein per gram of fresh weight; range, 1.26 to 5.04). All data were statistically analyzed with least-squares regression analysis of variance, and significant differences ($P < .05$) were determined by the least significant difference (*LSD*) procedure ($N = 2$).

specific. However, our results indicate that both adaptations may occur in the same species, with streamside *Spartina* utilizing the first type and the inland form utilizing the second.

In the streamside zone, at soil redox potentials (+200 mV) indicating moderate reduction, root respiration was primarily aerobic, as evidenced by the high ATP levels and low ADH activity (Fig. 1, c and d). As the soil redox potential became more negative within the streamside zone, malate concentrations significantly increased while ADH activity remained low (Fig. 1c), indicating that in the absence of alcoholic fermentation the possible importance of malate in the metabolic adjustment of the plant to anaerobiosis. The ATP concentration and energy charge ratio decreased because there was no net ATP synthesis. The second type of adaptation was operative in the inland zone where soil redox potentials were low, ADH activity was induced, and ethanol was the primary end product of anaerobic respiration but was lost through diffusion from the roots; the energy supply was maintained by an increased rate of glycolysis (Fig. 1, c and d).

The specific metabolic adaptation to anoxia used by *Spartina* appears to be environmentally induced by the intensity of soil reduction. In contrast to what has been previously thought, the aerenchyma tissue in *Spartina* does not conduct sufficient oxygen to the roots for complete aerobic respiration in highly reduced substrates. Therefore, hydrological modifications of salt marshes that cause increased soil waterlogging may affect root respiration and, ultimately, plant productivity.

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References and Notes

1. *Spartina alterniflora*, the dominant angiosperm in regularly flooded salt marshes of the Atlantic and Gulf coasts of the United States, often occurs as relatively distinct height forms along a complex gradient of environmental factors landward from tidal creek banks. Along the Louisiana Gulf Coast, this gradient in *Spartina* productivity is identified by two forms: a high vigor streamside (~1 m) form and a low vigor inland (60 to 80 cm) form. However, the low vigor inland form often grades into an even less vigorous form (< 50 cm) whose habitat is characterized by small open bodies of water, continuous flooding, and *Spartina* dieback [W. G. Smith, *Coastal Stud. Bull.* **5**, 89 (1970)].
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16 September 1980; revised 19 December 1980

Metals in Estuarine Sediments: Factor Analysis and Its Environmental Significance

Abstract. *Q-mode factor analysis has been used to partition the variability of environmentally active metals in Delaware Bay sediments. Three factors, identified as a natural background source, an oceanic or seawater source, and an estuarine source, account for 96 percent of the metal variability.*

Trace metals may enter an estuary in dissolved, colloidal, or suspended forms, but most seem to be associated with suspended particulate matter (*1*) which is distributed to an estuary by the hydrodynamics of the system. In a model case, then, there should be definite gradients in the properties of suspended and bottom sediments along the estuary. In practice, however, poorly understood secondary processes modify the properties of the suspended particles and bottom sediments to such an extent that physical or chemical gradients are rarely obvious. Factor analysis can be a valuable tool for the identification of important metal gradients of estuarine bottom sediments, providing insight into the overall processes responsible for these gradients and into the identification of bottom areas containing high concentrations of environmentally active and potentially toxic materials.

Applications of *Q*-mode factor analysis to geological problems have included the classification of Bahama Bank sediments, the analysis of heavy-mineral suites in the Gulf of California, the extraction of paleoclimatological information from microfossil assemblages in ocean sediments, and comparison of the chemistries of surface waters and country rock in river basins (*2*). The results of *Q*-mode factor analysis are written as two small matrices, the F_S-matrix and the *B*-matrix, the product matrix of which is an approximation of the original data matrix. The percentage of variance explained is a measure of the degree to which this product matrix approximates the original data matrix.

The data set consists of analyses of the fine-grained fraction (< 63 μm) for environmentally active (*3*) metals and particulate organic matter from Delaware Bay sediments. A wide range of concentrations occurred between and within the analyzed variables, even though the samples represent only the fine fraction of the bottom sediments from the central region of the Delaware estuary.

We have applied factor analysis to the data (*4*) on each of 18 variables from 119 samples. The analysis reveals that three factors account for 96 percent of the variance in the original matrix. The F_S-matrix (Table 1) is a listing of combinations of the 18 variables that define the compositional end-members for each of the three factors. Factor I is dominated by Fe, Mg, K, Li, and Al, and none of these five metals is strongly dominant over the others. Factor I accounted for 50.9 percent of the total variance in the data bank. Factor II is dominated by Sr, Mg, Ca, and Na and accounted for 24.1 percent of the total variance in the data bank. Factor III includes those metals usually considered to be potentially hazardous. It is dominated by Cu, Cr, Pb, Hg, and organic material, with some affinity shown by Cd, Ni, Zn, and Fe. Factor III accounted for 21 percent of the total data variance.

The *B*-matrix is a matrix of weights or loadings for each of the factors at each

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