

## NUTRIENT AND GROWTH RESPONSES OF *LEERSIA ORYZOIDES*, RICE CUTGRASS, TO VARYING DEGREES OF SOIL SATURATION AND WATER NITROGEN CONCENTRATION

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□ *Leersia oryzoides* (rice cutgrass) is an obligate wetland plant common to agricultural drainage ditches. The objective of this greenhouse study was to expose plants to various flooding and aqueous nitrogen (N) concentrations and then to quantify the allocation of nutrients and biomass to plant components. Plants in the continuously flooded treatment (CF) had the highest tissue concentrations of copper (Cu), sulfur (S), zinc (Zn), potassium (K), sodium (Na), and manganese (Mn) in one or more plant components. Plants in the partially flooded treatment (PF) had the highest concentrations of magnesium (Mg) in leaves. The N input affected phosphorus (P) and S concentrations in roots. Leaf, stem, and root biomass were highest in PF plants. Rhizome biomass was the lowest in CF plants. These results indicate that *L. oryzoides* may significantly affect elemental concentrations in surface waters by its ability to uptake various elements and subsequent sequestration in various biomass components.

**Keywords:** agricultural runoff, elemental concentrations, drainage ditch, N pollution, variable flooding, wetland plants, vegetated buffer, buffer strip

### INTRODUCTION

Human production of reactive nitrogen (N) is greater than that produced by all natural terrestrial systems (Galloway et al., 2003). Synthetic fertilizer provides close to half of N to crops (Smil, 2002) and is one of the primary determinates of global cropland yields (Galloway et al., 2003). Most of the 120 teragrams of N added annually to global croplands is lost

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to soil, air and water (Smil, 2001, 2002). Nitrogen applied to soil will eventually become nitrate, a mobile cation, which is available for movement in water. Because of their efficiency in removing N, wetlands are frequently constructed (Galloway et al., 2003). Water quality improvements have been documented in areas planted with grass-shrub buffers as narrow as 8 m (26.25 ft) wide (Mankin et al., 2007), which become more effective over time due to accumulated organic matter and root system development (Schultz et al., 1995). The function and effects of plants are species-specific; thus, heterogeneity in the system may provide the potential for maximum benefits.

Agricultural drainage ditches and their associated vegetative buffers can function as wetlands linking the agricultural surface and subsurface flow to receiving waters, effectively channeling the water from the saturated zone along fields directly into streams and rivers, and eventually the oceans (Goolsby et al., 2001; Moore et al., 2001; Kröger et al., 2007). Ephemeral and intermittent drainage ditches and ditch slopes often have wetland components (Kröger et al., 2007) defined as fluctuating hydrology, unique soil conditions that differ from adjacent uplands, and hydrophytic vegetation (Mitsch and Gosselink, 2007). Multi-species vegetated drainage ditches and riparian buffer strips can actively provide natural services (Schultz et al., 1995; Cooper et al., 2004) including increased distribution of beneficial organisms, providing habitat for wildlife, increased water availability and filtration, and improved bank stability and soil fertility (Mitsch and Gosselink, 2007).

Plants can both respond to and change their environments. Grasses, in particular, can form dense stands that benefit soil structure by enhanced water percolation, increased aggregation, improved aeration, increased carbon storage, and improved subsurface cohesion (Schultz et al., 1995; Mankin et al., 2007). Well-developed root systems and rapid growth rates are structural aspects that increase water infiltration, which is strongly linked to efficient sediment removal and contaminant uptake (Mankin et al., 2007). Increased root surface area supplies oxygen to heterotrophic microorganisms in the rhizosphere (Brix, 1997). Recently, research has focused on the efficacy of aquatic and wetland plants to mitigate and diffuse agricultural runoff in primary agricultural drainage ditches to better manage and protect fresh water (Pierce et al., 2009; Pierce and Pezeshki, 2010). These plants diversify the homogenous agricultural landscape by trapping and removing nonpoint source pollutants, such as excess nutrients in the waterway through direct assimilation and immobilization (Kröger et al., 2007; Pierce et al., 2009).

Many obligate wetland plant species, such as rice cutgrass [*Leersia oryzoides* (L.) Sw.], are found in agricultural ditches (Bouldin et al., 2004). *L. oryzoides* is an erect, perennial grass (Poaceae) that can form dense colonies (Hayden, 1919; USDA, 2006) and is often an early successional species of

open freshwater wetlands (Farnsworth and Myerson, 2003). Densities of *L. oryzoides* up to 34% are found in as many as 80% of edge-of-field waterways in the Mississippi River Delta landscape (Bouldin et al., 2004). *L. oryzoides* persists in all drainage size classes, reflecting its tolerance of variable water levels and nutrient regimes (Bouldin et al., 2004). *L. oryzoides* contributes to seed banks, especially in areas subject to sedimentation and fluctuating hydrology (Galatowitsch and van der Valk, 1996; Le Page and Keddy, 1998; Peterson and Baldwin, 2004), while also forming extensive systems of belowground rhizomes for vegetative propagation (Darris and Barstow, 2006). Exposed rhizomes facilitate increased numbers of shoots that may allow plants to endure or avoid environmental stresses (Pierce et al., 2007). *L. oryzoides* can grow readily during several days of soil saturation, demonstrating its ability to survive and potentially affect water quality (Pierce et al., 2007; Koontz and Pezeshki, 2011), such as the reduction of effluent nutrient concentrations through uptake and sequestration (Deaver et al., 2005; Pierce et al., 2009).

The objective of this greenhouse study was to quantify the compartmentalization of various nutrients and biomass allocation to different *L. oryzoides* components (leaves, stems, rhizomes, and adventitious roots). Plants were subjected to various soil moisture and aqueous N input regimes to gain a better understanding of how this species responds to changes similar to those found in agricultural drainage ditch environments. Plants grown in partially flooded treatments with higher N inputs were expected to be productive because this environment is the most common habitat in which this species is found. Furthermore, it was hypothesized that enhanced growth would also lead to higher total elemental tissue concentrations than other treatment combinations.

## MATERIALS AND METHODS

### Experimental Materials

*L. oryzoides* plants were collected from wild populations at the University of Mississippi Field Station near Abbeville, Mississippi. Plants were grown in a greenhouse under natural light with supplemental lighting to maintain a 14-hour photoperiod. The median temperature was 24.5°C (76 °F). Plants were separated and then cut to 5 cm (2 in) with equal parts of both stems and rhizomes. The fresh weights of each were 0.27 g ± 0.05. Individuals were planted in plastic plant trays and allowed to grow. After eight weeks, individual plants were randomly selected and replanted in 60 cm (2 ft) deep, 15 cm polyvinyl chloride (PVC) pipe pots. Caps were glued to the bottom of each pot to prevent water loss. Drainage was controlled by two sets of three holes on the side of the PVC pipe. One set of holes was at 15 cm (5.9 in) below the soil surface and another set at 55 cm (21.7 in). The holes were plugged with rubber stoppers to control the water level within each pot.

Each pot was filled with soil. The soil was Bruno loamy sand (NRCS, 2011) obtained from Craighead County, Arkansas. The Bruno series consists of very deep, excessively drained, rapidly permeable soils (NRCS, 2011). Repotted plants were allowed to establish for a period of six weeks prior to treatment initiation. Prior to treatment, each plant was well-watered and well-drained. Plants were fertilized every two weeks with 2 L (67.6 oz) of tap water mixed with 1.25 g L<sup>-1</sup> 20–20–20 water-soluble fertilizer.

## Experimental Procedure

The experiment was a complete 3×3 factorial design with three levels of soil moisture and three concentrations of aqueous ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>-N) addition. A completely randomized design was employed. The study concluded six weeks after flood treatment initiation.

The three levels of soil moisture were: 1) well-watered, well-drained, the control treatment (C), 2) water maintained at 15 cm (5.9 in) below the soil surface, the partially flooded treatment (PF), and 3) water maintained at 5 cm (2 in) above the soil surface, the continuously flooded treatment (CF). After flooding initiation, plants were watered daily with 2 L (67.6 oz) of tap water. Once a week, flooded treatments were drained of water overnight and refreshed with 7 L (246.4 oz) of tap water the following morning. These variable hydrologic conditions were intended to replicate conditions of both the ditch slope and trough, represented by PF and CF, respectively.

The NH<sub>4</sub>NO<sub>3</sub>-N addition was given in two separate exposures at concentrations of 15 mg L<sup>-1</sup>, 50 mg L<sup>-1</sup>, or 100 mg L<sup>-1</sup>. The NH<sub>4</sub>NO<sub>3</sub>-N doses were given at two and four weeks following the initiation of flooding treatments. The treatments consisted of 2 L (67.6 oz) of NH<sub>4</sub>NO<sub>3</sub>-N solution containing the desired concentration and tap water. The NH<sub>4</sub>NO<sub>3</sub>-N doses were representative of those found in surface water runoff from agricultural fields (Stuntembeck et al., 2011). Concentrations up to 10 mg L<sup>-1</sup> of nitrate is considered the maximum contaminant level (MCL) in drinking water (USEPA, 2009). Levels at 100 mg N L<sup>-1</sup> causes lethal and sub-lethal effects in amphibians (Rouse et al., 1999).

## Soil Measurements

The soil used in this study was classified as loamy sand (81.0% sand, 10.5% silt, and 8.5% clay) with low organic matter content (0.4%). The Mehlich-3 test was used to obtain soil elemental results. The soil element concentrations were 0.4 ppm boron (B), 1019 ppm calcium (Ca), 0.9 ppm copper (Cu), 111 ppm iron (Fe), 83 ppm potassium (K), 113 ppm magnesium (Mg), 50 ppm manganese (Mn), 29 ppm sodium (Na), 40 ppm phosphorus (P), 6 ppm sulfur (S), and 2.6 ppm zinc (Zn). Soil N was determined using a LECO FP-528 nitrogen analyzer (Leco Corp., St. Joseph, MI, USA). There

was 0.5642% soil N with 6.2736 mg L<sup>-1</sup> nitrate (NO<sub>3</sub>-N) and 4.1443 mg L<sup>-1</sup> ammonium (NH<sub>4</sub>-N). The calculated cation exchange capacity was 7.3 meq 100 g<sup>-1</sup>. The calculated cation saturation values were 69.8% for Ca, 13.2% for hydrogen (H), 2.9% for K, 12.9% for Mg, and 1.7% for Na. The K: Mg ratio was 0.23. The initial soil pH was 6.4.

Soil redox potential (Eh, mV) was measured using platinum-tipped electrodes, a mV meter (Orion, Model 250A), and a calomel reference electrode (Thermo Orion, Beverly, MA, USA). Two platinum-tipped electrodes were placed in an individual pot at depths of 10 cm and 30 cm (3.9 in and 11.8 in) below the soil surface. Electrodes remained in place for the duration of the experiment. The redox potential values were measured according to methods established by Patrick and DeLaune (1977). Baseline measurements of soil redox potential were taken two weeks prior to flood treatment initiation and every two weeks subsequently, equaling four sampling dates. An Eh value of +350 mV represents the approximate level at which soil becomes anoxic.

### Nutrient Analyses

All samples were finely milled in a Cyclone Sample Mill to a uniform size. Plant tissue samples (0.1 g ± 0.005 g) (0.0035 oz) were digested using the wet ash method with nitric acid (HNO<sub>3</sub>) (10 ml) (0.34 oz), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (2 mL) (0.7 oz), and hydrochloric acid (HCl) (10 mL) (0.34 oz) using a block heater and element. Total N was analyzed using a LECO FP-528. Aluminum (Al), B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Elemental Iris II).

### Plant Growth

Plants were harvested and partitioned into leaf, stem, rhizome, and adventitious root components. Plant parts were placed in separate bags and oven-dried at 65°C (149°F) for 48 hours, or until a constant weight was reached. Plant parts were weighed to the nearest 0.01 g (0.0035 oz).

### Data Analyses

Repeated measures multivariate analysis of variance (MANOVA) (SPSS Inc., Chicago, IL, USA) with three levels of soil moisture and four levels of sampling dates was used to test the differences in means of soil redox measurements (Eh) at 10 cm and 30 cm depth. Differences in means of nutrient tissue concentrations, including total N, Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn, and differences in means of biomass measurements, including leaf, stem, rhizome, and adventitious root components, were individually analyzed and tested with two-way analysis of variance (ANOVA) (SYSTAT Inc.,

Chicago, IL, USA) with three levels of soil moisture and N concentrations as independent factors. Significant results were followed by Tukey's post-hoc analysis (Hinkle et al., 2003). Differences were considered significant at  $\alpha < 0.05$ .

## RESULTS

### Soil Measurements

Soil pH averaged  $7.25 \pm 0.15$  at the conclusion of the experiment. Prior to the flooding treatments, soil was aerated in all pots at both the 10 cm ( $E_h = +482 \pm 110$  mV) and 30 cm depth ( $+455 \pm 105$  mV). Following flood treatments, soil remained aerated at 10 cm ( $E_h = +579 \pm 94$  mV) and 30 cm depths ( $+538 \pm 90$  mV) in C and at 10 cm deep ( $E_h = +534 \pm 101$  mV) in PF during the course of the experiment. Soil in flooded treatments became reduced 2 weeks following soil moisture treatment. After four weeks, soil  $E_h$  was in the anoxic range in flooded treatments: 30 cm depth in PF ( $E_h = +194.00 \pm 139$  mV,  $F = 24.980$ ,  $P < 0.001$ ), 10 cm depth in CF ( $E_h = +285 \pm 102$  mV,  $F = 12.084$ ,  $P < 0.001$ ), and 30 cm depth in CF ( $+172 \pm 148$  mV,  $F = 19.240$ ,  $P < 0.001$ ). These results indicate that soil became anoxic due to  $E_h$  dropping below  $+350$  mV.

### Nutrient Tissue Concentrations

Elemental tissue concentrations were compared between plant components (Table 1). The leaves had the highest concentrations of B, Ca, Mg, Mn, N, and S. The stems had the highest concentrations of K and Zn. The adventitious roots had the highest concentrations of Al, Cu, Fe, Mn, and Na. The rhizomes had the highest concentrations of P. The leaves had the lowest elemental concentrations of Cu. The stems had the lowest elemental concentrations of Al, B, Fe, N, and Na. The adventitious roots had the lowest elemental concentrations of K, P, and S. The rhizomes had the lowest elemental concentrations of Ca, Mg, Mn, and Zn.

Flooding did affect elemental concentrations of Cu, Mg, Mn, K, Na, S, and Zn (Table 2). Plants grown in the CF treatment had significantly higher concentrations of Cu, S, and Zn in leaves, Zn in stems, Cu, Mn, S, and Zn in adventitious roots, and Cu, K, and Na in rhizomes. The PF treatment had significantly higher concentrations of Mg in leaves and of Na in adventitious roots. The flooding treatments did not significantly affect Al, B, Ca, Fe, N, and P tissue concentrations in any plant module.

Higher  $\text{NH}_4\text{NO}_3$ -N additions led to significantly less P and S adventitious root tissue concentrations (Table 3). Ammonium nitrate-N addition did not significantly affect elemental concentrations of Al, B, Ca, Cu, Fe, Mg, Mn, N, K, Na, and Zn in any plant module. A significant interaction between

**TABLE 1** Mean biomass and nutrient tissue concentrations allocated to leaves, stems, rhizomes and adventitious roots of *Leersia oryzoides*. Each value is the mean of 135 replications ( $\pm$ SE) for biomass and 36 replications ( $\pm$ SE) for nutrient tissue concentrations. Significant differences between plant components are indicated by using different lower-case letters, according to Tukey's post-hoc. Differences were considered significant at  $\alpha < 0.05$

	Biomass (g)	Al ( $\mu\text{g g}^{-1}$ )	B ( $\mu\text{g g}^{-1}$ )	Ca ( $\mu\text{g g}^{-1}$ )	Cu ( $\mu\text{g g}^{-1}$ )	Fe ( $\mu\text{g g}^{-1}$ )	K ( $\mu\text{g g}^{-1}$ )
Leaf	8.19 $\pm$ 0.39 <sup>a</sup>	866.10 $\pm$ 40.73 <sup>a</sup>	20.54 $\pm$ 0.69 <sup>a</sup>	6696.70 $\pm$ 150.97 <sup>a</sup>	19.56 $\pm$ 0.67 <sup>a</sup>	964.52 $\pm$ 56.49 <sup>a</sup>	11522.82 $\pm$ 306.19 <sup>a</sup>
Stem	11.96 $\pm$ 0.62 <sup>b</sup>	840.15 $\pm$ 41.75 <sup>a</sup>	9.52 $\pm$ 0.67 <sup>b</sup>	2477.38 $\pm$ 129.13 <sup>b,c</sup>	25.28 $\pm$ 0.83 <sup>b</sup>	716.52 $\pm$ 47.90 <sup>a</sup>	13287.80 $\pm$ 331.32 <sup>b</sup>
Rhizome	6.60 $\pm$ 0.40 <sup>a</sup>	1070.18 $\pm$ 77.45 <sup>a</sup>	11.13 $\pm$ 0.89 <sup>b</sup>	2079.77 $\pm$ 142.02 <sup>c</sup>	25.78 $\pm$ 0.79 <sup>b</sup>	1388.41 $\pm$ 100.10 <sup>a</sup>	10699.87 $\pm$ 363.18 <sup>a,c</sup>
Root	7.19 $\pm$ 0.49 <sup>a</sup>	3559.81 $\pm$ 336.36 <sup>b</sup>	14.87 $\pm$ 0.65 <sup>c</sup>	2955.54 $\pm$ 128.62 <sup>b</sup>	34.45 $\pm$ 1.75 <sup>c</sup>	8086.69 $\pm$ 505.79 <sup>b</sup>	9820.46 $\pm$ 391.64 <sup>c</sup>
	Mg ( $\mu\text{g g}^{-1}$ )	Mn ( $\mu\text{g g}^{-1}$ )	Total N (%)	Na ( $\mu\text{g g}^{-1}$ )	P ( $\mu\text{g g}^{-1}$ )	S ( $\mu\text{g g}^{-1}$ )	Zn ( $\mu\text{g g}^{-1}$ )
Leaf	2401.47 $\pm$ 88.74 <sup>a</sup>	697.79 $\pm$ 34.54 <sup>a</sup>	1.91 $\pm$ 0.06 <sup>a</sup>	352.49 $\pm$ 22.77 <sup>a</sup>	1864.44 $\pm$ 66.19 <sup>a</sup>	2086.07 $\pm$ 64.07 <sup>a</sup>	57.53 $\pm$ 2.32 <sup>a</sup>
Stem	1926.52 $\pm$ 55.23 <sup>b</sup>	539.10 $\pm$ 19.95 <sup>b</sup>	0.88 $\pm$ 0.03 <sup>b</sup>	321.40 $\pm$ 18.96 <sup>a</sup>	2232.22 $\pm$ 75.31 <sup>b</sup>	1735.09 $\pm$ 59.56 <sup>b</sup>	82.59 $\pm$ 2.91 <sup>b</sup>
Rhizome	875.05 $\pm$ 20.98 <sup>c</sup>	218.47 $\pm$ 13.74 <sup>c</sup>	1.02 $\pm$ 0.03 <sup>b,c</sup>	577.58 $\pm$ 36.53 <sup>b</sup>	2327.43 $\pm$ 67.26 <sup>b</sup>	1731.20 $\pm$ 50.65 <sup>b</sup>	42.83 $\pm$ 1.15 <sup>c</sup>
Root	1650.64 $\pm$ 58.73 <sup>d</sup>	729.20 $\pm$ 45.22 <sup>a</sup>	1.02 $\pm$ 0.03 <sup>c</sup>	1011.74 $\pm$ 61.88 <sup>c</sup>	1189.84 $\pm$ 35.34 <sup>c</sup>	1464.59 $\pm$ 65.53 <sup>c</sup>	70.25 $\pm$ 3.62 <sup>d</sup>

**TABLE 2** Mean biomass and nutrient tissue concentrations allocated to leaves, stems, rhizomes and adventitious roots of *Leersia oryzoides* across soil moisture treatments (control, C; partially flooded, PF; and continuously flooded, CF). Each value is the mean for 45 replications ( $\pm$ SE) for biomass measurements and 12 replications ( $\pm$ SE) for elemental concentrations. Significant differences across treatments are indicated by using different lower-case letters, according to Tukey's post-hoc. Differences were considered significant at  $\alpha < 0.05$

	C	PF	CF
Biomass (g)			
Leaf	6.79 $\pm$ 0.57 <sup>a</sup>	10.71 $\pm$ 0.80 <sup>b</sup>	7.08 $\pm$ 0.46 <sup>a</sup>
Stem	9.80 $\pm$ 0.89 <sup>a</sup>	15.52 $\pm$ 1.25 <sup>b</sup>	10.54 $\pm$ 0.83 <sup>a</sup>
Rhizome	7.31 $\pm$ 0.73 <sup>a</sup>	8.72 $\pm$ 0.74 <sup>a</sup>	3.77 $\pm$ 0.34 <sup>b</sup>
Root	5.34 $\pm$ 0.57 <sup>a</sup>	9.97 $\pm$ 1.10 <sup>b</sup>	6.26 $\pm$ 0.63 <sup>a</sup>
Al ( $\mu$ g g <sup>-1</sup> )			
Leaf	892.44 $\pm$ 66.63	814.31 $\pm$ 71.58	891.56 $\pm$ 76.84
Stem	901.36 $\pm$ 71.58	746.31 $\pm$ 68.50	872.76 $\pm$ 74.74
Rhizome	885.73 $\pm$ 84.80	1298.43 $\pm$ 189.65	1026.38 $\pm$ 78.08
Root	3644.10 $\pm$ 626.39	3591.97 $\pm$ 593.40	3443.35 $\pm$ 577.50
B ( $\mu$ g g <sup>-1</sup> )			
Leaf	20.03 $\pm$ 1.50	20.68 $\pm$ 1.05	20.91 $\pm$ 1.11
Stem	10.50 $\pm$ 1.22	8.24 $\pm$ 0.87	9.81 $\pm$ 1.34
Rhizome	10.84 $\pm$ 1.08	10.52 $\pm$ 0.69	12.04 $\pm$ 2.41
Root	14.95 $\pm$ 1.29	15.14 $\pm$ 1.12	14.53 $\pm$ 1.04
Ca ( $\mu$ g g <sup>-1</sup> )			
Leaf	6872.48 $\pm$ 292.73	6944.27 $\pm$ 235.94	6273.35 $\pm$ 226.96
Stem	2511.94 $\pm$ 214.87	2427.23 $\pm$ 247.35	2492.97 $\pm$ 226.84
Rhizome	1969.26 $\pm$ 248.32	2117.07 $\pm$ 200.28	2153.00 $\pm$ 298.39
Root	3045.62 $\pm$ 226.31	2920.84 $\pm$ 166.91	2900.15 $\pm$ 278.76
Cu ( $\mu$ g g <sup>-1</sup> )			
Leaf	16.90 $\pm$ 1.07 <sup>a</sup>	19.70 $\pm$ 1.06 <sup>a,b</sup>	22.08 $\pm$ 0.89 <sup>b</sup>
Stem	24.89 $\pm$ 1.86	23.62 $\pm$ 1.08	27.33 $\pm$ 1.16
Rhizome	23.52 $\pm$ 1.28 <sup>a</sup>	24.17 $\pm$ 0.80 <sup>a</sup>	26.64 $\pm$ 1.25 <sup>b</sup>
Root	26.92 $\pm$ 1.76 <sup>a</sup>	31.97 $\pm$ 2.49 <sup>a</sup>	44.44 $\pm$ 2.30 <sup>b</sup>
Fe ( $\mu$ g g <sup>-1</sup> )			
Leaf	1041.63 $\pm$ 92.03	854.11 $\pm$ 90.44	997.83 $\pm$ 109.84
Stem	787.93 $\pm$ 71.73	593.80 $\pm$ 66.34	767.81 $\pm$ 101.41
Rhizome	1101.15 $\pm$ 101.69	1677.76 $\pm$ 244.09	1386.31 $\pm$ 103.01
Root	7233.87 $\pm$ 691.01	9638.86 $\pm$ 956.49	7387.35 $\pm$ 848.53
K ( $\mu$ g g <sup>-1</sup> )			
Leaf	10865.85 $\pm$ 583.10	11595.53 $\pm$ 358.30	12107.07 $\pm$ 596.41
Stem	13276.46 $\pm$ 603.34	13445.14 $\pm$ 652.89	13141.79 $\pm$ 503.32
Rhizome	9589.46 $\pm$ 478.98 <sup>a</sup>	10708.98 $\pm$ 698.01 <sup>a,b</sup>	11801.18 $\pm$ 565.73 <sup>b</sup>
Root	9714.09 $\pm$ 651.53	10009.68 $\pm$ 712.78	9737.60 $\pm$ 725.68
Mg ( $\mu$ g g <sup>-1</sup> )			
Leaf	2525.38 $\pm$ 153.66 <sup>a,b</sup>	2576.28 $\pm$ 151.44 <sup>a</sup>	2102.74 $\pm$ 113.95 <sup>b</sup>
Stem	2045.55 $\pm$ 90.97	1990.70 $\pm$ 89.23	1743.30 $\pm$ 90.35
Rhizome	837.66 $\pm$ 22.41	909.45 $\pm$ 47.11	878.04 $\pm$ 35.44
Root	1628.38 $\pm$ 113.57	1627.93 $\pm$ 95.76	1695.61 $\pm$ 102.86
Mn ( $\mu$ g g <sup>-1</sup> )			
Leaf	666.53 $\pm$ 69.16	672.67 $\pm$ 42.56	754.17 $\pm$ 66.01
Stem	511.11 $\pm$ 40.96	542.34 $\pm$ 27.04	563.85 $\pm$ 35.54
Rhizome	189.26 $\pm$ 17.96	220.52 $\pm$ 28.34	245.63 $\pm$ 23.04
Root	601.51 $\pm$ 74.00 <sup>a</sup>	694.56 $\pm$ 59.59 <sup>a,b</sup>	891.53 $\pm$ 80.66 <sup>b</sup>
N (%)			
Leaf	1.84 $\pm$ 0.08	1.98 $\pm$ 0.12	1.92 $\pm$ 0.10

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**TABLE 2** Mean biomass and nutrient tissue concentrations allocated to leaves, stems, rhizomes and adventitious roots of *Leersia oryzoides* across soil moisture treatments (control, C; partially flooded, PF; and continuously flooded, CF). Each value is the mean for 45 replications ( $\pm$ SE) for biomass measurements and 12 replications ( $\pm$ SE) for elemental concentrations. Significant differences across treatments are indicated by using different lower-case letters, according to Tukey's post-hoc. Differences were considered significant at  $\alpha < 0.05$  (Continued)

	C	PF	CF
Stem	0.88 $\pm$ 0.06	0.86 $\pm$ .06	0.89 $\pm$ 0.07
Rhizome	1.07 $\pm$ 0.05	1.00 $\pm$ 0.04	0.98 $\pm$ 0.05
Root	1.06 $\pm$ 0.05	1.01 $\pm$ 0.08	1.00 $\pm$ 0.04
Na ( $\mu\text{g g}^{-1}$ )			
Leaf	315.12 $\pm$ 22.96	389.23 $\pm$ 56.53	353.12 $\pm$ 31.23
Stem	269.01 $\pm$ 19.04	318.42 $\pm$ 37.04	376.78 $\pm$ 34.15
Root	750.21 $\pm$ 65.73 <sup>a</sup>	1293.98 $\pm$ 101.68 <sup>b</sup>	991.03 $\pm$ 91.58 <sup>a</sup>
Rhizome	405.43 $\pm$ 31.29 <sup>a</sup>	608.88 $\pm$ 44.27 <sup>b</sup>	718.44 $\pm$ 72.18 <sup>b</sup>
P ( $\mu\text{g g}^{-1}$ )			
Leaf	1720.73 $\pm$ 110.54	1832.29 $\pm$ 91.22	2040.29 $\pm$ 128.45
Stem	2249.33 $\pm$ 160.52	2279.32 $\pm$ 131.05	2168.01 $\pm$ 102.90
Rhizome	2418.13 $\pm$ 142.58	2260.14 $\pm$ 103.60	2304.02 $\pm$ 104.47
Root	1162.29 $\pm$ 58.53	1170.42 $\pm$ 48.53	1236.82 $\pm$ 76.40
S ( $\mu\text{g g}^{-1}$ )			
Leaf	1911.93 $\pm$ 93.61 <sup>a</sup>	1984.37 $\pm$ 91.15 <sup>a</sup>	2361.91 $\pm$ 107.24 <sup>b</sup>
Stem	1679.03 $\pm$ 104.95	1641.57 $\pm$ 100.12	1884.66 $\pm$ 98.50
Rhizome	1764.81 $\pm$ 104.92	1578.87 $\pm$ 82.73	1849.92 $\pm$ 56.07
Root	1386.64 $\pm$ 109.32 <sup>a,b</sup>	1307.85 $\pm$ 94.95 <sup>a</sup>	1699.28 $\pm$ 110.61 <sup>b</sup>
Zn ( $\mu\text{g g}^{-1}$ )			
Leaf	49.17 $\pm$ 3.19 <sup>a</sup>	54.98 $\pm$ 3.04 <sup>a</sup>	68.46 $\pm$ 3.75 <sup>b</sup>
Stem	69.09 $\pm$ 2.98 <sup>a</sup>	87.41 $\pm$ 2.69 <sup>b</sup>	91.26 $\pm$ 6.25 <sup>b</sup>
Rhizome	42.03 $\pm$ 2.52	41.66 $\pm$ 1.55	44.80 $\pm$ 1.82
Root	55.13 $\pm$ 3.45 <sup>a</sup>	66.07 $\pm$ 4.24 <sup>a</sup>	89.54 $\pm$ 6.27 <sup>b</sup>

flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition was detected for Cu concentrations only. The following results summarize the concentration measurements and treatment effects of each individual element.

### Aluminum

Results indicate a greater concentration of Al was found in adventitious roots than other plant parts ( $F_{3, 140} = 56.98$ ,  $P < 0.001$ ) (Table 1). Al concentrations in leaves, stems, adventitious roots, and rhizomes showed no significant effects resulting from an interaction between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. No effect on Al concentrations from flooding on leaves, stems, adventitious roots, and rhizomes ( $F_{2, 33} = 2.68$ ,  $P = 0.083$ ) (Table 2). There was no effect on Al concentrations from  $\text{NH}_4\text{NO}_3\text{-N}$  addition to leaves, stems, adventitious roots, or rhizomes (Table 3).

### Boron

Leaves had the greatest concentration of B, followed by adventitious roots, rhizomes and stems ( $F_{3, 140} = 44.79$ ,  $P < 0.001$ ) (Table 1). Plant tissue

**TABLE 3** Mean biomass and elemental concentrations for *Leersia oryzoides* across aqueous  $\text{NH}_4\text{NO}_3$ -N addition treatments (15 mg  $\text{L}^{-1}$ , 50 mg  $\text{L}^{-1}$ , or 100 mg  $\text{L}^{-1}$ ). Each value is the mean for 45 replications ( $\pm$ SE) for biomass measurements and 12 replications ( $\pm$ SE) for elemental concentrations. Significant differences across treatments are indicated by using different lower-case letters, according to Tukey's post-hoc. Differences were considered significant at  $\alpha < 0.05$

	15	50	100
Biomass (g)			
Leaf	7.65 $\pm$ 0.69	8.54 $\pm$ 0.70	8.39 $\pm$ 0.64
Stem	11.09 $\pm$ 1.05	12.87 $\pm$ 1.14	11.90 $\pm$ 1.02
Rhizome	6.60 $\pm$ 0.71	6.68 $\pm$ 0.66	6.52 $\pm$ 0.75
Root	7.04 $\pm$ 0.88	7.83 $\pm$ 0.80	6.70 $\pm$ 0.89
Al ( $\mu\text{g g}^{-1}$ )			
Leaf	905.85 $\pm$ 86.20	884.53 $\pm$ 65.61	807.93 $\pm$ 60.14
Stem	912.50 $\pm$ 54.43	857.06 $\pm$ 89.90	750.88 $\pm$ 66.09
Rhizome	948.82 $\pm$ 87.09	1119.03 $\pm$ 198.14	1142.69 $\pm$ 91.53
Root	3368.90 $\pm$ 551.60	4101.00 $\pm$ 728.46	3209.53 $\pm$ 451.62
B ( $\mu\text{g g}^{-1}$ )			
Leaf	21.61 $\pm$ 1.44	20.98 $\pm$ 1.23	19.04 $\pm$ 0.83
Stem	10.00 $\pm$ 1.31	9.41 $\pm$ 1.27	9.14 $\pm$ 0.95
Rhizome	10.10 $\pm$ 0.81	11.97 $\pm$ 2.39	11.32 $\pm$ 1.00
Root	14.88 $\pm$ 1.22	16.24 $\pm$ 1.19	13.49 $\pm$ .88
Ca ( $\mu\text{g g}^{-1}$ )			
Leaf	6935.46 $\pm$ 259.72	6597.21 $\pm$ 244.20	6557.43 $\pm$ 287.43
Stem	2502.38 $\pm$ 198.41	2466.68 $\pm$ 236.38	2463.08 $\pm$ 252.68
Rhizome	2058.53 $\pm$ 230.75	2170.88 $\pm$ 285.66	2009.92 $\pm$ 237.58
Root	2980.88 $\pm$ 249.75	3013.48 $\pm$ 215.58	2872.25 $\pm$ 219.24
Cu ( $\mu\text{g g}^{-1}$ )			
Leaf	20.29 $\pm$ 1.19	20.07 $\pm$ 1.20	18.33 $\pm$ 1.11
Stem	26.53 $\pm$ 1.28	25.12 $\pm$ 1.60	24.20 $\pm$ 1.47
Rhizome	27.14 $\pm$ 1.37	24.98 $\pm$ 1.16	25.20 $\pm$ 1.55
Root	33.70 $\pm$ 2.58	35.30 $\pm$ 1.89	34.34 $\pm$ 4.36
Fe ( $\mu\text{g g}^{-1}$ )			
Leaf	1034.27 $\pm$ 114.67	988.29 $\pm$ 100.32	871.01 $\pm$ 77.20
Stem	769.18 $\pm$ 51.27	734.13 $\pm$ 109.12	646.24 $\pm$ 81.46
Rhizome	1228.62 $\pm$ 114.85	1422.51 $\pm$ 263.82	1514.08 $\pm$ 94.83
Root	7790.33 $\pm$ 666.29	8871.20 $\pm$ 737.15	7598.55 $\pm$ 1170.06
K ( $\mu\text{g g}^{-1}$ )			
Leaf	11667.18 $\pm$ 667.12	11319.51 $\pm$ 440.77	11581.76 $\pm$ 499.61
Stem	13694.66 $\pm$ 521.16	12240.61 $\pm$ 667.09	13928.12 $\pm$ 423.30
Rhizome	11501.40 $\pm$ 667.70	10513.81 $\pm$ 625.63	10084.41 $\pm$ 570.81
Root	10337.41 $\pm$ 685.66	9371.11 $\pm$ 581.06	9752.85 $\pm$ 783.10
Mg ( $\mu\text{g g}^{-1}$ )			
Leaf	2330.63 $\pm$ 113.76	2302.48 $\pm$ 132.79	2571.30 $\pm$ 193.06
Stem	1955.73 $\pm$ 79.75	1828.58 $\pm$ 94.82	1995.23 $\pm$ 111.29
Rhizome	871.84 $\pm$ 34.49	909.80 $\pm$ 47.86	843.50 $\pm$ 22.78
Root	1673.23 $\pm$ 111.22	1771.15 $\pm$ 84.64	1507.53 $\pm$ 100.81
Mn ( $\mu\text{g g}^{-1}$ )			
Leaf	698.42 $\pm$ 51.87	753.43 $\pm$ 57.83	641.52 $\pm$ 69.15
Stem	556.37 $\pm$ 79.75	558.49 $\pm$ 29.45	502.44 $\pm$ 43.34
Rhizome	210.69 $\pm$ 27.24	226.12 $\pm$ 26.98	218.61 $\pm$ 17.91
Root	734.98 $\pm$ 83.33	759.38 $\pm$ 53.89	693.23 $\pm$ 97.30
N (%)			
Leaf	1.86 $\pm$ 0.09	1.79 $\pm$ 0.09	2.08 $\pm$ 0.10
Stem	0.83 $\pm$ 0.06	0.85 $\pm$ 0.05	0.95 $\pm$ 0.06

(Continued on next page)

**TABLE 3** Mean biomass and elemental concentrations for *Leersia oryzoides* across aqueous NH<sub>4</sub>NO<sub>3</sub>-N addition treatments (15 mg L<sup>-1</sup>, 50 mg L<sup>-1</sup>, or 100 mg L<sup>-1</sup>). Each value is the mean for 45 replications (±SE) for biomass measurements and 12 replications (±SE) for elemental concentrations. Significant differences across treatments are indicated by using different lower-case letters, according to Tukey's post-hoc. Differences were considered significant at  $\alpha < 0.05$  (*Continued*)

	15	50	100
Rhizome	0.95 ± 0.05	1.05 ± 0.05	1.06 ± 0.04
Root	1.04 ± 0.04	0.96 ± 0.03	1.07 ± 0.09
Na (μg g <sup>-1</sup> )			
Leaf	366.72 ± 44.23	343.71 ± 30.16	347.03 ± 45.32
Stem	337.14 ± 31.44	298.39 ± 35.57	328.68 ± 33.18
Rhizome	573.95 ± 54.41	568.21 ± 70.00	590.59 ± 69.65
Root	922.23 ± 71.83	927.98 ± 107.09	1185.01 ± 125.78
P (μg g <sup>-1</sup> )			
Leaf	1891.66 ± 124.39	1779.88 ± 88.93	1921.77 ± 132.01
Stem	2275.10 ± 117.04	2188.15 ± 145.26	2233.41 ± 137.80
Rhizome	2464.62 ± 99.91	2285.71 ± 137.52	2231.96 ± 107.68
Root	1299.11 ± 65.10 <sup>a</sup>	1201.48 ± 46.93 <sup>a,b</sup>	1068.93 ± 55.26 <sup>b</sup>
S (μg g <sup>-1</sup> )			
Leaf	2192.13 ± 112.54	1942.16 ± 110.17	2123.93 ± 106.54
Stem	1852.10 ± 84.10	1611.88 ± 106.63	1741.29 ± 113.07
Rhizome	1813.20 ± 90.99	1581.71 ± 88.72	1798.70 ± 72.68
Root	1683.06 ± 113.07 <sup>a</sup>	1315.93 ± 105.89 <sup>b</sup>	1394.78 ± 101.02 <sup>a,b</sup>
Zn (μg g <sup>-1</sup> )			
Leaf	59.76 ± 3.63	53.18 ± 2.87	59.66 ± 5.23
Stem	87.34 ± 5.40	79.04 ± 3.96	81.38 ± 5.71
Rhizome	45.24 ± 2.69	41.24 ± 1.52	42.01 ± 1.48
Root	76.55 ± 7.44	68.91 ± 5.33	65.28 ± 5.98

concentrations of B in leaves, stems, adventitious roots, and rhizomes showed no significant effects due to an interaction between flooding and NH<sub>4</sub>NO<sub>3</sub>-N addition. No effect on B tissue concentrations from increased flooding was detected, and no treatment effects from NH<sub>4</sub>NO<sub>3</sub>-N addition, on leaves, stems, adventitious roots, or rhizomes (Tables 2 and 3) were found.

**Calcium**

Leaves contained the highest concentration of Ca ( $F_{3,140} = 237.46$ ,  $P < 0.001$ ), followed by adventitious roots, stems, and rhizomes (Table 1). Concentrations of Ca in leaves, stems, adventitious roots, and rhizomes showed no significant effects due to an interaction between flooding and NH<sub>4</sub>NO<sub>3</sub>-N addition. No effect on Ca tissue concentrations from increased flooding and no treatment effect of NH<sub>4</sub>NO<sub>3</sub>-N addition to leaves, stems, adventitious roots, or rhizome (Tables 2 and 3) were observed.

**Copper**

Adventitious roots had the highest concentration of Cu ( $F_{3,140} = 31.21$ ,  $P < 0.001$ ) (Table 1). Stems and rhizomes had similar concentrations and

leaves had the lowest concentration of Cu. Measurements of Cu concentrations in leaves, stems, and rhizomes did not indicate an effect due to an interaction between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. An interactive effect on Cu concentrations in adventitious roots was detected ( $F_{2, 2, 4, 27} = 5.33$ ,  $P = 0.003$ ). No detectable differences were found in Cu tissue concentrations in stems grown in different flooding treatments. Leaves ( $F_{2, 33} = 6.56$ ,  $P = 0.004$ ), adventitious roots ( $F_{2, 33} = 16.77$ ,  $P < 0.001$ ), and rhizomes ( $F_{2, 33} = 8.85$ ,  $P = 0.001$ ) had significantly higher Cu concentrations in CF (Table 2). No treatment effect on Cu concentrations from  $\text{NH}_4\text{NO}_3\text{-N}$  addition to leaves, stems, adventitious roots, and rhizomes (Table 3) were observed.

### ***Iron***

The greatest concentration of Fe was found in adventitious roots ( $F_{3, 140} = 185.02$ ,  $P < 0.001$ ) (Table 1), while leaves, stems, and rhizomes contained significantly less. Plant tissue concentrations and allocation of Fe in leaves, stems, adventitious roots, and rhizomes showed no significant effects due to an interaction between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. No significant effects of flooding on Fe tissue concentrations were found in leaves, stems, adventitious roots ( $F_{2, 33} = 2.58$ ,  $P = 0.091$ ), or rhizomes ( $F_{2, 33} = 3.10$ ,  $P = 0.059$ ) (Table 2).  $\text{NH}_4\text{NO}_3\text{-N}$  addition had no detectable effects on Fe tissue concentrations in leaves, stems, adventitious roots, or rhizomes (Table 3).

### ***Magnesium***

All plant modules contained significantly different concentrations of Mg ( $F_{3, 140} = 112.99$ ,  $P < 0.001$ ) (Table 1). The highest concentration was found in the leaves, followed by stems, adventitious roots, and rhizomes. Plant tissue concentrations of Mg in leaves, stems, adventitious roots, and rhizomes showed no significant effects due to an interaction between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. No detectable effects of flooding were detected in stems ( $F_{2, 33} = 3.19$ ,  $P = 0.054$ ), adventitious roots, or rhizomes; however, leaves in PF had higher concentrations ( $F_{2, 33} = 3.41$ ,  $P = 0.045$ ) (Table 2). No treatment effects on Mg concentrations from  $\text{NH}_4\text{NO}_3\text{-N}$  addition to leaves, stems, adventitious roots, or rhizomes were observed (Table 3).

### ***Manganese***

Adventitious roots and leaves had higher concentrations of Mn than stems, while the lowest concentrations were found in rhizomes ( $F_{3, 140} = 57.15$ ,  $P < 0.001$ ) (Table 1). Plant tissue concentrations of Mn in leaves, stems, adventitious roots, and rhizomes showed no significant effects due to an interaction between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. No detectable effects due to flooding on Mn concentrations in leaves, stems, or rhizomes were seen; however, adventitious roots had increased concentrations with

increased flooding ( $F_{2, 33} = 4.24$ ,  $P = 0.023$ ) (Table 2). No treatment effects on Mn concentrations from  $\text{NH}_4\text{NO}_3\text{-N}$  addition to leaves, stems, adventitious roots, or rhizomes were detected (Table 3).

### ***Nitrogen***

The highest concentration of total N was found in leaves while significantly less was found in stems, adventitious roots and rhizomes ( $F_{3, 140} = 144.12$ ,  $P < 0.001$ ) (Table 1). Plant tissue concentrations and allocation of total N in leaves, stems, adventitious roots, and rhizomes showed no significant effects due to an interaction between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. No effects on total N concentrations due to flooding in leaves, stems, adventitious roots, or rhizomes were found (Table 2). No treatment effect on N concentrations from  $\text{NH}_4\text{NO}_3\text{-N}$  addition to leaves, stems, adventitious roots, or rhizomes were observed (Table 3).

### ***Potassium***

The highest concentration of K were in the stems followed by leaves, rhizomes, and adventitious roots ( $F_{3, 140} = 17.86$ ,  $P < 0.001$ ) (Table 1). Plant tissue concentrations of K in leaves, stems, adventitious roots, and rhizomes showed no significant effects due to an interaction between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. Flooding did not show a detectable effect on K concentrations in leaves, stems, or adventitious roots; however, K concentrations increased with flooding in rhizomes ( $F_{2, 33} = 3.54$ ,  $P = 0.041$ ) (Table 2). No effect on K concentrations due to  $\text{NH}_4\text{NO}_3\text{-N}$  addition was found in leaves, stems, adventitious roots, or rhizomes (Table 3).

### ***Phosphorus***

Higher concentrations of P were found in rhizomes and stems than in leaves, with the lowest concentrations found in adventitious roots ( $F_{3, 140} = 67.28$ ,  $P < 0.001$ ) (Table 1). Plant tissue concentrations of P in leaves, stems, adventitious roots, and rhizomes showed no significant effects due to an interaction between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. No significant effects on P tissue concentrations due to flooding on leaves, stems, adventitious roots or rhizomes were detected (Table 2). Concentrations of P were not affected by  $\text{NH}_4\text{NO}_3\text{-N}$  addition to leaves, stems, or rhizomes; however, in adventitious roots, P concentrations decreased with increases in  $\text{NH}_4\text{NO}_3\text{-N}$  addition ( $F_{2, 33} = 4.22$ ,  $P = 0.023$ ) (Table 3).

### ***Sodium***

The highest concentrations of Na were found in adventitious roots, followed by rhizomes, leaves and stems ( $F_{3, 140} = 67.16$ ,  $P < 0.001$ ) (Table 1). Plant tissue concentrations of Na in leaves, stems, adventitious roots, and rhizomes showed no significant effects due to an interaction between flooding

and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. Flooding had no detectable effect on concentrations in leaves and stems ( $F_{2,33} = 3.01$ ,  $P = 0.063$ ). Flooding affected adventitious roots with the highest concentrations found in PF ( $F_{2,33} = 9.67$ ,  $P < 0.001$ ) and rhizomes where N concentrations increased with flooding ( $F_{2,33} = 9.29$ ,  $P = 0.001$ ) (Table 2). No detectable effects of  $\text{NH}_4\text{NO}_3\text{-N}$  addition on Na concentrations in the leaves, stems, adventitious roots or rhizomes were found (Table 3).

### **Sulfur**

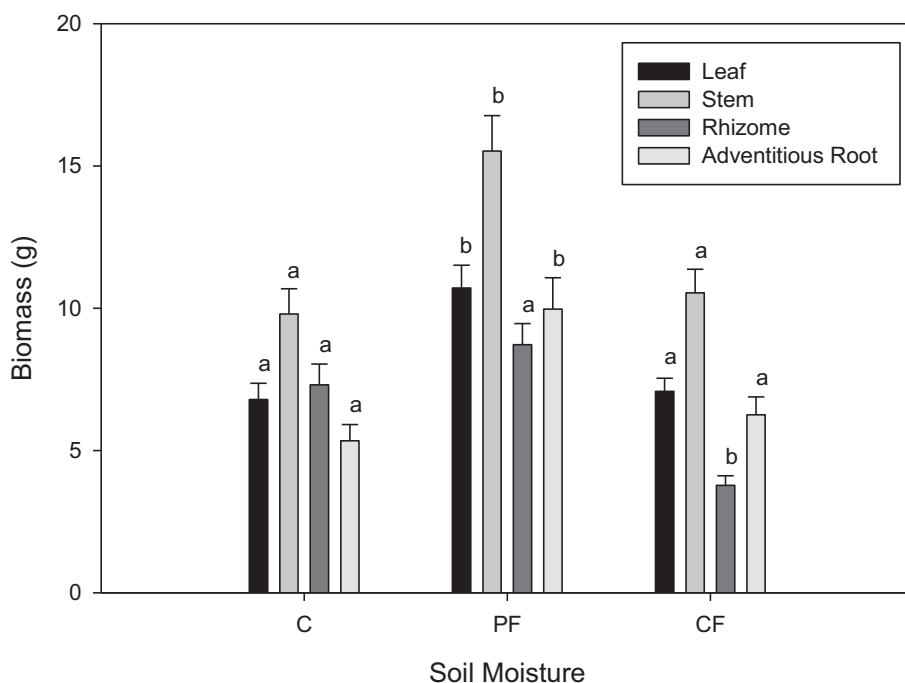
The highest concentration of S was found in the leaves, followed by stems, rhizomes and adventitious roots ( $F_{3,140} = 17.91$ ,  $P < 0.001$ ) (Table 1). Plant tissue concentrations of S in leaves, stems, adventitious roots, and rhizomes showed no significant effects due to an interaction between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. No detectable effects on S concentrations from flooding on stems and rhizomes were observed ( $F_{2,33} = 2.75$ ,  $P = 0.079$ ) (Table 2). Flooding affected S concentrations in leaves ( $F_{2,33} = 6.13$ ,  $P = 0.005$ ) and adventitious roots ( $F_{2,33} = 3.87$ ,  $P = 0.031$ ), with concentrations being highest in CF. No detectable effects of  $\text{NH}_4\text{NO}_3\text{-N}$  addition to leaves, stems, or rhizomes (Table 3).  $\text{NH}_4\text{NO}_3\text{-N}$  addition at  $15 \text{ mg N L}^{-1}$  had the highest concentration of S in adventitious roots, with decreased amounts associated with the  $100 \text{ mg N L}^{-1}$  treatment, and the least amount associated with the  $50 \text{ mg N L}^{-1}$  treatment ( $F_{2,33} = 3.28$ ,  $P = 0.050$ ).

### **Zinc**

The highest concentration of Zn was found in stems, followed by adventitious roots, leaves and rhizomes ( $F_{3,140} = 41.14$ ,  $P < 0.001$ ) (Table 1). Plant allocation of Zn in leaves, stems, adventitious roots and rhizomes showed no significant effects due to an interaction between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition. Flooding did not significantly affect the Zn concentrations in rhizomes; however, tissue concentrations increased with flooding in leaves ( $F_{2,33} = 8.79$ ,  $P = 0.001$ ), stems ( $F_{2,33} = 7.63$ ,  $P = 0.002$ ), and adventitious roots ( $F_{2,33} = 13.40$ ,  $P < 0.001$ ) (Table 2). No effects on tissue Zn concentrations due to  $\text{NH}_4\text{NO}_3\text{-N}$  addition to leaves, stems, adventitious roots or rhizomes were detected (Table 3).

### **Plant Growth**

Interactive effects between flooding and  $\text{NH}_4\text{NO}_3\text{-N}$  addition were not detectable with any measurements of plant biomass components. Flooding increased leaf ( $F_{2,132} = 12.17$ ,  $P < 0.001$ ), stem ( $F_{2,132} = 9.59$ ,  $P < 0.001$ ), and adventitious root ( $F_{2,132} = 9.32$ ,  $P < 0.001$ ) biomass in PF (Table 2, Figure 1). Rhizome ( $F_{2,132} = 16.24$ ,  $P < 0.001$ ) biomass in CF was significantly



**FIGURE 1** Leaf, stem, rhizome, and adventitious root biomass of *Leersia oryzoides* across soil moisture treatments. The treatments were control (C), partial flooded (PF), and continuously flooded (CF). Plants were harvested 6 weeks following the treatment initiation. Each value is the mean for 45 replications ( $\pm$ SE). Significant differences across treatments are indicated by using different lower-case letters, according to Tukey's post-hoc. Differences were considered significant at  $\alpha < 0.05$ .

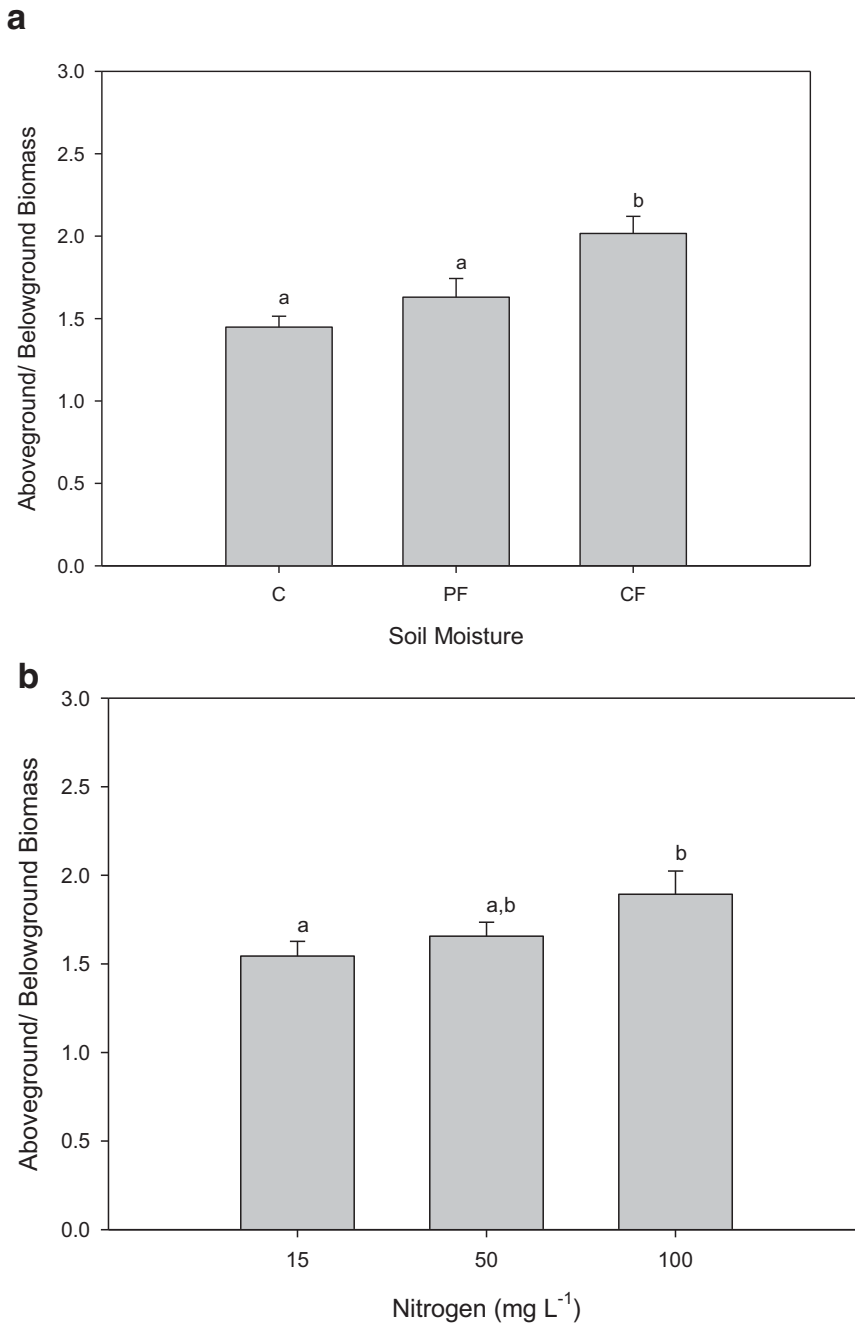
lower than other treatments (Table 2, Figure 1). Ammonium nitrate- N addition had no significant effects on module biomass (Table 3).

Aboveground-to-belowground ratio was significantly greater in CF ( $2.02 \pm 0.10$ ) than other soil moisture treatments ( $1.45 \pm 0.07$  and  $1.63 \pm 0.11$ ) (C and PF respectively) ( $F_{2, 132} = 9.03$ ,  $P < 0.001$ ) (Figure 2a). In addition, aboveground-to-belowground ratio was significantly greater in 100 mg N  $L^{-1}$  ( $1.89 \pm 0.13$ ) treatments than in 15 mg N  $L^{-1}$  treatments ( $1.54 \pm 0.08$ ) ( $F_{2, 132} = 3.14$ ,  $P = 0.05$ ) (Figure 2b). No significant treatment effects on leaf-to-stem ratio were found. Adventitious root-to-rhizome ratios were significantly greater in CF ( $1.76 \pm 0.13$ ) than other soil moisture treatments ( $0.75 \pm 0.05$  and  $1.16 \pm 0.08$ , C and PF, respectively) ( $F_{2, 132} = 30.18$ ,  $P < 0.001$ ) (Figure 2c).

## DISCUSSION

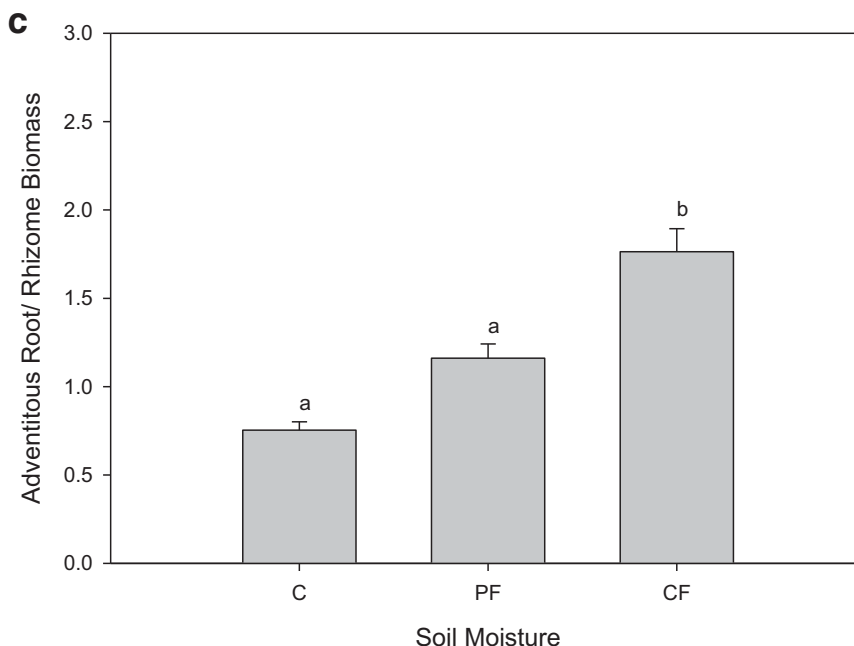
### Nutrient Tissue Concentrations

Anion macronutrients utilized by plants are soluble in water and may be moved by mass flow and leached from the soil following irrigation, high



**FIGURE 2** Aboveground/ belowground biomass ratio of *Leersia oryzoides*. Plants were harvested 6 weeks following the flooding treatment initiation. A) Soil moisture treatments were control (C), partial flooded (PF), and continuously flooded (CF) B) Aqueous  $\text{NH}_4\text{NO}_3\text{-N}$  addition treatments, were given in two separate doses of concentrations at  $15 \text{ mg L}^{-1}$ ,  $50 \text{ mg L}^{-1}$ , and  $100 \text{ mg L}^{-1}$ . c) Adventitious root/rhizome biomass ratio of *Leersia oryzoides* across soil moisture treatments. Each value is the mean for 45 replications ( $\pm \text{SE}$ ). Significant differences across treatments are indicated by using different letters, according to Tukey's post-hoc. Differences were considered significant at  $\alpha < 0.05$ . (Continued)





**FIGURE 2** (Continued)

precipitation events, or erosion. These nutrients are of special concern because excessive application can cause an imbalance in receiving waters (Galloway et al., 2003). The anions, N, P, and S are in the forms of  $\text{NO}_3^-$ , dihydrogen phosphate ( $\text{H}_2\text{PO}_4^-$ ), hydrogen phosphate ( $\text{HPO}_4^{2-}$ ), and sulfate ( $\text{SO}_4^{2-}$ ).

All growth processes in plants require large amounts of N. Plants absorb N in the form of  $\text{NO}_3^-$  or  $\text{NH}_4^+$  from the soil (Epstein, 1972). In this study, leaves had the highest concentrations of N, where as much as 70% of total leaf N may be in chloroplasts (Stocking and Ongun, 1962). In addition, chlorophyll contains Mg compounds (Fitter and Hay, 2002). The highest Mg tissue concentrations occurred in the leaves of the PF treatment, which is supported by the observation that the chlorophyll content of leaves of *L. oryzoides* have been measured to be highest in partially flooded plants (Koontz and Pezeshki, 2011). Stems had the lowest concentrations of N, indicating that when measuring N tissue concentrations in *L. oryzoides*, consideration of concentrations in leaves is the most important. Leaves are the primary component tested for analysis of nutrient concentrations, based on the assumption that changes in leaf nutrient concentrations are correlated with changes in physiological activities in the leaf (Fitter and Hay, 2002).

Highest tissue concentrations of S were in the leaves. S is a constituent of some proteins involved in photosynthesis (Epstein, 1972). The lowest S tissue concentrations were found in adventitious roots. Concentrations of S

were highest in the CF treatment of leaves and adventitious roots. Flooded conditions promote slow leaching of S, making it available to the plant for uptake (Mitsch and Gosselink, 2007). Tissue concentrations of S were lowest in the 50 mg N L<sup>-1</sup> addition. Excessive available N in low organic matter soils may cause S to occur below sufficient levels. The majority of total S is found in the soil organic matter.

Rhizomes contained the highest concentrations of P, while the adventitious roots contained the lowest. Rhizomes may act as a sink for excess P which could then be mobilized for new growth. HPO<sub>4</sub><sup>2-</sup> is incorporated into adenosine triphosphate (ATP), which is required in all living cells for energy transfer and metabolism (Epstein, 1972). The greatest loss of applied HPO<sub>4</sub><sup>2-</sup> is from erosion. The low amount of organic matter in the soil used in this study may have had an indirect influence on the amount of P available in the soil due to the lack of microbial activity. As the NH<sub>4</sub>NO<sub>3</sub>-N addition concentration increased to 100 mg N L<sup>-1</sup>, significantly less concentrations of P were observed in adventitious roots. The high concentration of NH<sub>4</sub>NO<sub>3</sub>-N may have affected certain physiological and biological conditions of the soil, resulting in insoluble and unavailable forms, such as phosphates of Fe, Al, or Ca (Vymazal et al., 1989), which explains the lowest concentrations of P being found in the adventitious roots.

Plants in wetland " soils experience a lack of oxygen represented by reducing conditions. The flooded soil used in the study had an Eh of less than +350 mV, which reflects anoxic conditions of limited oxygen availability. Wetland plants can decrease oxygen consumption in response to low oxygen concentrations to avoid internal anoxia (Geigenberger, 2003). Under hypoxia, ethylene-dependent death and lysis of cells occurs behind the apex, where cells are fully expanded, resulting in gas-filled channels becoming aerenchymous to convey oxygen from the leaves (Drew, 1997). This passage reduces internal respiring tissue and enhances the potential for oxygen to reach underground portions (Pezeshki, 2001). Internal plant tissue oxygen concentrations may be effected by highly competitive oxygen demand within the plant along with an oxygen demand in the sediment and oxygen leakage into the rhizosphere (Pezeshki, 2001). Phosphorus, Fe, and Al become more soluble and, therefore, available under flooded conditions. Water conservation under flooded conditions reduces the rate that soil toxins diffuse toward the root and increases the probability of detoxification as they move through the oxidized rhizosphere (Mitsch and Gosselink, 2007). The PF plants had the highest concentrations of Fe in adventitious roots and rhizomes, and highest concentrations of Al were in rhizomes, although the differences were not significant. Excessive moisture, such as found in the CF treatment, reduces aeration, root extension, and eventually nutrient uptake (Pezeshki, 2001).

Copper, Mn, and Zn, were found in higher concentrations in adventitious roots of the CF plants. These elements function as a part of or

with enzyme systems involved with plant growth, regulation, and restoration (Fitter and Hay, 2002). Some studies have shown interdependence between Cu and Mn (O'Sullivan, 1969). In addition, as flooding treatments intensified in the CF treatment, K concentrated in the rhizome. When K is dissolved into the soil solution, it tends to leach more quickly out of sandy soils, making elemental uptake easier (Mitsch and Gosselink 2007). Soil erosion will remove K from agricultural fields (O'Geen and Schwankl, 2006).

## Growth

The mean total biomass of plants per pot was  $33.94 \pm 1.75$  g. The average percent allocated to each module across all treatments was approximately 35% to stems ( $11.96 \pm 0.61$  g), 24% to leaves ( $8.19 \pm 0.39$  g), 21% to adventitious roots ( $7.19 \pm 0.49$  g), and 20% to rhizomes ( $6.60 \pm 0.40$  g). The lack of significant effects on growth due to the  $\text{NH}_4\text{NO}_3$ -N addition indicates that plants acquired all required nitrogen without reaching a toxic level.

Soil moisture treatments had an effect on plant growth. PF treatment plants produced the most overall biomass ( $44.92 \pm 3.62$  g) of a plant per pot compared to other treatments ( $29.24 \pm 2.56$  g and  $27.64 \pm 2.06$  g) (C and CF, respectively). The potential rate of nutrient uptake by plants is limited by growth rate and plant tissue concentration (Vymazal *et al.*, 1989). Although tissue concentrations of N and P were not significantly different, plants in PF were able to immobilize higher amounts of these elements due to greater biomass accumulation.

Resource allocation strategies differed between the C and CF treatments, even though total biomass measurements were not significantly different. Plants in CF had the highest aboveground-to-belowground ratio when compared to other treatments. Plants may reallocate resources to leaf and root tissues depending on their relative needs. These needs change with plant age, growth, and development, and are primarily controlled by the internal mechanisms that balance the relative redistribution of resources to root and leaf modules based on the most optimal balance for the plant as an independent organism (Bazzaz and Grace, 1997; Lovett-Doust *et al.*, 1983). During the growing season, fine-root respiration rates and longevity are closely linked to photosynthesis rates and whole-plant source-sink relationships (Eissenstat and Yanai, 2002). CF plants had higher adventitious root-to-rhizome ratios when compared to other treatments, altering their growth patterns to tolerate reduced conditions. Rhizomes of CF plants had half the biomass of rhizomes in C and PF treatment plants. Morphologically, the plants in CF had adventitious roots that grew to 5 cm up toward the surface of the water, likely in order to allow the diffusion of oxygen into the plant. This may reflect a strategy to maintain the plant until conditions improved.

Roots may acclimate metabolically " to the gradual loss of oxygen supply in flooded soils, improve their tolerance to anoxia, or partially avoid oxygen deficiency by structural changes that aid internal transfer of oxygen to the roots via shoots (Drew, 1990). Metabolic changes in the plant under hypoxia help maintain cell survival in the root apical meristems, which is important for future development (Drew, 1997). A high capacity for producing adventitious roots compensates for the decay of original roots under soil anaerobiosis (Kozłowski and Pallardy, 2002). Increased root surface area has been shown to increase the probability of root interception of ions (Colmer and Greenway, 2010). Adventitious root function is superior during flooded conditions, although diminished nutrient delivery exists; moderate shoot growth is still observed.

Changes in the soil environment may change the nutrient uptake efficiency of the root and, hence, the optimal longevity (Eissenstat and Yanai, 2002). Root life span responds to soil fertility, but measurements of growth have been inconsistent (Eissenstat and Yanai, 2002). Increased N availability has been associated with decreased root life span in some studies (Majdi and Kangas, 1997; Pregitzer et al., 2000) and with greater root life spans in other studies (Pregitzer et al., 1993; Fahey and Hughes, 1994; Wells, 1999; Burton et al., 2000). This suggests that roots are maintained as long as the benefit they provide (nutrients) outweighs the cost of keeping them alive (Burton et al., 2000). Suboptimal root function leads to nutrient deficiencies in shoots (Colmer and Greenway, 2010).

The time and character of growth should be considered when interpreting this study. Plants were harvested late in the growing season after a second growth peak, possibly contributing to the low N content in the leaves; some of the plants were beginning to show early signs of senescence. Comparing the nutrient content of *L. oryzoides* over the course of a growing season would be helpful in understanding how nutrient contents of the plant changes throughout the year. Because both N and P are released back into the system (Vymazal et al., 1989), removal of vegetation could be considered. A need exists for long-term field studies to determine this species' potential to survive and mitigate contaminants such as N in agroecosystems. Wetland plants, such as *L. oryzoides*, are a viable option for removing contaminants in agricultural runoff. By assessing plant function, a better understanding may be gained of the specific services a particular species may offer, such as the ability to remove nitrogen from runoff. Soil conservation is enhanced when the banks of drainage ditches are vegetated (Herzon and Helenius, 2008). Land management systems need to balance both natural and anthropogenic processes, however, the composition, structure and function of the surrounding ecosystem must be considered. In order to develop a sustainable plan, the anthropogenic effects on biodiversity and ecosystem functions must be examined, and results used toward mitigating excess fertilizers.

## CONCLUSIONS

*Leersia oryzoides* has the ability to adapt and persist in environmental conditions, encountered in drainage ditches and associated buffers. This grass is capable of reallocating resources in response to fluctuations in hydrology and of tolerating excess aqueous N. PF plants had the greatest accumulation of biomass. These conditions facilitate the plants to be able to immobilize excess nutrients, such as N and P, typically found in agricultural runoff. Results indicate that *L. oryzoides* may affect elemental concentrations in surface waters by its ability to uptake various elements and subsequent sequestration in various biomass components. Optimal allocation strategies in plants under partially flooded conditions indicate that *L. oryzoides* may be considered for planting in areas that experience such dynamic and diverse hydraulic regimes, such as agricultural drainage ditches and the surrounding vegetated buffers. Managers should consider removal of the senesced above-ground plant tissue through mowing or burning to reduce the re-release of nutrients into the waterway while making efforts to minimize the soil impacts in such operation.

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