

## Ditch plant response to variable flooding: A case study of *Leersia oryzoides* (rice cutgrass)

S.C. Pierce, S.R. Pezeshki, and M.T. Moore

**Abstract:** Vegetated drainages are an effective method for removal of pollutants associated with agricultural runoff. *Leersia oryzoides* (rice cutgrass), a plant common to agricultural ditches, may be particularly effective in the remediation process; however, responses of *L. oryzoides* to flooding are undocumented. The objective of this greenhouse study was to characterize responses of *L. oryzoides* to various soil moisture regimes representative of agricultural ditches, including four treatments ranging from well drained to saturated. Over the eight weeks of the study, plants were subjected to four flooding regimes that included a (1) well-watered, well-drained control; (2) well-watered, well-drained intermittently flooded treatment; (3) partially flooded treatment; and (4) continuously flooded treatment. Decreases in photosynthesis occurred only when soil redox potential (Eh) dropped below +350 mV (all flooded treatments). Although flooding reduced belowground:aboveground biomass ratios, overall productivity and root development were unaffected. Results indicated that management practices increasing retention time in agricultural ditches would not reduce productivity in *L. oryzoides* until soil oxygen is depleted for sufficient duration to induce reduction.

**Key words:** drainage ditch—*Leersia oryzoides*—primary productivity—variable flooding

### Nonpoint source water pollution related to agriculture has become an issue of great environmental concern.

Pesticides and excess nutrients have been implicated as potential environmental hazards, both to humans and the environment at large (Arcury et al. 2006; Dudgeon et al. 2006; Mueller et al. 2004). A large body of literature exists addressing the utility of vegetation as a filtration system for municipal and agricultural waters (Mitch and Gosselink 2000). Recently, the use of vegetated agricultural ditches for water remediation has received attention for removal of contaminants such as pesticides (Cooper et al. 2004; Bennett et al. 2005) and excess nutrients (Kröger et al. 2004). Ongoing research is evaluating nutrient cycling of common ditch plants (Kröger et al. 2007) and the effectiveness of native wetland plants as accumulators of nutrients in constructed wetlands (Hunt et al. 2002; Kao et al. 2003).

A recent survey of 36 agricultural ditches in the Mississippi and Arkansas deltas found

that in small edge-of-field ditches, species richness was less than half that found in larger sized drainages, with the most prominent species in smaller ditches belonging to the genus *Leersia* (cutgrass) (Bouldin et al. 2004). Anecdotal evidence suggests a majority of *Leersia* found in these ditches is probably *L. oryzoides* [L.] Sw (rice cutgrass), often a major component of wetland seed banks, especially those subject to sedimentation and variation in water level (Galatowitsch and van der Valk 1996; Le Page and Keddy 1998; Peterson and Baldwin 2004). Colonization of ditches by *L. oryzoides* may result from a combination of hydrologic regime, dredging, and dispersal ability leading to an environment where it can become quickly established in the absence of major competition.

In areas prone to flooding, water depth and persistence can have major impacts on plant species composition (Blom 1999; Cassanova and Brock 2000; Voeselek et al. 2004) and plant productivity (Pezeshki 2001). Agricultural ditches are one such

area. A number of plants found in agricultural ditches are obligate wetland species (Bouldin et al. 2004). However, even plants commonly found in wetland areas may be negatively affected by the long-term effects of flooding. Flooding effectively stops diffusion of oxygen into the soil resulting in an anoxic, reduced soil environment (Gambrell et al. 1991). This transition to a reduced root environment results in a number of metabolic and morphological changes that diminish root functions (Pezeshki 2001; Gibbs and Greenway 2003; Greenway and Gibbs 2003).

Limitations imposed on roots by flooding often lead to stomatal closure (Kozłowski 1984a, 1984b; Pezeshki 1994; Pezeshki et al. 1996a, 1996b), which may serve to regulate water imbalances resulting from decreased water uptake capacity of roots (Pezeshki and Chambers 1985). Stomatal closure limits leaf gas exchange, which along with metabolic (nonstomatal) inhibition results in decreased rates of photosynthesis (Pezeshki 1993). Nonstomatal limitations include reduction in leaf chlorophyll content (McKelvin et al. 1995) and reduction in Photosystem II activity, which may be estimated using leaf chlorophyll fluorescence measurements (Maxwell and Johnson 2000).

After initial reduction, partial recovery of net photosynthesis (Pn) and stomatal conductance (gs) occur in many species related to the duration and intensity of reduction (Pezeshki 1993; Pezeshki and Anderson 1997; Brown and Pezeshki 2000). However, even with substantial recovery of gas exchange, plant survival and growth may be impacted (Pezeshki and Anderson 1997), which is reflected in reduced plant biomass, particularly belowground biomass (DeLaune et al. 1983; Pezeshki 1991; Kludze and DeLaune 1995) and root penetration depth (Pezeshki 1991). The threshold level of soil reduction (redox potential, Eh in mV) for these responses differs among wetland species, ranging from +300 mV to -200 mV (Pezeshki 1991; Will et al. 1995). The upper level of this threshold corresponds roughly to

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the onset of oxygen disappearance from the soil, which begins to occur at Eh of +350 mV (DeLaune et al. 1990).

Water regimes present in agricultural ditches are highly variable, and are likely to result in fluctuating levels of soil Eh. The majority of studies on plant response to flooding have focused on continuously saturated conditions; however, research has shown that temporal variation in flooding, referred to as “periodic flooding” or “intermittent flooding,” may have varying consequences on productivity among several taxa of wetland plants by reducing Pn (Pezeshki et al. 1989; Pezeshki et al. 1998; Anderson and Pezeshki 1999; Pezeshki and Anderson 1999; Li et al. 2004a). Various degrees of flooding can result in a host of morphological responses, depending upon the plant species, with flood-related responses in root morphology present in *Salix nigra* (black willow) even when water level was located below the soil surface and only part of the roots were flooded (Li et al. 2006).

In environments where either spatial or temporal heterogeneity of resources play a major role in survival, plants may utilize various stress avoidance strategies. In flooded conditions, oxygen can quickly become the limiting resource. For example, submergence induces internode elongation in river bank species, allowing such plants to reach the surface of the water and “snorkel” air to submerged portions (Voeselek et al. 2004). Clonal plants, in particular, have the ability to spread over areas that vary in resources, a trend that may be increased by moderate variation in resource availability (Pitelka and Ashmun 1985). In *Phalaris arundinacea*, a species common in agricultural ditches and municipal stormwater drainages, high temporal variation in flooding resulted in increased numbers of shoots (Miller and Zedler 2003). It is possible that increased numbers of shoots in conjunction with potential for clonal integration allowed *P. arundinacea* to utilize variable resources as they became available.

Like many grasses, *L. oryzoides* utilizes an extensive system of belowground rhizomes and aboveground stolons for vegetative propagation (Darris and Bartow 2006) and potentially carbohydrate storage. While it may be expected that *L. oryzoides* would show a similar pattern in aboveground stem production to *P. arundinacea*, currently little information is available regarding how

flooding affects belowground rhizome development in this species, or grasses in general. Although given the trend for plants to show an increase in the ratio of belowground: aboveground growth in response to flooding (Pezeshki 2001), a pattern of decreased rhizome soil penetration, with increased biomass allocation to aboveground stems would be expected.

The purpose of this study was to examine responses of *L. oryzoides* to four water regimes common to drainage ditches: a well-watered, well-drained control; an intermittently flooded treatment; a partially flooded treatment; and a continuously flooded treatment. We hypothesized that carbon assimilation and allocation will be affected by flooding regimes, with a general shift toward reduction in belowground:aboveground ratios of production. We predicted three generalized responses to these flooding treatments:

1. Belowground:aboveground biomass ratios, root penetration depth, and rhizome penetration depth would be decreased in flooded plants, with this effect being most pronounced in the Continuously Flooded treatment.
2. Number of shoots would increase under the Intermittently Flooded treatment.
3. Reduction of root function and belowground carbon sink capacity due to flooding would lead to decreased net carbon assimilation rates.

### Methods and Materials

Plants were collected from wild populations found in wetland cells at the Jamie L. Whitten Plant Materials Center in Coffeeville, Mississippi, which is located in Major Land Resource Area 134A, the Southern Coastal Plain (USDA Natural Resources Conservation Service 2006). Temperatures in the immediate vicinity of Coffeeville range from lows of 1°C (33°F) during winter months to average highs of 32°C (90°F) during summer months, with mean annual precipitation of 152 cm (60 in) (30-year average, Grenada Dam, National Atmospheric and Oceanographic Administration).

Plants were grown under natural light in the Life Sciences Greenhouse at the University of Memphis. Plants were grown in pots 60 cm (23.6 in) high constructed of 15 cm (5.9 in) PVC pipe filled with a 60:40 (v/v) mixture of washed play sand and field soil to allow for adequate drainage. Field soil

was obtained from the A<sub>p</sub> horizon of the Waverly Silt Loam Series (Soil Conservation Service 1989). Although the high sand content of the sand/soil mixture used in this study is not representative of agricultural ditches in the region, the hydraulic conductivity of common ditch soils is so low that a well-drained control was essentially impossible given the time scale of the study.

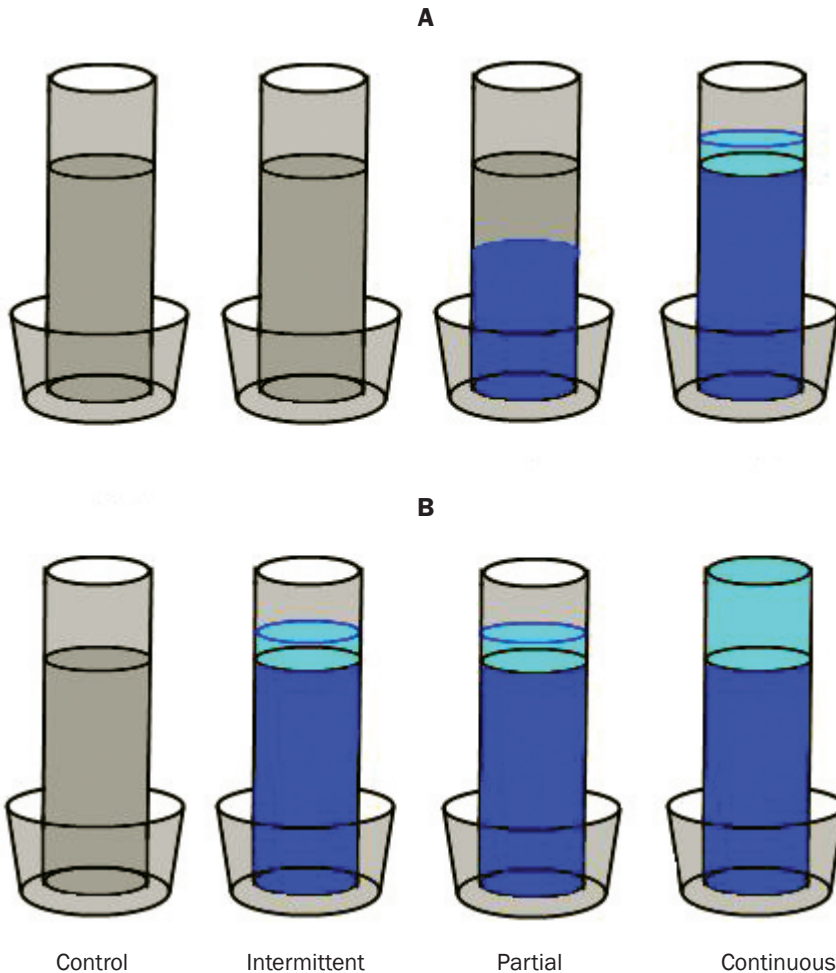
After placement in PVC pipes, plants were well watered and well drained for a period of three weeks prior to treatment initiation. During this time, plants were fertilized weekly with 500 mL (16.91 US fluid ounces) of 20-20-20 Peter's fertilizer mixed with tap water at 1.25g L<sup>-1</sup> (1.47 ounces/ fluid ounce). The study was terminated 56 days after treatment initiation.

**Soil Moisture Treatments.** A completely randomized design was employed, examining stress response of *L. oryzoides* [L.] Sw across four soil moisture regimes. Each treatment was replicated 12 times, with individual plants being treated as replicates. After treatment initiation, plants were watered daily with approximately two liters of a nutrient solution containing a maximum of 12 mg L<sup>-1</sup> (1.4 x 10<sup>-2</sup> ounces/ fluid ounce) ammonium nitrate and a maximum of 5 mg L<sup>-1</sup> (6 x 10<sup>-3</sup> ounces/ fluid ounce) sodium phosphate. These concentrations approximate the upper expected concentrations for agricultural ditches of the Mississippi River Delta in Arkansas and Mississippi (Bouldin et al. 2004).

Treatments included a (1) well-watered, well-drained control; (2) well-watered, well-drained intermittently flooded treatment; (3) partially flooded treatment; and (4) continuously flooded treatment. Water level was manipulated by placing pots in polyethylene bags and raising or lowering the level of the bag to the appropriate distance from the top of the soil. Control plants were well watered and allowed to drain freely. The intermittently flooded treatment was well watered and well drained except on days 7, 14, 19, 28, 35, and 45, when pots were flooded to 5 cm (2 in) above soil surface for a period of 48 hours. In the partially flooded treatment, water was maintained at 15 cm (5.9 in) below soil surface. Water level within the soil was checked periodically using an internal gauge constructed from 1.9 cm (0.75 in) perforated PVC pipe. In the continuously flooded treatment, water was maintained at 5 cm above the soil surface.

**Figure 1**

(A) Water level during the main portion of the study. (B) The same treatments during the 48-hour pulse flooding phase of the experiment, where water-level is raised to 5 cm above soil surface level in the 48-hour and partially flooded treatment, and to 20 cm above soil surface level in the saturated treatment.



On days 7, 14, 19, 28, 35, and 45, partially flooded and continuously flooded pots were flooded an additional 15 cm (5.9 inches) of water for 48 hours (figure 1). Standing water in the three flooded treatments was refreshed with nutrient solution once a week prior to the 48-hour intermittent flood. This design was intended to mimic variable hydrologic conditions on both ditch slope and trough as represented by the partially flooded and continuously flooded treatments, respectively.

**Soil Redox Potential Measurements.** Soil redox potential (Eh) was monitored using platinum-tipped electrodes, a Model 250 A ORION redox meter and a calomel reference electrode (Thermo Orion, Beverly, Massachusetts) as described by Patrick and DeLaune (1977). Redox electrodes (one at

each depth) were placed at 10 cm (3.9 in) and 30 cm (11.8 in) below the soil surface. Measurements were replicated at least eight times per measurement day on days 0, 1, 9, 16, and 30, with measures in each pot at each depth being considered replicates.

**Plant Photosynthetic Responses.** Measurements of plant photosynthetic responses were made prior to treatment initiation and on days 8, 20, and 29. All photosynthetic measurements were conducted between the hours of 9:00 a.m. and 2:00 p.m. local time. Measurements of net photosynthesis ( $P_n$ ) and stomatal conductance ( $g_s$ ) were obtained on the same eight plants per treatment, one measurement per plant, on each sampling day using a portable infrared gas analyzer (CIRAS1, PP Systems,

Haverhill, Massachusetts).

By the third week after treatment initiation, shoots included recumbent stolons hanging outside of the pot; however, gas exchange measurements were confined to the tallest upright shoots using leaves on the second or third node from the tip. On day 30, chlorophyll fluorescence was measured using an OS-100 Modulated Fluorometer (Opti-Sciences, Tynsboro, Massachusetts). Specifically, efficiency of excitation capture of open Photosystem II (Fv/Fm) was measured following guidelines of Maxwell and Johnson (2000).

**Plant Growth.** Immediately prior to treatment initiation, 10 plants were randomly harvested and separated into aboveground and belowground portions, dried and weighed to estimate initial biomass. At the study conclusion, the longest shoot, root, and penetration depth of rhizomes were measured. Aboveground shoots were counted for each pot. Plants were separated into aboveground and belowground portions and dried in a glasshouse for two weeks. Plants were then dried and weighed to determine biomass allocation. Biomass measures were then compared to initial biomass estimated using the following formula from Hoffmann and Poorter (2002):

$$r = \frac{\overline{\ln(M_2)} - \overline{\ln(M_1)}}{t_2 - t_1},$$

where  $\overline{\ln(M_1)}$  is the sample mean for the natural log of the final measured values,  $\overline{\ln(M_2)}$  is the sample mean for the natural log of the initial measured values, and  $t_2 - t_1$  is the length of time between sampling times.

**Statistical Analyses.** Soil Eh was analyzed for four flooding treatments across five sample dates at 10 cm and 30 cm sample depths using the general linear model for ANOVA. Leaf gas-exchange measures ( $P_n$  and  $g_s$ ) were analyzed using a repeated measures MANOVA (Hotelling's Trace) with four levels of soil moisture. Because of interactions between treatment and sample date, treatment effect was analyzed at each sample date using the General Linear Model MANOVA. Chlorophyll fluorescence (Fv/Fm) and all final measures of plant growth were analyzed using the general linear model for ANOVA. If a treatment effect was significant, univariate post hoc comparisons were made with Tukey's HSD. Because of non-normality, number of shoots was log transformed prior

**Table 1**

Summary of results giving mean values and standard deviation for each sampling date, as well as treatment effects for each parameter.

Measures	Sample day	Control Mean $\pm$ sd	Intermittent treatment		Partial treatment		Continuous treatment	
			Mean $\pm$ sd	Treatment effect	Mean $\pm$ sd	Treatment effect	Mean $\pm$ sd	Treatment effect
Pn ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	0	15.5 $\pm$ 1.6	15.1 $\pm$ 1.2	0	15.3 $\pm$ 1.8	0	15.5 $\pm$ 2.0	0
gs ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	0	208.2 $\pm$ 42.0	212.1 $\pm$ 29.1	0	222.2 $\pm$ 41.6	0	216.8 $\pm$ 39.1	0
Eh (mV) 10 cm	0	572 $\pm$ 76	561 $\pm$ 77	0	563 $\pm$ 68	0	498 $\pm$ 76	0
Eh (mV) 30 cm	0	573 $\pm$ 38	576 $\pm$ 20	0	577 $\pm$ 39	0	569 $\pm$ 20	0
Eh (mV) 10 cm	1	556 $\pm$ 78	436 $\pm$ 142	0	501 $\pm$ 120	0	456 $\pm$ 131	0
Eh (mV) 30 cm	1	568 $\pm$ 55	402 $\pm$ 126	0	465 $\pm$ 109	0	434 $\pm$ 138	0
Pn ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	8	14.2 $\pm$ 2.9	14.0 $\pm$ 2.3	0	12.4 $\pm$ 2.0	0	14.6 $\pm$ 2.8	0
gs ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	8	258.0 $\pm$ 67.4	262.2 $\pm$ 43.4	0	236.5 $\pm$ 46.2	0	253.8 $\pm$ 51.6	0
Eh (mV) 10 cm	9	590 $\pm$ 68	554 $\pm$ 139	0	348 $\pm$ 101	-	272 $\pm$ 209	-
Eh (mV) 30 cm	9	580 $\pm$ 70	442 $\pm$ 160	0	250 $\pm$ 165	-	207 $\pm$ 211	-
Eh (mV) 10 cm	16	589 $\pm$ 69	532 $\pm$ 86	0	557 $\pm$ 95	0	329 $\pm$ 130	0
Eh (mV) 30 cm	16	528 $\pm$ 158	379 $\pm$ 185	0	260 $\pm$ 103	-	263 $\pm$ 268	0
Pn ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	20	7.4 $\pm$ 3.5	7.6 $\pm$ 3.2	0	8.4 $\pm$ 2.6	0	7.0 $\pm$ 1.3	0
gs ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	20	139.6 $\pm$ 62.7	141.6 $\pm$ 52.2	0	155.4 $\pm$ 51.4	0	141.2 $\pm$ 48.9	0
Pn ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	29	9.7 $\pm$ 1.3	7.1 $\pm$ 1.0	-	6.5 $\pm$ 2.2	-	7.3 $\pm$ 1.8	-
gs ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	29	198.3 $\pm$ 25.0	149.8 $\pm$ 23.5	0	133.8 $\pm$ 38.3	-	160.5 $\pm$ 43.6	0
Eh (mV) 10 cm	30	600 $\pm$ 64	236 $\pm$ 67	-	213 $\pm$ 129	-	272 $\pm$ 57	-
Eh (mV) 30 cm	30	523 $\pm$ 84	136 $\pm$ 157	-	142 $\pm$ 205	-	96 $\pm$ 113	-
Fv/Fm day 30	30	0.748 $\pm$ 0.081	0.737 $\pm$ 0.033	0	0.752 $\pm$ 0.061	0	0.723 $\pm$ 0.069	0
Aboveground mass (g)	56	33.8 $\pm$ 13.0	37.4 $\pm$ 8.8	0	35.9 $\pm$ 7.8	0	47.2 $\pm$ 12.3	+
Belowground mass (g)	56	24.8 $\pm$ 8.6	22.1 $\pm$ 6.3	0	20.4 $\pm$ 8.3	0	20.4 $\pm$ 5.5	0
Number of shoots	56	48.3 $\pm$ 18.8	53.7 $\pm$ 20.8	0*	48.2 $\pm$ 15.3	0*	65.1 $\pm$ 11.2	0*
Shoot length (cm)	56	142.1 $\pm$ 24.3	154.9 $\pm$ 22.1	0	147.9 $\pm$ 23.2	0	143.1 $\pm$ 21.6	0
Root depth (cm)	56	47.3 $\pm$ 6.5	46.7 $\pm$ 4.9	0	45.5 $\pm$ 6.6	0	45.8 $\pm$ 4.3	0
Rhizome depth (cm)	56	44.8 $\pm$ 6.2	41.7 $\pm$ 6.9	0	43.8 $\pm$ 4.5	0	36.3 $\pm$ 5.5	-

Notes: For treatment effects, + indicates an increase compared with control, - indicates a decrease compared with control, and 0 indicates no significant difference between treatment and control. Treatment effects for Eh values were not considered significant on the basis of statistical analysis alone. Rather, their significance was determined on the basis of probable changes in bioavailable oxygen or other pertinent soil chemistry parameters as estimated by soil Eh. Although an overall treatment effect on number of shoots was observed, pairwise comparisons showed no statistical differences between individual treatments at  $\alpha = 0.05$ .

to data analyses. All statistical analyses were made using SPSS 14.0.

## Results

A summary of data is recorded in table 1, with mean values and standard deviations presented across all sample days, as well as an indication of positive or negative significant differences from the control.

**Soil Eh.** Prior to treatment initiation, all treatments were well aerated; mean soil Eh across all treatments were +549 ( $\pm$  76) mV and +573 ( $\pm$  29) mV at 10 cm and 30 cm, respectively. Immediately following treatment initiation, flooded treatments showed a decrease in soil Eh ( $p = 0.006$ ). In general, soil Eh continued to decrease in flooded treatments throughout the study. By day 30, soil Eh was near or below the critical oxygen

threshold at 10 cm depth and 30 cm depth across all flooded treatments (figure 2). With the exception of the continuously flooded treatment, which had mean soil Eh values near or below +350 mV after day nine of the study (table 1), Eh differences between treatments prior to day 30 may be largely disregarded, as even large changes in soil Eh have little effect on soil chemistry or wetland plant response if oxygen is readily available (Pezeshki 2001).

An overall treatment effect was demonstrated across sampling depths and sampling times (table 2). However, there was a significant interaction for sampling date and treatment, as well as depth and treatment for soil Eh. Further analysis showed the former interaction to be a general decline in soil Eh for all flooded treatments, while the control

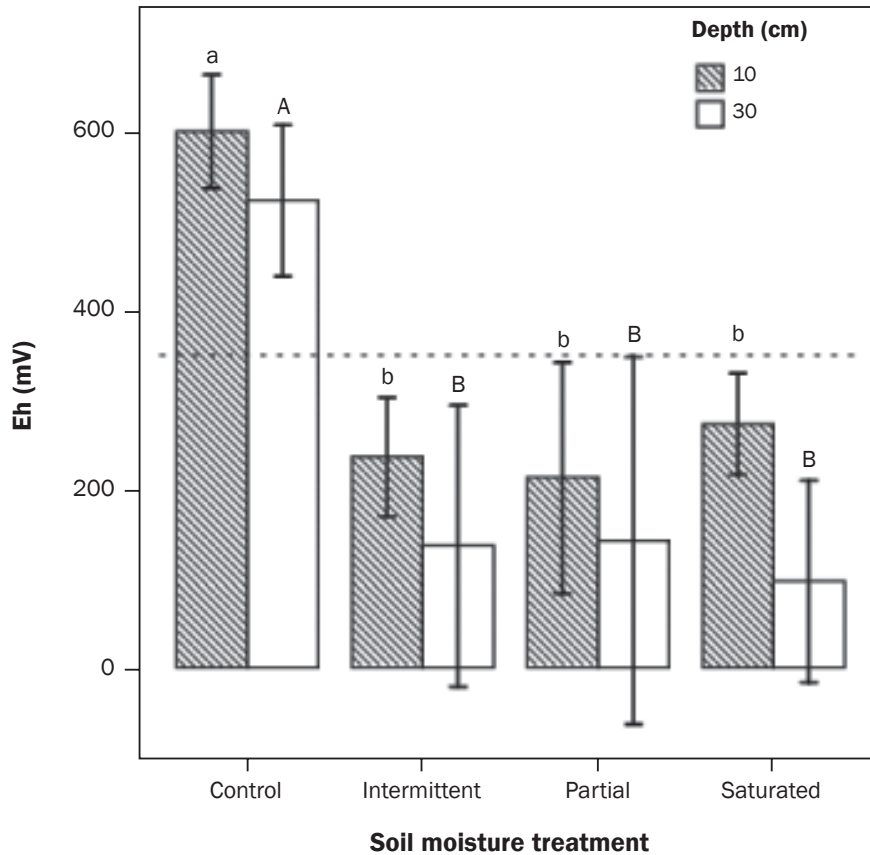
maintained a mean Eh of +580 ( $\pm$  69) mV at the 10 cm depth and +556 ( $\pm$  93) mV at the 30 cm depth throughout the study (table 1). The interaction between depth and treatment resulted from variations in treatment differences between 10 cm sample depth and 30 cm sample depths. While Eh was generally lower at 30 cm than 10 cm in all treatments, these differences would not be relevant for most wetland plants, as soils were either aerated at both depths or anoxic at both depths (Pezeshki 2001). Additionally, due to the lack of precision associated with soil redox measures, and the fact that only single electrodes were used within each replicate, any generalizations of these data should be used with caution.

**Plant Gas Exchange.** A significant time and treatment interaction was found for



**Figure 2**

Mean ( $\pm$  sd) soil redox potential (Eh) under various moisture regimes at 10 cm depth and 30 cm depth on day 30.



Notes: The dashed line at Eh = 350 mV indicated the approximate critical oxygen threshold. Significant differences across soil moisture treatment within each sampling depth are indicated by different letters.

Pn and  $g_s$  ( $p = 0.015$ ,  $F = 1.953$ ). Days 1, 8, and 20 showed no significant treatment effect (table 1). Only on day 29 did gas exchange show any response to flooding ( $p = 0.04$ ,  $F = 5.666$ ). All flooded treatments differed significantly from the control, while flooded treatments did not differ significantly from each other (figure 3). With regard to  $g_s$

(figure 4), a treatment effect was observed ( $p = 0.015$ ,  $F = 4.117$ ), with only partially flooded plants showing a significant response ( $p = 0.011$ ), while intermittently flooded plants showed a marginal response ( $p = 0.076$ ). Because gas exchange measures such as  $g_s$  and Pn reveal short-term stress effects related to carbon fixation, these data suggest that flooding would negatively affect productivity only during times of moderate to severe soil reduction ( $Eh < +350$  mV). No difference was found in Fv/Fm on day 30 (mean Fv/Fm = 0.7402,  $p = 0.695$ ; table 1), implying rate-limiting mechanisms of Photosystem II were not affected by flooding conditions imposed in this study.

Interestingly, although Pn was eventually decreased in all flooded treatments, apparently in response to soil anoxia, the continuously flooded treatment showed no decrease in Pn on days 8 and 20, in spite of having mean Eh values at 10 cm of 272  $\pm$  208 mV on day 9 and 329  $\pm$  130 mV on day 16 (table 1). While the large variance in Eh and lack of specific soil data make any generalizations of soil chemistry difficult, it is possible that factors other than oxygen depletion, such as reduction of nitrate, are responsible for the observed decrease in Pn.

**Plant Morphology.** There were no differences in shoot length among treatments ( $p = 0.459$ ; table 1). A significant treatment effect on number of shoots was found in response to flooding showing a general effect of flooding on shoot number ( $p = 0.043$ ,  $F = 2.959$ ), with the continuously flooded treatment having the most shoots, followed by intermittently flooded, control, and partially flooded (see table 1). The continuously flooded treatment had an average of 17 more shoots per plant; however, a post hoc analysis comparing the continuously flooded treatment to the control found only marginal statistical differences ( $p = 0.056$ ).

Rhizome penetration depth differed significantly between treatments ( $p = 0.021$ ,  $F = 3.715$ ; table 1), with continuously flooded plants having significantly shallower rhizomes than control plants ( $p = 0.017$ ) and marginally shallower rhizomes than partially flooded plants ( $p = 0.057$ ). Root penetration depth was not affected by flooding ( $p = 0.858$ ; table 1). A comparison of root depth and rhizome penetration depth is shown in figure 5. Because rhizomes are belowground stems, it is interesting to note that the continuously flooded treatment,

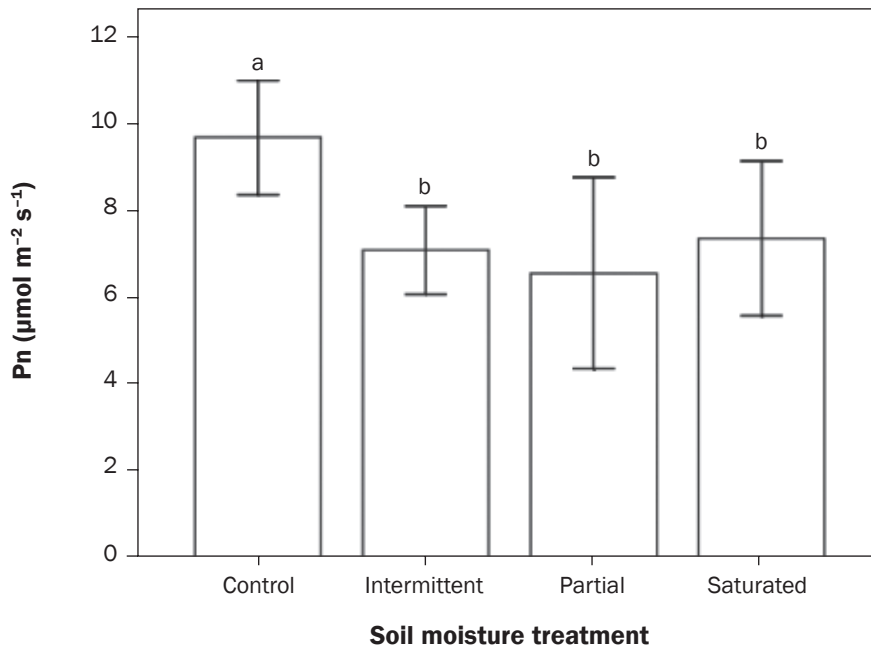
**Table 2**

ANOVA table showing the main effects and interactions on Eh.

Source	df	F	Significance
Flooding treatment	3	46.190	0.000
Depth of sample	1	19.347	0.000
Sampling day	4	43.279	0.000
Flooding treatment $\times$ depth	3	1.115	0.343
Flooding treatment $\times$ sampling day	12	7.160	0.000
Sampling depth $\times$ sampling day	4	4.412	0.002
Flooding treatment $\times$ sampling depth $\times$ sampling day	12	0.819	0.631
Error	260		
Total	300		
Corrected total	299		

**Figure 3**

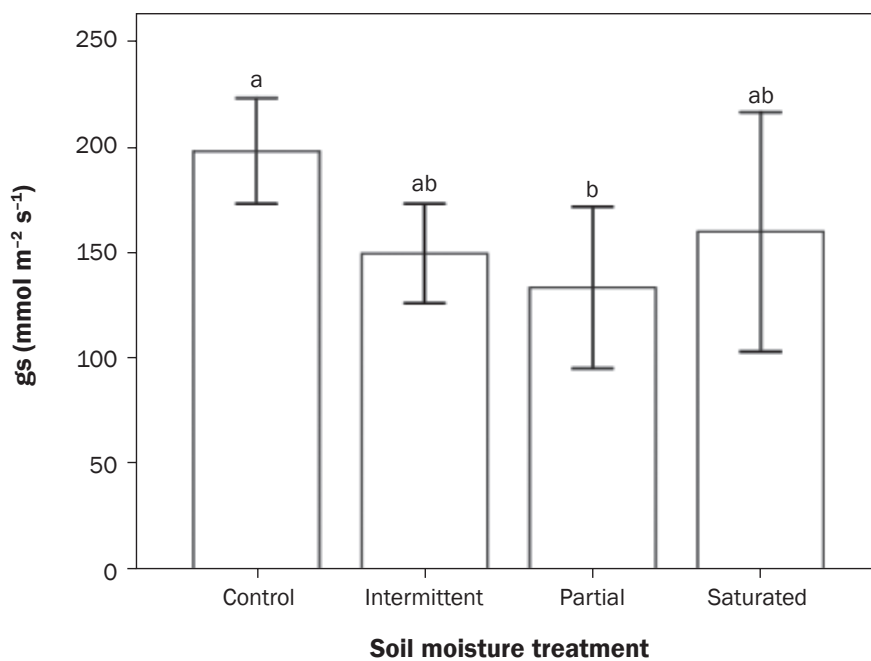
Mean ( $\pm$  sd) net photosynthesis (Pn) of *L. oryzoides* across various soil moisture regimes on day 29, showing a significant difference between the control and all flooded treatments.



Notes: Significant differences are indicated by different letters.

**Figure 4**

Mean ( $\pm$  sd) stomatal conductance (gs) of *L. oryzoides* across various soil moisture regimes on day 29, demonstrating a difference between the control and the partially flooded treatment.



Note: Significant differences are indicated by different letters.

which showed decreased belowground stem development, also showed the greatest number of aboveground stems, indicating a trend towards aboveground development even under only moderate soil reduction.

A significant treatment effect ( $p < 0.001$ ) was observed in root:shoot biomass ratios demonstrating a typical wetland plant response to flooding by allocating carbon preferentially aboveground rather than belowground ( $p < 0.001$ ,  $F = 7.86$ ). No differences were observed in aboveground:belowground ratios among flooded treatments, but all of these treatments differed significantly from the control (intermittently flooded:  $p = 0.041$ , partially flooded:  $p < 0.001$ , continuously flooded:  $p = 0.017$ ). Further analyses revealed treatment differences lie in increased aboveground mass for flooded treatments ( $p = 0.024$ ,  $F = 3.501$ ) rather than reduced belowground mass, with only continuously flooded plants differing significantly from control ( $p = 0.022$ ; figure 6, table 1). Therefore, although reduction of soil Eh caused a measurable decrease in Pn, this decrease was not reflected in total productivity. Specifically, although the soil in continuously flooded treatment was moderately reduced throughout much of the study, and Pn decreased near the end of the study, aboveground biomass was 23% greater in the continuously flooded treatment than in the control.

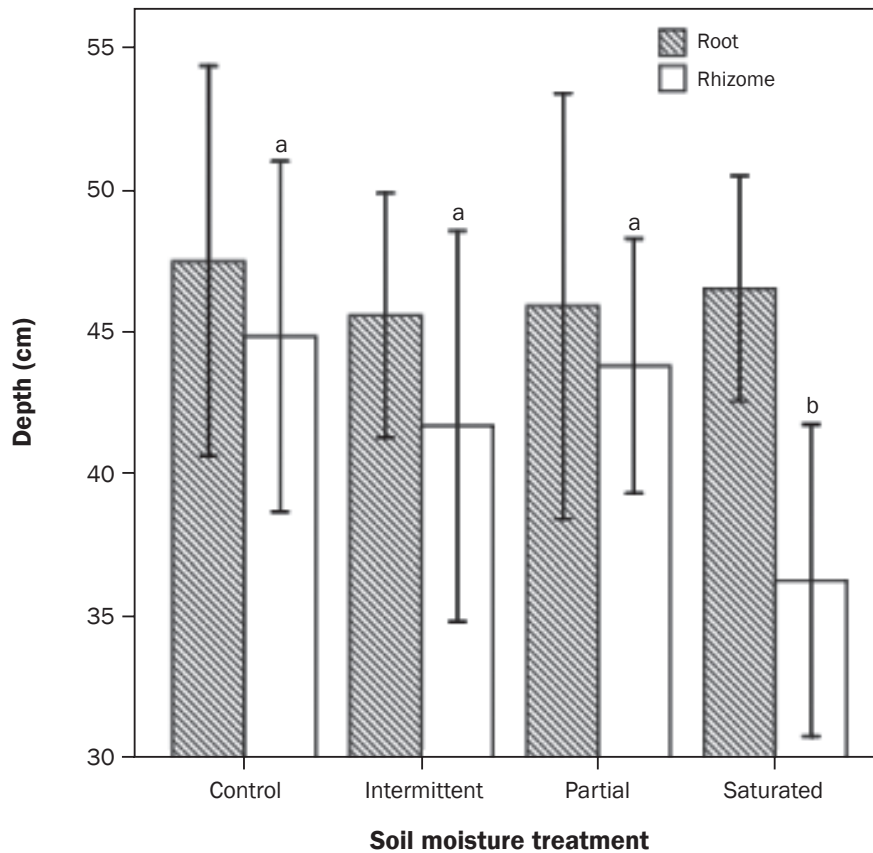
Trends in relative growth rate ( $\Delta \text{g}^{-1} \text{g}^{-1}$  week,  $\Delta \text{cm cm}^{-1} \text{week}^{-1}$ ) were essentially the same as the trends for measured values ( $\text{cm [g]}$ ). Mean root penetration depth actually decreased over the course of the experiment for all treatments. Calculated mean values for relative growth rate of belowground and aboveground biomass, as well as root penetration depth and shoot length, are recorded in table 3.

### Discussion

Photosynthesis in *L. oryzoides* decreased under flooded conditions when soil was reduced below the critical oxygen threshold ( $E_h = +350 \text{ mV}$ ; table 1, figures 2, 3, and 4). However, mechanisms for this decrease may differ depending upon whether flooding is continuous, intermittent, or partial. Although all flooded treatments eventually showed decreases in Pn (table 1, figure 2), plants in the continuously flooded treatment showed no difference in gs (figure 3), implying that photosynthetic rates may be

**Figure 5**

Mean ( $\pm$  s.d.) soil penetration depth of longest root and longest rhizome in *L. oryzoides* across various moisture regimes, demonstrating a flood response for the rhizomes under continuous flooding.



Notes: No comparable response was seen in roots. Significant differences in rhizome penetration across soil moisture treatments are indicated by different letters.

limited by nonstomatal (metabolic) factors in this species when repeatedly exposed to extended periods of flooding.

This nonstomatal limitation is not reflected in Photosystem II (PSII) activity, as demonstrated by Fv/Fm data (table 1). Because Fv/Fm represents the capacity of Photosystem II, rather than actual energy absorbed, it is

indicative of photochemical processes that are regulated over a longer time period than gas exchange measures such as Pn (Maxwell and Johnson 2000). Comparisons of Fv/Fm data to gas exchange data indicated that while soil Eh below the critical oxygen threshold results in a short-term stress response, damage to the photosynthetic apparatus for this

species is negligible under short-term moderate soil reduction (table 1).

The ability of vegetation to remove nutrients and pesticides from water is related to plant productivity, which is directly dependent upon carbon fixation. Increased productivity is generally accompanied by greater total nutrient sequestration, and the larger plants resulting from greater productivity provide a larger surface area for mechanical and biological interactions with the water (Cronk and Fennessy 2001). Therefore, reduced photosynthesis occurring at high soil water volumes may limit the ability of vegetation to uptake contaminants, especially excess nutrients (Pezeshki et al. 1998; DeLaune et al. 1998). This relationship is especially important when stomatal limitations related to loss of root function and low stomatal conductance limits the volume of water moving through the plant.

Belowground biomass was unaffected by the moderate soil reduction in this study, while aboveground biomass was significantly greater in continuously flooded plants than control plants (table 1, figure 6). The increase in aboveground biomass was anomalous. Li et al. (2004b) showed enhanced biomass production in continuously flooded *Typha latifolia* (cattail); however, in that study, Pn was also elevated allowing for greater carbon fixation. It is possible that weekly draining and replenishment of water slowed soil reduction sufficiently for continuously flooded plants to recover from initial flooding and for Pn rates to recover and exceed rates found in control plants. Although previous studies have shown similar findings, the recovery period is usually several days to weeks (Li et al. 2004b; Pezeshki 2001). In order to further test this possibility, laboratory studies should be conducted in which daily measurements of initial flood stress and recovery responses are feasible.

Root penetration depth was unaffected by flooding (figure 5). In agricultural areas, erosion is often a major environmental problem linked not only to localized ecosystem changes, but potentially to downstream turbidity and eutrophication (Lal 1998). Vegetation establishment has been recommended as an effective method for reducing erosion in agricultural areas (Lovell and Sullivan 2006) and adjacent riparian areas (Shields et al. 1995). The fact that moderate soil reduction had no effect on root penetra-

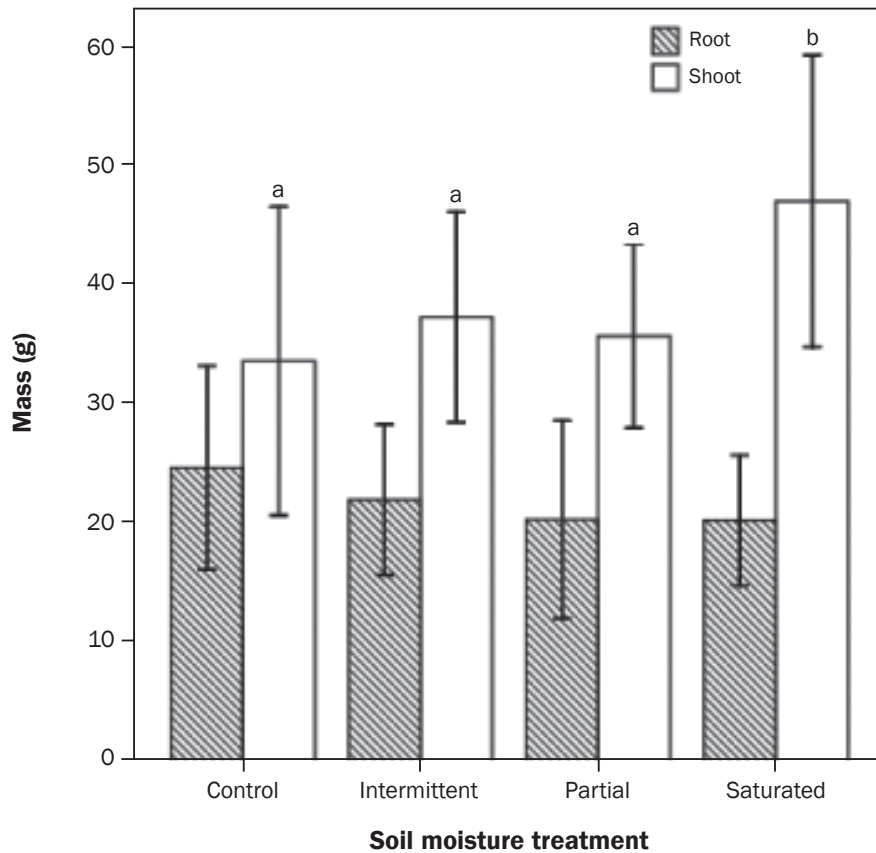
**Table 3**

Relative growth rate for *Leersia oryzoides* under various soil moisture regimes: control, intermittent flooding, partial flooding, and continuous flooding.

	Mass ( $\Delta$ g <sup>-1</sup> g <sup>-1</sup> week)		Length ( $\Delta$ cm cm <sup>-1</sup> week <sup>-1</sup> )	
	Aboveground	Belowground	Shoot	Root
Control	0.207	0.281	0.093	-0.018
Intermittent	0.226	0.268	0.104	-0.019
Partial	0.221	0.257	0.098	-0.023
Continuous	0.255	0.259	0.095	-0.022

**Figure 6**

Mean ( $\pm$  sd) biomass of *L. oryzoides* across various moisture regimes, demonstrating a flood response for aboveground biomass under continuous flooding.



Notes: No comparable response was seen in belowground biomass. Significant differences for each biomass component across treatments are indicated by different letters.

tion and biomass in *L. oryzoides*, when taken in consideration with the dense, fast growing colonies formed by this species (Darris and Bartow 2006), suggests that it may serve a valuable role in stabilizing soils in and adjacent to agricultural ditches.

While root penetration depth was unaffected by flooding, rhizome penetration depth was decreased in continuously flooded plants (figure 5). In other words, belowground stems were not elongating into the anoxic zone. It is well established that root penetration depth can differ between flooded and nonflooded plants (Pezeshki 1991, 2001; Will et al. 1995); however, differences in depth of rhizome penetration have not been previously reported.

The observed difference in rhizome penetration depth due to flooding has two potential implications with regard to the ecology of *L. oryzoides*. First, continuous flooding of *L. oryzoides* may reduce below-

ground carbon storage capacity. Our data fail to support this notion, as belowground biomass was unaffected by flooding (table 1, figure 6). Alternately, the rhizome may be functioning primarily for vegetative propagation rather than for storage, with rhizomes preferentially growing toward more favorable conditions when the mother plant is stressed (Jónsdóttir and Watson 1997). Such a pattern of rapid vegetative spread would be advantageous for early successional species in an environment where much of the ground is bare. Further study of this species is needed to determine what portion of the belowground biomass is composed of root tissue versus rhizomatous tissue under different water regimes and the role that rhizomes play in storage and/or processing of the carbohydrate pool, as well as the functioning of clonal integration under variable flooding.

Although wetland plants have numerous mechanisms to acclimate to flooding, under

more intense soil reduction resulting from longer periods of saturation or increased soil organic carbon content, it is likely that the Pn in flooded plants would continue to decline, eventually leading to decreased productivity (Pezeshki 2001). Additionally, even though wetland plants may grow in standing water, unlike aquatic plants they have only limited tolerance for submergence (Voeselek 2004). This consideration is especially important early in the growing season when shoots of ditch plants may be submerged by only a few centimeters of water.

### Summary and Conclusions

Data from this study have important ramifications with regard to the role that *L. oryzoides* can play in occupying agricultural ditches and the effect they can have on water quality in such habitats. While flooding had no initial effect on leaf gas exchange, when soil Eh dropped below the critical oxygen threshold for several days, Pn was reduced. These findings indicate that periods of saturation greater than those in this study, or saturation of soils that are more readily reduced because of lower sand content, may negatively affect productivity for *L. oryzoides*. However, as previously noted, relating the hydrologic regimes in this study to those in the field is difficult due to the comparatively high sand content of the soil used in this study. The relationship between Eh and productivity, while imprecise, does provide a sound basis for further experimentation and field observation.

This study demonstrated that *L. oryzoides* grew readily when subjected to fluctuating levels in soil water, and that periods of saturation for several days may even improve productivity. However, evidence of reduced carbon assimilation (Pn) suggests that prolonged or repeated soil saturation resulting in moderately to highly reduced soils may reverse this trend. Such trends should be taken into account when considering management practices which alter the natural “flashy” hydrology of agricultural ditches. Efforts to increase hydraulic retention time, such as use of weirs, may decrease productivity in dominant species, resulting in a short-term decrease in functioning, and potentially long-term changes in community composition.



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