

Oxidation of the Root Zone by Aquatic Plants Growing in Gravel-Nutrient Solution Culture

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ABSTRACT

The root zone oxidation state was monitored over a period of 87 d for alpine rush (*Juncus alpinus* Vill.), canarygrass (*Phalaris arundinacea* L.), and cattail (*Typha latifolia* L.) growing in gravel-nutrient solution culture. The dissolved oxygen concentration in the root zone of cattail and canarygrass was ≤ 1 mg L⁻¹, whereas in alpine rush it ranged from 0 to 2 mg L⁻¹. All planted treatments consistently had a dissolved oxygen concentration 1 to 2 mg L⁻¹ lower than gravel without plants. Redox potentials in the root zone of alpine rush were normally between 400 and 700 mV, indicating an aerobic root zone. The root zone of cattail also tended to be aerobic, although redox potentials of <400 mV were obtained 40% of the time. Canarygrass had the most reduced root zone with 85% of the redox potential measurements <400 mV. Dissolved oxygen concentrations and redox potentials in the root zone did not change significantly on a diurnal basis for any of the plant species. The results show that there was a plant species effect on the oxidation state of the root zone as measured by dissolved oxygen and redox potential.

THE REED BED WETLAND SYSTEM has potential as an effective, low-cost method of wastewater treatment. Aquatic plants are believed to be an integral part of this system because of biological and chemical activities that occur in the rhizosphere. Many aquatic plants are known to transport oxygen to the roots. Oxygen released from the roots may create an oxidized rhizosphere (Armstrong, 1979), which facilitates processes in waste degradation such as N removal (Gersberg et al., 1986).

Evidence for oxygen transport to the root zone of aquatic plants has been obtained from the measurement of internal plant gas pressure and composition (Dacy, 1980; Dacy and Klug, 1982), from oxygen diffusion rates from individual roots (Armstrong et al., 1990), and from oxygen transport through the plant (Moorhead and Reddy, 1988). However, these measurements say little about the general effect, if any, of plant oxygenation on the bulk growing media surrounding the roots. In a study of 91 plant species, Justin and Armstrong (1987) concluded that unless root densities were very high, root oxidation of the rhizosphere would be very localized and would not greatly affect redox potentials of the bulk growing media. Oxygen transported to the roots and not consumed by root respiration may be released into the rhizosphere and immediately consumed by microbial respiration or chemical processes (Howeler and Bouldin, 1971; Bedford et al., 1991). Thus, the oxidation state of the overall root zone will be a reflection of the growing media, plant, and microorganism species. This was alluded to by Armstrong (1967), who found that oxidizing activity measured by a dye technique was substantially higher than oxygen measured diffusing from roots of

two aquatic plants to solution. Reddy et al. (1989) also found that dissolved oxygen concentration measurements alone were an insufficient measure of oxygen transport to the root zone by aquatic plants, as it did not account for respiration by microorganisms.

Oxygen transport into constructed wetlands has been studied primarily from the standpoint of biological oxygen demand or N removal. In that context the role of plants has been studied from the perspective of waste treatment (Gersberg et al., 1986; Brix, 1990), and not from the perspective of how the plants influence the constructed wetlands environment. While the former question may be the ultimate goal of constructed wetlands research, the latter question must be addressed to understand the long-term viability of both the wetland and the plants contained therein. Gravel is often used as the substrate in constructed wetlands, yet there is little information about how aquatic plants affect a gravel-based culture system. Our purpose was to determine the effect of plant oxygenation on the oxidation state of a gravel-nutrient solution culture system. A nutrient solution without oxygen demanding substances was used so that any C compounds present were derived from the plants. The objectives were (i) describe the root zone oxidation state for three aquatic plant species, and (ii) develop a method of categorizing the oxidation state of the root zone of aquatic plants growing in gravel-nutrient solution culture.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse at the Tennessee Valley Authority Constructed Wetlands Research and Development Facility in Muscle Shoals, AL, May to September 1992. Environmental conditions in the greenhouse were: air temperature 25 to 40°C, quantum flux 500 to 1200 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$, and relative humidity 40 to 90%. No supplemental lighting was used.

Each experimental system consisted of plants growing in gravel-nutrient solution culture in 1.2 m by 0.6 m by 0.3 m translucent polyethylene tanks. The tanks were filled to a depth of 230 to 245 mm with pea gravel (5–10 mm diam.). Before filling the tanks, the gravel was thoroughly washed with tap water to remove loose dirt and debris. It was then washed with deionized water. Three pairs of 13 mm polyvinyl chloride (PVC) access tubes were placed in the gravel at 305-mm intervals down the center of the tank (Fig. 1). A 50-mm access tube was placed in the gravel 458 mm from either end. The portion of the access tubes below the water level were constructed out of PVC screen (1-mm slits spaced at 5-mm intervals). This section was joined to regular PVC, which extended several inches above the gravel. A stillwell (not shown in Fig. 1), made out of 102-mm diam. PVC screen was placed in one corner of the tank. It was used for removing used nutrient solution and for monitoring nutrient solution temperature.

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Abbreviations: PVC, polyvinyl chloride; redox, oxidation-reduction; Eh, redox potential.

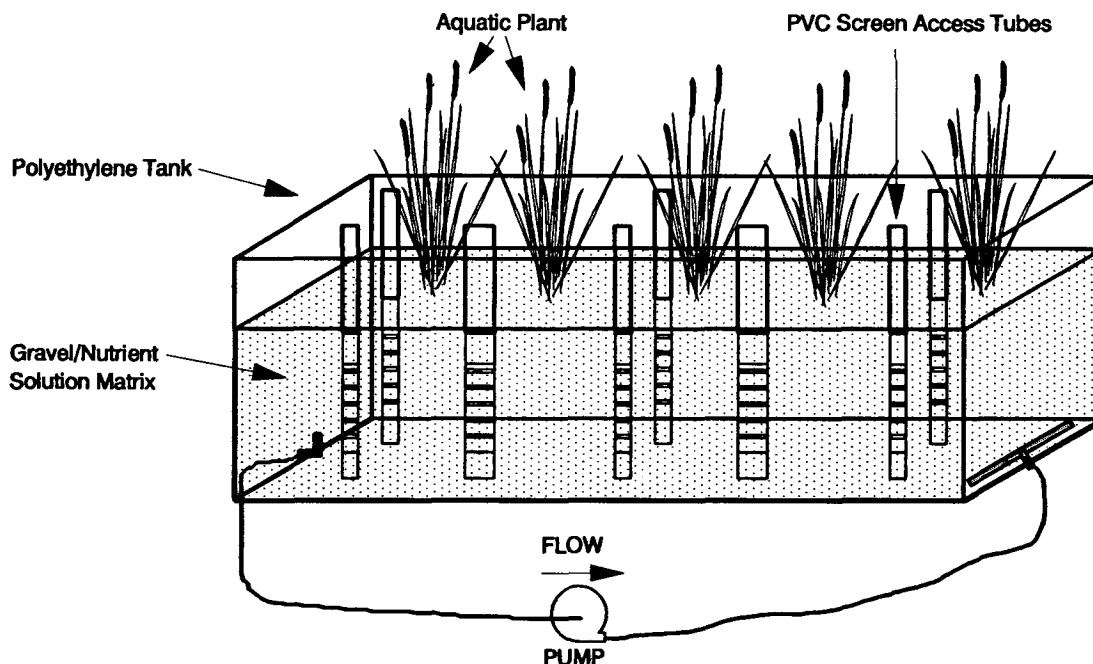


Fig. 1. Schematic diagram of experimental system showing the polyethylene tank, nutrient recirculation system, gravel, and PVC access tubes.

Nutrient solution in the tanks was continuously recirculated at a flow rate of 230 mL or 115 mL min^{-1} . It was pumped into the tank through a 13-mm i.d. PVC pipe laid across the bottom of one end of the tank (Fig. 1). Small holes were drilled every 25 mm to evenly diffuse the solution across the width of the tank. Nutrient solution flowed out of the opposite end of the tank through a pipe 120 mm from the bottom. An outlet spigot was also fitted with clear plastic tubing for monitoring the water level in the tanks (not shown in Fig 1).

The nutrient solution contained: 2.0 mM NH_4NO_3 , 0.4 mM CaCl_2 , 0.2 mM KH_2PO_4 , 0.25 mM K_2SO_4 , 0.5 mM MgSO_4 , 0.1 mM NaCl , 1.0 μM H_3BO_3 , 1.0 μM CoCl_2 , 1.0 μM MnSO_4 , 0.2 μM Na_2MoO_4 , 0.2 μM ZnSO_4 , 0.1 μM CuSO_4 , and 167.4 mM Fe as FeNaEDDHA (ethylenediamine di(*o*-hydroxyphenylacetic acid)) (6% Fe). Every 3 wk, nutrient solution was changed in all tanks. The tanks were filled with nutrient solution to a depth of 25 mm below the gravel surface, and were maintained at that level by daily additions of deionized water.

Cattail (*Typha latifolia* L.) and alpine rush (*Juncus alpinus* Vill.) were planted 5 May 1992; canarygrass (*Phalaris arundinaceae* L.) was planted on 15 May 1992. All plants were at least 6 mo old and had been previously growing in gravel-nutrient solution culture. There were three tanks per species with eight plants per tank. To ensure that the access tubes would be located in the root zone, the planting configuration was two rows of four plants, one row on each side of the row of access tubes. Three tanks were filled with gravel only and served as controls.

Platinum oxidation-reduction (redox) electrodes were made by welding 12 mm of 20-gauge platinum wire to copper wire. The wire was sealed into 5- to 10-mm diam. glass tubing with 2 to 5 mm of platinum extending from the tip. A saturated calomel reference electrode was used to complete the cell when redox measurements were made; potential differences were adjusted by 244 mV to obtain standard redox (Eh) values (Stumm and Morgan, 1981).

Measurements from redox electrodes made in this manner represent the mixed potential of several redox couples under nonequilibrium conditions. In spite of the fact that the measure-

ment is not a true measure of Eh, it can be used to describe the intensity of oxidizing or reducing conditions (Gambrell and Patrick, 1978). The reliability of redox measurements is related to the concentration of redox couples, both being low in oxidized systems and higher in reduced systems (Bohn, 1971). In general, systems with high concentrations of oxidizing agents give a greater Eh value than systems low in oxidizing agents (Stumm, 1966). Oxidized soil systems have been found to have Eh values greater than 400 mV (Gambrell and Patrick, 1978).

The redox potential at oxygen depletion in the gravel-nutrient solution matrix without plants was measured using a system similar to that described by Patrick et al. (1973). Nutrient solution or gravel-nutrient solution were placed in a reaction kettle containing redox, pH, and temperature sensors. Nitrogen gas was bubbled through the kettle until the redox potential had fallen to a stable value, usually 3 to 5 d. Measured redox potentials were corrected to pH 7 by -59 mV per unit pH change (Gambrell and Patrick, 1978).

Redox, pH, and dissolved oxygen concentration were measured biweekly in each of the three pairs of small access tubes in each tank beginning 16 June 1992 (Calendar Day 167) and continuing until 11 Sept. 1992 (Calendar Day 254). To measure redox in the tanks the platinum electrodes were placed in situ in one of the small access tubes per pair. Every 3 wk all redox probes were checked using a redox standard solution (Standard Practice, 1991), cleaned if necessary, and placed back in the tanks. Dissolved oxygen and pH measurements were made by dipping probes into the remaining access tube of each pair. The dissolved oxygen probe (Orion model 840) was accurate to $\pm 1\%$ of the measured value. During the measurement process the dissolved oxygen probe was gently moved up and down to facilitate adequate flow past the electrode. This technique was determined to be adequate by previously comparing mechanical stirring with the hand movement in the laboratory. All measurements were made 60 mm from the bottom of the tank.

Root zone pH was consistently 6.9 ± 0.1 in tanks with plants, and 7.3 ± 0.1 in tanks without plants. Acidification of the root zone has been commonly observed when NH_4^+ is

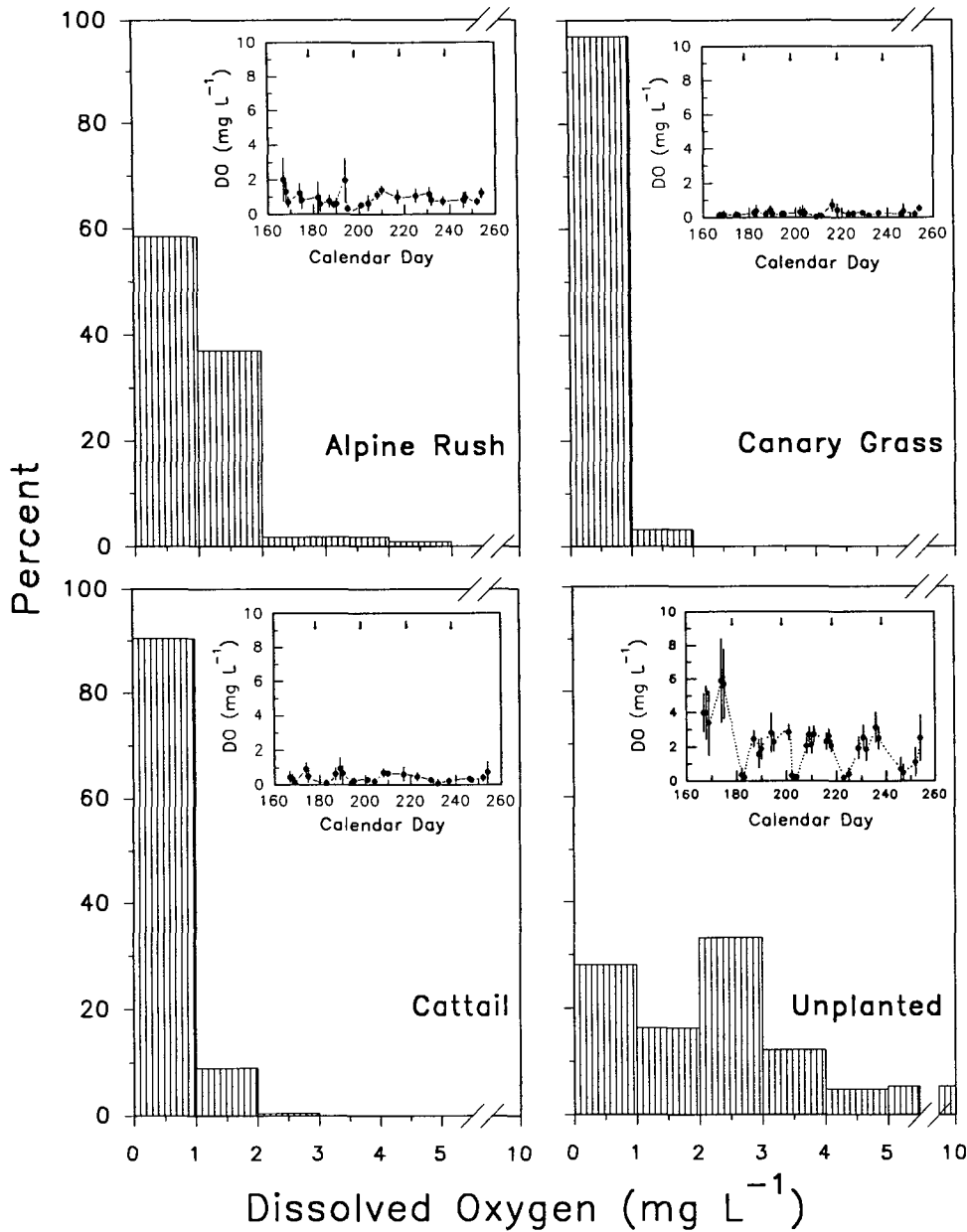


Fig. 2. Percentage of dissolved oxygen (DO) measurements falling within a given range over the duration of the experiment. Inset: DO means ± 1 SD for each day measurements were taken ($n = 9$), nutrient solution changes = \downarrow .

used as the N source (Marschner et al., 1982). Our nutrient solution contained both NO_3^- -N and NH_4^+ -N. Because the pH in planted and unplanted tanks was very near pH 7, redox potentials measured in the tanks were not corrected for pH.

Diurnal measurements of redox, pH, dissolved oxygen, and water temperature were made for at least one complete 24-h period using in situ probes placed in the large and small access tubes in the center of each tank. Air temperature and quantum flux above the tanks were also measured. The redox probes were those described previously. The pH sensor (model 399, Rosemount Analytical) was placed in one of the two larger access tubes. The dissolved oxygen probe (model 430-03, Rosemount Analytical) was placed in a separate PVC canister (50 mm diam.; 120 mm length) sealed at each end. Nutrient solution was pumped at a flow rate of 115 mL min^{-1} from the remaining large access tube through the canister and returned to the tank to ensure adequate movement of solution past the probe. Temperature measurements were made using thermo-

couples and quantum flux was measured with a LICOR LI190SA quantum flux sensor (LICOR, Lincoln, NE). The probe signals were logged hourly and sent via 4-20 mA transmitters (Rosemount Analytical, Irvine, CA) to an automated data acquisition and control system ('Tactician,' Eurotherm, Reston, VA) running on a personal computer.

Redox and dissolved oxygen data were not normally distributed, nor were variances homogenous between treatments. The F -test was used to test for equality of two variances. Treatment means were compared using the non-parametric Wilcoxon rank sum test or Mann-Whitney test (Snedecor and Cochran, 1967).

RESULTS

Dissolved oxygen concentrations measured biweekly during the 87-d experimental period are shown in Fig. 2 and summarized in Table 1. In tanks without plants 90% of the dissolved oxygen concentration measure-

Table 1. Mean and variance of redox potentials and dissolved oxygen concentrations in the rhizosphere of three aquatic plant species for a 3-mo period.

Treatment	Redox, mV			Dissolved oxygen conc., mg L ⁻¹		
	Mean	Variance	SD	Mean	Variance	SD
Alpine rush	489a†	13063b‡	114.3	0.97b	0.41b	0.64
Canarygrass	113c	70728d	266.0	0.26d	0.07d	0.27
Cattail	427b	38495c	196.2	0.45c	0.14c	0.38
Unplanted	486a	8812a	94.0	2.12a	2.74a	1.65

† Mean separation using Wilcoxon test ($p = 0.01$).

‡ Variance separation by F -test ($p = 0.01$).

ments ranged between 0 and 4 mg L⁻¹, averaging around 2 mg L⁻¹. Concentrations of less than 1 mg L⁻¹, which accounted for 28% of the measurements, corresponded to the several days immediately after a change in nutrient solution. The dissolved oxygen concentration in planted tanks ranged between 0 and 2 mg L⁻¹, with small differences between species. Canarygrass and cattail had the lowest dissolved oxygen concentrations, with 97 and 91% of all readings ≤ 1 mg L⁻¹. Dissolved oxygen in the root zone of alpine rush was slightly higher with 40% of readings ≥ 1 mg L⁻¹.

The redox potential at oxygen depletion in the gravel-nutrient solution matrix without plants was 333 ± 15 mV ($n = 5$), respectively. This value is similar to the oxygen depletion range of 320 to 340 mV reported for soils (Gambrell and Patrick, 1978). The percentage of redox potential measurements that were less than 300 mV was 74% in canarygrass, 14.5% in cattail, 0.4% in alpine rush, and 3.1% in tanks without plants (Fig. 3). Mean redox potentials were similar for the control and alpine rush, but significantly lower in cattail and canary grass when redox data was summarized over the experimental period (Table 1). The normal variation of redox potentials within tanks and species was significantly different for all planting treatments (Table 1); it was approximately 100 mV greater for canary grass and cattail than for alpine rush or the control (Fig. 3, *inset*).

The measurement regime was begun in mid-June, approximately 30 d after the tanks were planted and continued until mid-September. During this time there was little evidence of a long-term change in dissolved oxygen concentrations in any tank, or in redox potentials measured in alpine rush, cattail, or tanks without plants (Fig. 2 and 3, *inset*). In canarygrass, the average redox potential appeared to decrease over time; however, because of the large normal variation in redox potentials in this species, this change may not be significant.

There was no diurnal pattern in redox potentials measured in the root zones of any of the three species or in the unplanted gravel (Fig. 4). For the 2-d period, redox potentials measured in alpine rush and cattail were not significantly different from the unplanted gravel, but were about 600 mV lower in canarygrass. The dissolved oxygen concentration in the planted tanks also did not vary diurnally. However, in unplanted gravel it did change diurnally, increasing with solar radiation and the temperature of the gravel-nutrient solution matrix during the day.

At the termination of the experiment the plants and

gravel were removed from the tanks and examined. In all tanks with plants, the roots had completely and densely filled the gravel medium, regardless of species. It was not possible to separate the roots from the gravel for root density or dry matter determination.

DISCUSSION

Our results show that there was a plant species effect on the oxidation state of the root zone as measured by redox and dissolved oxygen concentration. There were small but significant differences in dissolved oxygen between the planted treatments, and all were consistently lower than the control. A comparison of alpine rush, canarygrass, and cattail root zones shows that the total difference in the dissolved oxygen concentration between treatments was only 0.5 to 1.0 mg L⁻¹, while the variation in redox potentials between treatments was as much as 300 to 600 mV.

Redox potentials in the range of 400 to 700 mV generally denote oxidized conditions (Gambrell and Patrick, 1978). Redox measurements indicate that canarygrass maintained the most anaerobic conditions in the root zone; 85% of all readings were below 400 mV. Redox potentials of less than 400 mV were present 40% of the time in cattail, and 20% of the time in alpine rush or the control. We also found a large variation in redox measurements within species. In natural systems different redox potentials within the same locale are common because redox processes rarely reach equilibrium and are not readily coupled (Stumm and Morgan, 1981). The fact that canarygrass and cattail had a variation in redox potentials of approximately 100 mV greater than alpine rush or the control suggests a species specific interaction between the growing media, plant, and microorganism species.

There was little diurnal change in the level of dissolved oxygen or intensity of redox conditions in the planted tanks. Oxygen transport from the atmosphere to the roots in aquatic plants can occur by either diffusion or active transport (Schütz et al., 1991). The mechanism of oxygen transport in canarygrass and alpine rush is unknown, but in cattail it has been documented to occur by pressurized flow (Sebacher et al., 1985). The energy source for pressurized flow is solar radiation, which indicates that oxygen transport should be greater during the daytime. In fact, Armstrong et al. (1990) found oxygen loss from individual common reed [*Phragmites australis* (Cav.) Trin. ex Steud.] roots greater during day and attributed it to daytime flow through convection. Our data show that if oxygen transport to the root zone of cattail was greater in the daytime, it did not produce significant changes in the diurnal pattern of dissolved oxygen concentrations or redox in the bulk root zone. This result is similar to that of Dunbabin et al. (1988), who could only detect very small diurnal fluctuations in dissolved oxygen in the root zone of (*Typha domingensis* Pers.).

The dissolved oxygen concentration in the unplanted tanks was generally between 2 and 4 mg L⁻¹ higher than in the planted tanks, except immediately following a change in nutrient solution. After the addition of fresh

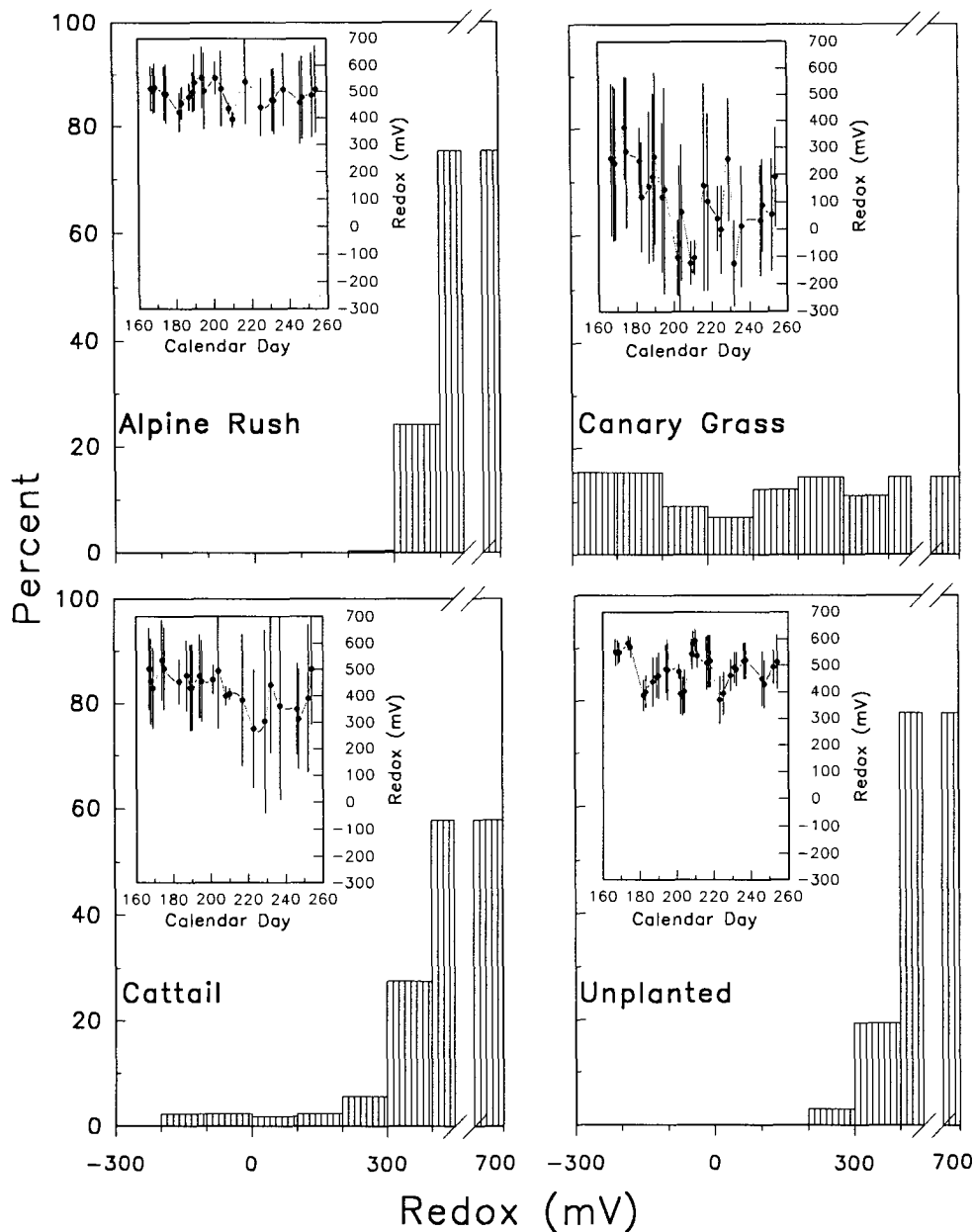


Fig. 3. Percentage of redox measurements falling within a given range over the duration of the experiment. Inset: Redox means ± 1 SD for each day measurements were taken ($n = 9$).

nutrient solution, the dissolved oxygen was low in all tanks, but increased after several days in unplanted gravel. The diurnal cycle of increasing dissolved oxygen concentrations with increased quantum flux and temperature of the gravel-nutrient solution in the unplanted tanks supports the presence of oxygen-producing microorganisms, as the solubility of oxygen in water decreases with increasing temperature. Although we observed a small amount of green algae growing on the surface of the gravel and on the sides of all tanks, the diurnal cycling of dissolved oxygen was only observed in the unplanted gravel.

If the solution in the tanks had been in equilibrium with the atmosphere the dissolved oxygen concentrations would have been in the range of 6.5 to 8 mg L⁻¹. The dissolved oxygen concentrations were considerably

below that in all tanks, indicating that a gradient existed for oxygen diffusion into the water from the atmosphere or from plant roots, even though the nutrient solution did not contain oxygen demanding substances. Furthermore, the plant roots and their attendant microorganisms tended to deplete the system of oxygen relative to the unplanted gravel.

Other reports in the literature concerning the ability of aquatic plants to oxygenate the root zone are contradictory. For example, Brix (1990) concluded that common reed roots contributed little to the oxygen balance of a soil-based constructed reed bed receiving domestic sewage during the winter. In that study, plant oxygenation of the root zone was inferred from gas transport in dead culms and respiratory oxygen consumption in the roots. During a 14-wk period, Dunbabin et al. (1988) found

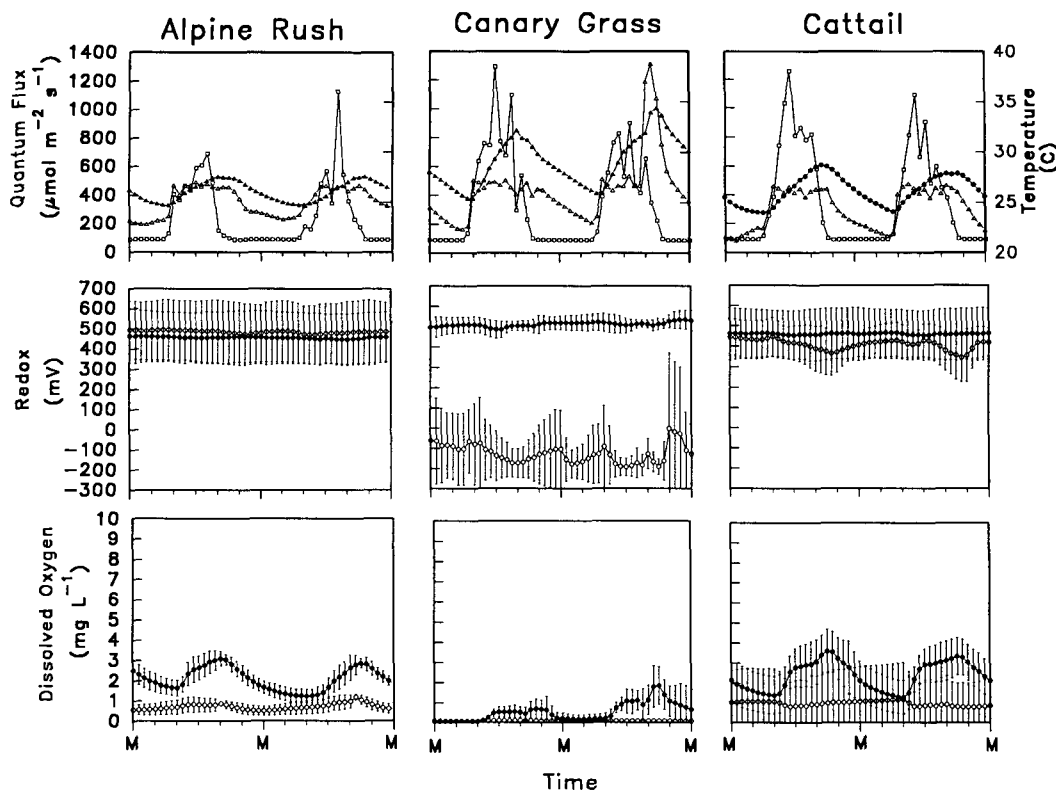


Fig. 4. Diurnal cycle of environmental conditions and the root zone oxidation state for two consecutive days (M = midnight). For redox and dissolved oxygen ($n = 3$): planted tanks (\circ), tanks without plants (control) (\bullet). Environmental parameters: quantum flux = (\square), air temperature = (\triangle), gravel-nutrient solution temperature = (\blacktriangle).

that the dissolved oxygen concentration measured in the root zone of *Typha domingensis* growing in gravel-nutrient solution culture was similar to unplanted gravel. When a C supplement was added to the system to increase the respiratory demand for oxygen, root zone oxygenation by cattail was sufficient to maintain the dissolved oxygen concentration at the same level, while that in unplanted gravel was significantly reduced. Reddy et al. (1989) found that nine different species of floating and emergent aquatic plants increased the dissolved oxygen concentration in the root zone above a control when incubated in sewage for 8 d. Bedford et al. (1991) suggests that these different conclusions may result from the variable time frames and sampling scales used in these studies.

Over a several-month period we measured relatively low dissolved oxygen concentrations in the presence of high redox potentials in the root zone of alpine rush and cattail. This suggests that oxygen transported to the root zone by the plants or diffusing from the atmosphere was immediately consumed by root respiration, microorganisms, or chemical processes present in the planted tanks. A similar conclusion was reached by Yamasaki (1984) who theorized that a correlation between redox potentials in the rhizosphere and oxygen concentrations inside the stems of both Manchurian wildrice [*Zizania latifolia* (Griseb.) Turcz. ex Stapf] and common reed was indicative of oxygen leaking from plant roots and being consumed in the process of rhizosphere oxidation. It is possible that the population of aerobic microorganisms in the root zone may increase up to the limit of the

oxygen supply, thus making steady state dissolved oxygen concentrations appear to be similar for different plant species and unchanging on a diurnal basis, while oxygen demand or utilization might be considerably different.

The results of this study and those of others indicate that the oxidation state of the root zone reflects the system as a whole, which includes the growing media, plants, and microorganisms living in the root zone. We found that the oxidation state cannot be accurately inferred from a single type of measurement since each measurement only defines one aspect of a complex system. In our study, a combination of dissolved oxygen and redox measurements was a better indicator of the oxidation state of the root zone than either measurement alone. Due to the range of variation in redox potentials and dissolved oxygen concentrations, both within and between plant species, a range of values or percentage of time values within a given range may be a better method of characterizing the oxidation state of a plant root zone or wetland system instead of comparing mean values.

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