

# Macronutrient (N, P, K) and Redoximorphic Metal (Fe, Mn) Allocation in *Leersia oryzoides* (Rice Cutgrass) Grown Under Different Flood Regimes

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**Abstract** Vegetated drainages are an effective method for removal of pollutants associated with agricultural runoff. *Leersia oryzoides*, a plant common to agricultural ditches, may be particularly effective in remediation; however, research characterizing responses of *L. oryzoides* to flooding are limited. Soil reduction resulting from flooding can change availability of nutrients to plants via changes in chemical species (e.g., increasing solubility of Fe). Additionally, plant metabolic stresses resulting from reduced soils can decrease nutrient uptake and translocation. The objective of this study was to characterize belowground and aboveground nutrient allocation of *L. oryzoides* subjected to various soil moisture regimes. Treatments included: a well-watered and well-drained control; a continuously saturated treatment; a 48-h pulse-flood treatment; and a partially flooded treatment in which water level was main-

tained at 15 cm below the soil surface and flooded to the soil surface for 48 h once a week. Soil redox potential (Eh, mV) was measured periodically over the course of the 8-week experiment. At experiment termination, concentrations of Kjeldahl nitrogen, phosphorus (P), potassium (K), iron (Fe), and manganese (Mn) were measured in plant tissues. All flooded treatments demonstrated moderately reduced soil conditions (Eh < 350 mV). Plant Kjeldahl nitrogen concentrations demonstrated no treatment effect, whereas P and K concentrations decreased in aboveground portions of the plant. Belowground concentrations of P, Mn, and Fe were significantly higher in flooded plants, likely due to the increased solubility of these nutrients resulting from the reductive decomposition of metal-phosphate complexes in the soil and subsequent precipitation in the rhizosphere. These results indicate that wetland plants may indirectly affect P, Mn, and Fe concentrations in surface waters by altering local trends in soil oxidation–reduction chemistry.

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## 1 Introduction

In recent years, eutrophication resulting from agricultural practices has become a major environmental concern (Birgand et al. 2007; Day et al. 2003; Needelman et al. 2007a), resulting in ongoing efforts

to establish best management practices to decrease water pollution (as cited by Cooper et al. 2004). Research suggests that the presence of vegetation in agricultural drainages could significantly impact water quality (Bouldin et al. 2004; Deaver et al. 2005; Kröger et al. 2007; Moore et al. 2005).

Several studies in constructed wetlands have demonstrated that vegetation substantially reduces nutrient load via a number of potential mechanisms, including direct uptake and immobilization (see Cronk and Fennessy 2001). Although in constructed wetlands designed for high nutrient loads, plants account for only a small fraction of nutrient removal; in low-load systems ( $0.4\text{--}2.0\text{ N m}^{-2}\text{ day}^{-1}$ ), plants can account for a substantial percentage of nitrogen (N) removal (Peterson and Teal 1996). Under flooded conditions, oxygen may diffuse from wetland plant roots (Armstrong and Beckett 1987), possibly counteracting the trend toward reduction in the immediate rhizosphere (Reddy et al. 1989).

While eutrophication of agricultural waters can have significant environmental repercussions, concentrations of macronutrients in row-crop ditches may be low compared to wetlands constructed for wastewater treatment (Bouldin et al. 2004). Information about nutrient immobilization and allocation in dominant plant species is crucial for understanding N and phosphorus (P) dynamics in these systems, both as sources and sinks for nutrients (Deaver et al. 2005; Kröger et al. 2007). *Leersia oryzoides* is an obligate wetland grass with a broad distribution throughout North America. This genus has been noted as particularly common in agricultural ditches draining row crops in the Upper Mississippi Embayment (Bouldin et al. 2004). The presence of *L. oryzoides* in ditches may be encouraged by regular disturbance, particularly periodic declines in water level due to decreased rainfall and increased evapotranspiration during the months of June–August. Populations of *L. oryzoides* have been demonstrated to respond favorably to summer drawdown (Meeks 1969).

With regard to water quality, this species may influence P in the water column by acting as a sink during the growing season (Deaver et al. 2005) or as a source following plant senescence (Kröger et al. 2007). The mechanism for this source/sink response is not known but is likely related to seasonal changes in hydrology, direct nutrient sequestration and release by the plant, and plant-mediated changes in soil and

water chemistry (Kröger et al. 2007; Meuleman and Beltman 1993; Needelman et al. 2007a, b; Jiang et al. 2007; Sharpley et al. 2007; Strock et al. 2007; Thiebaut and Muller 2003). Determining the effect of hydroperiod on nutrient relations is of particular importance given the recently proposed approach of installing controlled drainage structures to increase retention time for water-quality remediation in ditches (Dunne et al. 2007; Needelman et al. 2007a; Kröger et al. 2008) and the contrasting benefit of periodic soil oxidation (i.e., water drawdown) for P sequestration (Needelman et al. 2007b).

One often overlooked aspect of the effect of vegetation on water quality in wetlands is the effect of hydrology on plant tissue nutrient concentrations. Agricultural ditches in the Lower Mississippi Valley experience a high degree of variability in discharge through the growing season, with smaller ditches regularly drying out completely during the summer, followed by persistent soil saturation during winter and spring (R. Kröger, unpublished data). It has been suggested that periodic flooding and drying of agricultural ditches could increase P storage capacity of soil in agricultural ditches via increased mineralization (Needelman et al. 2007a, b). Additionally, the topographical heterogeneity of the ditches may result in “partial flooding,” where a portion of the root system at some distance below the soil surface is subjected to nearly continuous flooding by perched water tables in the soil that fluctuate more slowly than surface water (Kröger et al. 2007; Vadas et al. 2007). Because of low diffusion of oxygen through water, organisms quickly deplete soil oxygen, creating a reduced environment that does not favor the growth of aerobic organisms, including plants (Pezeshki 2001).

Although wetland plants employ a number of metabolic and morphological modifications that allow for growth in reduced soils, flooding can result in detrimental effects, such as decreased foliar gas exchange, decreased root development (Pezeshki 2001), and decreased vascular transport ability (Gibbs et al. 1998). *L. oryzoides* is fairly resilient to moderate soil reduction; however, stomatal conductance and net photosynthesis decrease when soil oxygen is depleted ( $E_h < +350\text{ mV}$ ; Pierce et al. 2007). This decline in stomatal conductance is linked to decreased capacity for water uptake from the roots, potentially affecting nutrient uptake. The decline in photosynthesis results in decreased translocation of assimilated carbon to the

roots (Pezeshki 2001). Declines in photosynthesis during flooding are usually indicative of low partial pressure of oxygen in root tissues and the subsequent necessity for decreased root metabolism, including organic assimilation of mineral nutrients (Drew 1997; Gibbs and Greenway 2003; Greenway and Gibbs 2003).

Chemical changes in the soil due to reduction may affect nutrient availability to plants either directly or indirectly. As oxygen is depleted, soil microorganisms begin to use other electron acceptors, including nitrate, the most common source of N for plants (Aerts and Chapin 2000). Reduction of nitrate to ammonia can result in decreased N uptake because ammonia, a positively charged ion, is more likely to be immobilized by the negatively charged surface of mineral soil peds (Cronk and Fennessy 2001). Although phosphorus species are not directly affected by soil reduction, their bioavailability may be affected by dissolution or decomposition of metal–P compounds under reduced soil conditions (Szilas et al. 1998; Richardson and Vepraskas 2001), which can subsequently increase plant tissue concentrations (Liang et al. 2006; Rubio et al. 1997). A portion of this P may result from precipitation with ferric iron (Fe(III)) in the rhizosphere and root cortex as a result of ferrous iron (Fe(II)) oxidation (Reddy and DeLaune 2008). Oxygen transport and subsequent diffusion of oxygen from the gas-filled lacunae into root tissues and the surrounding rhizosphere has been observed in wetland plants (as discussed by Pezeshki 2001).

In light of the above considerations, we hypothesize:

1. Belowground tissue nutrient concentrations would respond primarily to changes in plant nutrient availability (i.e., labiality) as a result of changes in soil redox conditions.
2. Aboveground tissue nutrient concentrations would decrease in response to flooding as a result of decreased water uptake and decreased organic assimilation of nutrients. These results will be most pronounced for nutrients that require organic assimilation for apical transport.

## 2 Methods

Plants were collected from wild populations found in wetland cells at the Jamie L. Whitten Plant Materials

Center in Coffeeville, MS, USA, and grown under natural light in the Life Sciences Greenhouse at the University of Memphis. Plants were grown in 60-cm-high pots constructed of 15-cm polyvinyl chloride (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) collected from the Brunswick site of the University of Memphis Edward J. Meeman Biological Field Station in Brunswick, TN, USA.

The high sand content of the sand/soil mixture used in this study was designed to allow simulation of the planned hydroperiods as well as achieving a well-drained control. After placement in PVC pipes, plants were well watered and well drained for a period of 3 weeks prior to treatment initiation. During this time, plants were fertilized weekly with 500 mL of 20–20–20 Peter's fertilizer mixed with tap water at 1.25 g/L. The study was terminated 56 days after treatment initiation.

### 2.1 Soil Moisture Treatments

A completely randomized design was employed, examining stress response of *L. oryzoides* across four soil moisture conditions. Each treatment was replicated 12 times, with individual plants being treated as replicates. After treatment initiation, plants were watered daily with approximately 2 L of a nutrient solution containing a maximum of 12 mg/L ammonium nitrate and a maximum of 5 mg/L 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).

Water level within individual mesocosms 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. The four treatments are described in detail below.

1. A control with plants that was well watered and allowed to drain freely
2. An intermittently flooded treatment that was well watered and well drained except on days 7, 14, 19, 28, 35, and 45, when pots were flooded to 5 cm above soil surface for a period of 48 h

3. A partially flooded treatment with water maintained at 15 cm below soil surface. Water level within the soil was checked periodically using an internal gauge constructed from 1.9-cm perforated PVC pipe
4. A continuously flooded treatment with water maintained at 5 cm above the soil surface

On days 7, 14, 19, 28, 35, and 45, partially flooded and continuously flooded pots were flooded an additional 15 cm of water for 48 h. Any excess water was held in overflow buckets and used to maintain water conditions as described above. Standing water in the three flooded treatments was drained overnight and all treatments were refreshed with 7 L of nutrient solution once a week prior to the 48-h 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. The pH of effluent draining from mesocosms was measured on days 30 and 45.

## 2.2 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, MA, USA) as described by Patrick and DeLaune (1977). Redox electrodes (one at each depth) were placed at 10 and 30 cm 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.

## 2.3 Plant Tissue Nutrient Analysis

Plant tissue analysis followed the general guidelines described in Kalra (1998). Ten randomly chosen plants per treatment were analyzed for nutrient analysis. Plants were washed, divided into aboveground and belowground portions, air-dried for 2 weeks, and weighed. Plants were then ground in a Wiley Mill and passed through a 1-mm-diameter mesh and frozen to await further processing. Subsamples of approximately 0.2 g were digested according to Quikchem Method 10-107-06-2-E (detection limit 0.018 mg/L) for Kjeldahl N and

QuikChem Method 10-115-01-1-C (detection limit 0.015 mg/L) for total P. The Kjeldahl digestion process stabilizes ammonium nitrogen and converts organic N into ammonium. Nitrate is not converted into ammonium. The digestion process converts all P to orthophosphorus. Ammonium and orthophosphorus are then quantified by colorimetry using Lachat Instruments Quickchem FIA+8000 series colorimeter.

Metal analyses followed procedures of Farmer et al. (2005); specifically, 1-g air-dried samples were fired for 8 h at 500°C then oxidized with 10 mL H<sub>2</sub>O<sub>2</sub> (35–37%), acidified with 4 mL HCl (to pH<1), and brought to 100-mL volume with deionized water. Samples were heated for 6 h at 70°C then filtered with a 45- $\mu$ m nitrocellulose filter. One hundred twenty-seven milligram CsCl (1,000 ppm Cs<sup>+</sup>) was added to reduce ionization interference in the analysis of potassium (K). Samples were analyzed using a Varian 220 220 atomic absorption spectrometer. Plant nutrient uptake was estimated by multiplying the measured nutrient concentration times the estimated increase in biomass during the study. Estimated biomass increase was determined by subtracting the dry mass of ten plants randomly selected immediately prior to treatment initiation from the final mass of experimentally manipulated plants.

## 2.4 Statistical Analyses

Unless otherwise indicated, statistical analyses used the general linear model for analysis of variance in SPSS 14, with the four levels of water treatment as independent fixed factors. Eh was analyzed as a repeated-measures multivariate analysis of variance (MANOVA), with Eh values at 15-cm depth and 30-cm depth analyzed as correlated dependent variables. Plant nutrient concentrations were evaluated with MANOVA, with aboveground values and belowground values as correlated dependent variables. Correlations in nutrient concentrations were analyzed using Pearson's correlation.

Because of time constraints and other considerations, soil Eh data were analyzed using smaller *n* values than desired. Because smaller *n* values greatly increase the likelihood of a type II error, the  $\alpha$  level was adjusted accordingly to  $\alpha=0.1$  to examine potential time  $\times$  treatment interactions (see Table 1). All other analyses used the standard  $\alpha=0.05$  value.

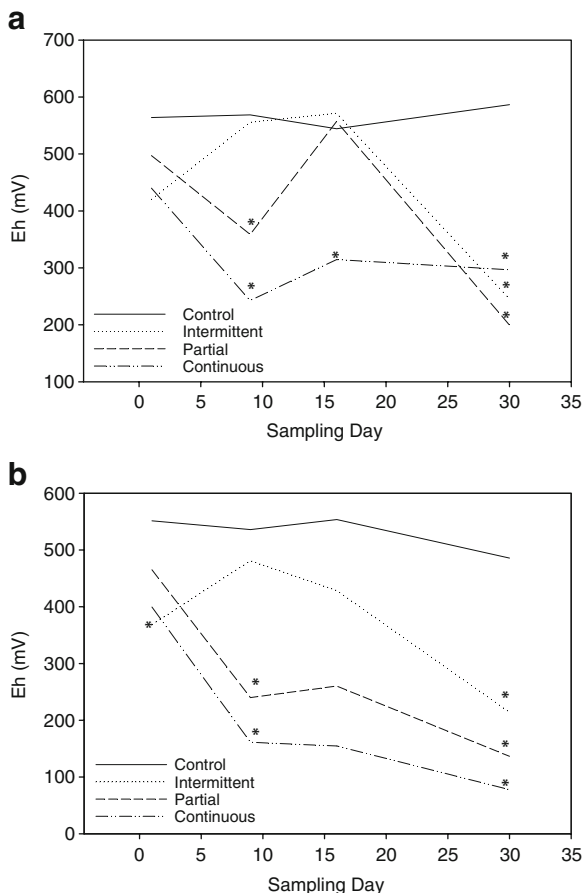
**Table 1** MANOVA (Hotelling's trace) table for soil Eh, analyzing Eh at 10-cm depth and 30-cm depth as correlated dependent variables

Effect		Hotelling's trace	<i>F</i>	Hypothesis <i>df</i>	Error <i>df</i>	<i>p</i> value
Between subject	Treatment	6.882	13.765	6	24	<0.0005
Within subject	Time	3.658	5.487	6	9	0.012
	Time × treatment	4.517	1.924	18	23	0.070

Because small sample size limited statistical power,  $\alpha=0.1$  was considered sufficient to justify further analysis based on a time × treatment interaction. All subsequent analyses used the standard  $\alpha=0.05$

### 3 Results

Soil Eh data are summarized in Fig. 1a, b. Initially, Eh values were all in the range expected for aerated soils (mean Eh averaged for 10 and 30 cm = 562 mV ± 58).



**Fig. 1** **a** Soil Eh at 10-cm depth through day 30. **b** Soil Eh at 30-cm depth through day 30. Asterisks indicate a significant difference from the control within the given sampling period. By day 30, all flooded treatments were anoxic

The control treatment remained aerated throughout the study. By day 9, the Eh had dropped below the critical oxygen threshold for anoxia.

Eh = 350 mV at both depths in partially flooded and continuously flooded treatments. By day 30, the intermittently flooded treatment had also become anoxic.

These differences were observed as a time × treatment interaction (Table 1). At 10-cm depth the partially flooded and continuously flooded differed from control ( $p=0.016$  and  $p=0.002$ , respectively). At 30-cm depth, Eh of all treatments differed from one another with the exception of the partially flooded and continuously flooded treatments.

pH was generally basic, with a mean of  $7.8 \pm 0.28$  standard deviation. A time × treatment interaction was observed ( $p=0.047$ ), as well as a significant time effect ( $p=0.026$ ). Only the continuously saturated treatment demonstrated a time effect, with a decrease in pH from  $7.86 \pm 0.276$  on day 30 to  $7.48 \pm 0.313$  on day 45 ( $p=0.04$ ,  $F=5.42$ ). Biomass was unaffected by flooding treatments (Table 2). Prior to treatment initiation, plant belowground mass was  $2.9 \pm 1.7$  and aboveground mass was  $6.6 \pm 3.1$ . This large initial error (approximately 50%) would be assumed to affect biomass throughout the study, resulting in the large error in biomass upon termination of the study (Table 2).

Belowground tissue concentrations of Fe increased progressively in response to flooding, with a fourfold increase in the continuously flooded treatment as compared to the control (Fig. 2). Belowground tissue concentrations of manganese (Mn) more than doubled in response to continuous flooding (Fig. 3). With respect to belowground tissue concentrations of Mn and Fe, only the intermittently flooded treatment did not differ from the control (Figs. 2 and 3). Belowground tissue P concentration was significantly



**Table 2** Mean biomass and total nutrient uptake across treatments

	Control	Intermittently flooded	Partially flooded	Continuously flooded
Biomass (g)				
Belowground	23.3±6.6	22.2±6.4	23±9.2	19.8±5.6
Aboveground	38.3±12	38.8±9.1	37.3±8.9	45.7±12
N (mg)				
Belowground	124.0±68	120.4±66	109.1±54	98.6±46
Aboveground	277.7±88	225±99	264±100	303±134
P (mg)				
Belowground	35.3±15	49.4±29	57.0±29	53.0±16
Aboveground	82.1±20	67.7±24	70.2±24	94.8±38
K (mg)				
Belowground	1,427±386	1,376±609	1,034±300	985±449
Aboveground	3,556±1,286	3,470±1,080	2,629±840	3,492±908
Fe (mg)				
Belowground	473±200	689±425	1,128±740	1,300±445 <sup>a</sup>
Aboveground	59.5±28	82.9±41	81.1±81.2	82.1±37
Mn (mg)				
Belowground	26.7±8.0	45.3±20	58.8±38	57.1±24.3
Aboveground	31.6±14	80.7±27	107±42 <sup>a</sup>	135±58 <sup>a</sup>

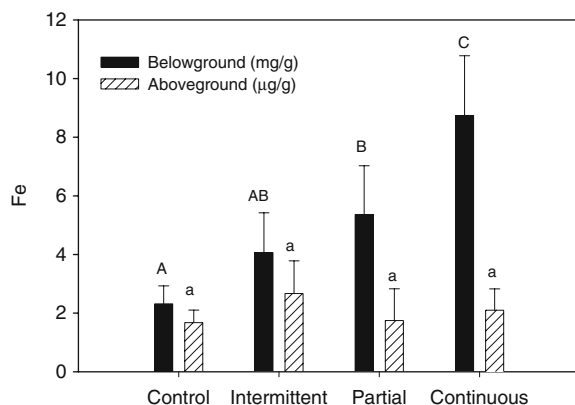
Only Fe and Mn demonstrated significant differences from the control

<sup>a</sup> Significant difference from the control for a given nutrient

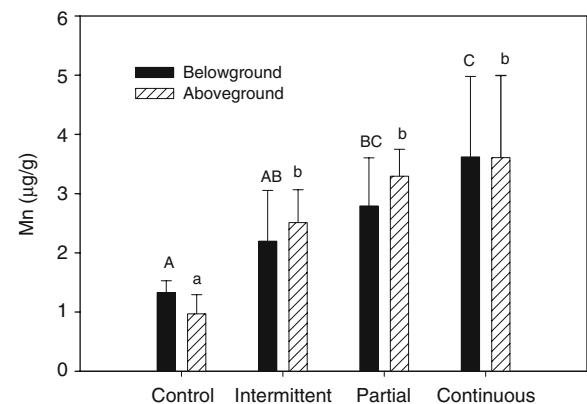
increased in response to flooding (Fig. 5,  $p < 0.0005$ ). Nutrient uptake followed the same patterns for Mn and Fe, although the statistical significance was lacking except in the most reduced treatments (Table 2). P uptake, however, did not increase; though

this lack of response was likely the result of natural variation in initial plant size and subsequent differences in development.

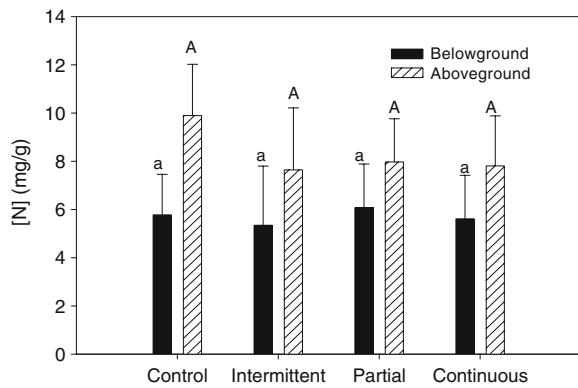
There was no overall treatment effect on tissue N concentration ( $p = 0.086$ ; see Fig. 4). P decreased in



**Fig. 2** Fe concentration in belowground tissues demonstrated progressive increases with intensity of flooding, whereas aboveground tissues demonstrated no detectable response. Different uppercase letters designate significant differences in belowground tissue concentrations across treatments. Different lowercase letters designate significant differences in aboveground tissue concentrations across treatments. Error bars indicate  $\pm$ one standard deviation

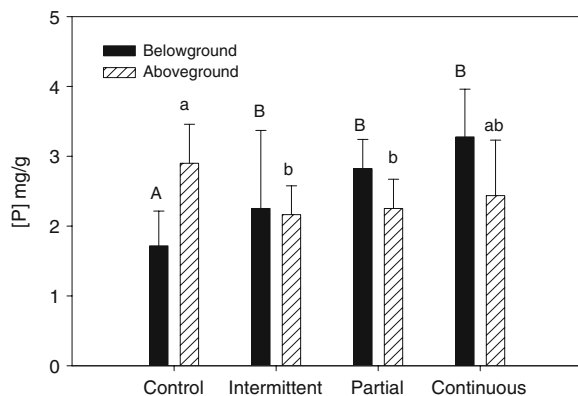


**Fig. 3** Mn concentration demonstrated a progressive increase in response to flooding in both belowground and aboveground tissues. Different uppercase letters designate significant differences in belowground tissue concentrations across treatments. Different lowercase letters designate significant differences in aboveground tissue concentrations across treatments. Error bars indicate  $\pm$ one standard deviation



**Fig. 4** N concentration demonstrated no detectable response to flooding. Different uppercase letters designate significant differences in belowground tissue concentrations across treatments. Different lowercase letters designate significant differences in aboveground tissue concentrations across treatments. Error bars indicate ±one standard deviation

response to flooding in aboveground tissue components (Fig. 5,  $p=0.022$ ). The aboveground tissue P concentrations in control were higher than the intermittently flooded treatment and the partially flooded treatment but not in the continuously flooded treatment (Fig. 5). K concentrations in aboveground tissue were significantly lower in the partially flooded and continuously flooded treatments than in the control ( $p=0.01$  and  $p=0.029$ , respectively; Fig. 6).



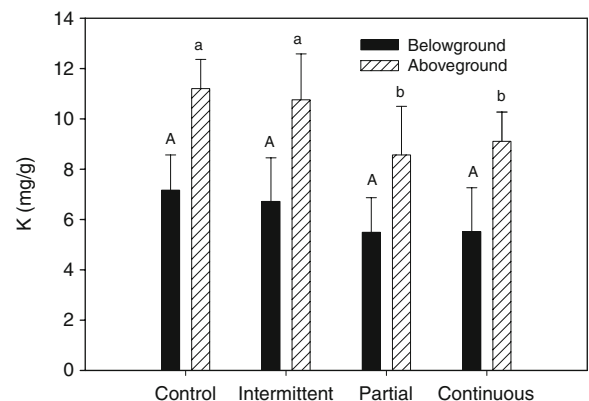
**Fig. 5** P concentration was significantly higher in belowground tissues of flooded plants, as compared to the control, whereas aboveground concentrations were decreased under intermittent and partial flooding. Different uppercase letters designate significant differences in belowground tissue concentrations across treatments. Different lowercase letters designate significant differences in aboveground tissue concentrations across treatments. Error bars indicate ±one standard deviation

Although tissue Fe concentration did not change in response to flooding in aboveground tissues, Mn concentrations and total uptake were higher in all flooded treatments (Figs. 2 and 3).

Correlational analyses revealed a number of significant relationships in nutrient concentration (Table 3). Belowground concentrations of K and Mn were indicative of their respective aboveground concentrations, with belowground concentrations explaining about 50% of the variance ( $r^2$ ) in aboveground concentrations. Additionally, a number of correlations were observed between different nutrients. Not surprisingly, belowground concentrations of Mn and Fe were most closely related ( $r^2=0.907$ ). Aboveground concentrations of Mn were significantly related to belowground concentrations of P, K, Fe, and Mn.

### 4 Discussion

As hypothesized, belowground concentrations of Fe, Mn, and P all increased in response to the expected increase in their availability. A similar pattern of uptake was observed in Fe and Mn but not in P. Although N was expected to decrease in belowground component due to the affinity of  $NH_4^+$  to negatively charged soil micelles, the high sand content of the soil mixture used may have limited this response. Previ-



**Fig. 6** K concentration was significantly lower in aboveground tissues of flooded plants, as compared to the control, whereas belowground tissues demonstrated no detectable response. Different uppercase letters designate significant differences in belowground tissue concentrations across treatments. Different lowercase letters designate significant differences in aboveground tissue concentrations across treatments. Error bars indicate ±one standard deviation

**Table 3** Correlation summary of nutrient concentrations in both aboveground and belowground tissues

N=28	Belowground tissue concentration					Aboveground tissue concentration					
	N	P	K	Fe	Mn	N	P	K	Fe	Mn	
Pearson correlation ( $r^2$ )											
Belowground tissue concentration	N		0.2633	0.1410	-0.0043	0.0893	0.1367	-0.1780	-0.0320	-0.0217	-0.1263
	P	0.2633		-0.0756	<i>0.5957</i>	<i>0.5303</i>	-0.0939	0.0031	-0.2753	0.0208	<i>0.7078</i>
	K	0.1410	-0.0756		-0.2662	-0.0885	0.3019	-0.0203	0.5148	-0.0500	-0.3679
	Fe	-0.0043	<i>0.5957</i>	-0.2662		<i>0.9070</i>	-0.2972	-0.2181	-0.3611	0.1602	<i>0.6449</i>
	Mn	0.0893	<i>0.5303</i>	-0.0885	<i>0.9070</i>		-0.1512	-0.3484	-0.3587	0.1851	<i>0.4642</i>
Aboveground tissue concentration	N	0.1367	-0.0939	0.3019	-0.2972	-0.1512		<i>0.5614</i>	0.0928	-0.4710	-0.2995
	P	-0.1780	0.0031	-0.0203	-0.2181	-0.3484	<i>0.5614</i>		0.2799	-0.4360	0.0449
	K	-0.0320	-0.2753	<i>0.5148</i>	-0.3611	-0.3587	0.0928	0.2799		-0.0444	-0.1168
	Fe	-0.0217	0.0208	-0.0500	0.1602	0.1851	-0.4710	-0.4360	-0.0444		0.0111
	Mn	-0.1263	<i>0.7078</i>	-0.3679	<i>0.6449</i>	0.4642	-0.2995	0.0449	-0.1168	0.0111	
Sig. (one-tailed, $p$ value)											
Belowground tissue concentration	N		0.0879	0.2371	0.4913	0.3256	0.2440	0.1824	0.4358	0.4563	0.2609
	P	0.0879		0.3511	<i>0.0004</i>	<i>0.0019</i>	0.3172	0.4939	0.0781	0.4582	<i>0.0000</i>
	K	0.2371	0.3511		0.0854	0.3271	0.0592	0.4591	<i>0.0025</i>	0.4003	<i>0.0271</i>
	Fe	0.4913	<i>0.0004</i>	0.0854		<i>0.0000</i>	0.0623	0.1324	<i>0.0295</i>	0.2078	<i>0.0001</i>
	Mn	0.3256	<i>0.0019</i>	0.3271	<i>0.0000</i>		0.2212	<i>0.0346</i>	<i>0.0304</i>	0.1728	<i>0.0064</i>
Aboveground tissue concentration	N	0.2440	0.3172	0.0592	0.0623	0.2212		<i>0.0009</i>	0.3192	<i>0.0057</i>	0.0608
	P	0.1824	0.4939	0.4591	0.1324	0.0346	<i>0.0009</i>		0.0746	<i>0.0102</i>	0.4103
	K	0.4358	0.0781	<i>0.0025</i>	<i>0.0295</i>	<i>0.0304</i>	0.3192	0.0746		0.4112	0.2770
	Fe	0.4563	0.4582	0.4003	0.2078	0.1728	<i>0.0057</i>	<i>0.0102</i>	0.4112		0.4777
	Mn	0.2609	<i>0.0000</i>	<i>0.0271</i>	<i>0.0001</i>	<i>0.0064</i>	0.0608	0.4103	0.2770	0.4777	

Statistically significant values are presented in italics. Pearson correlation values ( $r^2$ ). Corresponding  $p$  values.  $p$  values of 0 indicate  $p < 0.00005$ . Belowground tissue concentrations of Mn, Fe, and P were all significantly correlated, whereas aboveground tissue concentrations of N, P, and Fe were all significantly correlated. Only for K and Mn were belowground concentrations of the nutrient correlated with aboveground concentrations of the same nutrient

ous studies have found similar results for Fe and Mn, although P response varied (DeLaune et al. 1999; Rubio et al. 1997, Liang et al. 2006; Farmer et al. 2005; Chen et al. 2005). Some degree of variation in P response among the reported studies may be due to experimental design. For example, a study finding decreased P uptake (DeLaune et al. 1999) examined short-term uptake utilizing a hydroponic system, effectively negating any potential redox-related changes in soil P availability and the subsequent formation of Fe oxides and Fe phosphates in the rhizosphere (i.e., plaque). Studies with plants grown in soil have generally found increased belowground tissue P concentrations in response to waterlogging (Chen et al. 2005; Liang et al. 2006; Rubio et al. 1997), but this response may be limited to particular

soils or plant species (see Farmer et al. 2005; Pezeshki et al. 1999).

Macronutrients in aboveground tissues were generally lower in flooded treatments than in the control as hypothesized; however, only tissue concentration of K was significantly reduced by flooding. Even in the case of K, aboveground tissue concentrations may be impacted by belowground concentrations, as K was the only macronutrient in which aboveground and belowground tissue concentrations were correlated. This correlation indicates that the lack of differences in belowground concentrations of K may be due to insufficient statistical power. Plants grown in similar soils with a higher clay content would likely show a more pronounced K response to soil reduction, as Fe(III) reduction in flooded clay soils could



decrease availability of K to the plant (Chen et al. 1987).

The lack of significant decrease in aboveground N concentration in response to flooding may be the result of methodological limitations, as the analysis procedure used (Kjeldahl digestion) does not include nitrate. Although nitrate generally makes up a small percentage of the N content of plants, under conditions of excess nutrients or plant stress, a substantial quantity of nitrate (up to 25% of total N) may accumulate in select plant tissues (Chapin et al. 1990; Koch et al. 1988; Wang and Li 2004). The likely increase in  $\text{NH}_4^+$  would alter  $\text{NH}_4^+:\text{NO}_3^-$  uptake ratios in plant roots. Incorporation of  $\text{NH}_4^+$  into plant tissues is accompanied by the release of  $\text{H}^+$  that, in turn, leads to a decrease in rhizosphere pH (Neuman and Römhel 2002).

In a previous study on *Lepidium latifolium* (Chen et al. 2005) conducted under experimental conditions that were similar to this study, plants exhibited decreased phosphorus and nitrogen concentrations in the leaf tissue, whereas root tissue concentrations of phosphorus were significantly higher. These differences in nutrient allocation were attributed to decreased apical transport resulting from partial stomatal closure during the early stages of flooding.

With regard to the present study, flood-related decreases in stomatal conductance and photosynthesis were monitored in the same plants prior to harvesting for nutrient analysis (Pierce et al. 2007). Decreases in leaf gas exchange during flooding are related to decreased root functioning, including organic assimilation of nutrients and water uptake and transport (Pezeshki 2001). Although decreased root elongation in response to flooding has also been implicated as limiting nutrient uptake, Pierce et al. (2007) found no effects on root biomass or root penetration depth in *L. oryzoides* as a response to flooding. These measures, however, are a poor proxy for potential root interception of nutrients in comparison to measures of root surface area or specific root length (Jungk 2002).

The flood-induced decrease of P concentration in aboveground tissues was not observed in the continuously flooded treatment, possibly due to plant acclimation via production of adventitious roots or aerenchyma tissue. One of the most studied aspects of wetland plant response to flooding is the formation of lacunae consisting of porous aerenchyma tissue, presumably for conveyance of oxygen to tissues

experiencing anoxia. This process can result in rhizosphere oxidation of Fe(II). The subsequent release of protons from Fe(II) can cause rhizosphere acidification (Begg et al. 1994), potentially releasing P bound in Fe and Mn precipitates in the root cortex, thus facilitating nutrient translocation (Kirk and Du 1997; Saleque and Kirk 1995). Additionally, as rhizosphere acidification resulting from a predominance of  $\text{NH}_4^+$  nitrogen uptake (Thomson et al. 1993) can cause increase P solubility (Logan et al. 2000). This explanation is supported by a gradual decrease in pH in the continuously flooded treatment over the course of this study, possibly causing the slightly higher concentration of P in aboveground tissues (Fig. 4).

The increase in aboveground tissue Mn concentration and Mn uptake in response to flooding is in contrast to the lack of response for Fe. A similar trend was observed in *L. latifolium*, although in that study shoot Fe concentration was slightly increased by flooding, whereas Mn demonstrated more than a fourfold increase (Chen et al. 2005). Root Fe concentrations in Chen et al. (2005) were, however, more than an order of magnitude higher than those in the present study. Given the pH conditions in the present study, Mn should be soluble over the observed Eh range; however, Fe is only soluble in the most reduced conditions—based on Eh–Ph stabilities (Langmuir 1997). One explanation is that the Fe is soluble in the soil but is oxidized immediately within the rhizosphere so that it cannot bioaccumulate anywhere except near the roots. Mn, on the other hand, is soluble except in the more oxidized parts of the plant and thus has more freedom to bioaccumulate in aboveground tissues. This effect may be compounded by flood-induced rhizosphere acidification which would tend to stimulate Mn uptake in neutral and alkaline soils due to increased Mn availability (Neuman and Römhel 2002). It is likely that wetland plants have mechanisms for avoiding Fe uptake under flooded conditions, as Fe is one of the few metals that commonly reaches toxic concentrations in interstitial soil water under a range of pH values (Fitter and Hay 2002). Currently, there is limited information available to compare plant allocation of these metals in response to soil reduction.

The chemical processes governing nutrient assimilation and translocation are complex, involving multiple feedback mechanisms at multiple hierarchi-

cal levels within a plant (see Aerts and Chapin 2000). The high degree of permeability in the root cortex and predominance of oxidative adsorption reactions within the rhizosphere of flooded wetland plants (Armstrong and Beckett 1987; Reddy and DeLaune 2008) implies that to a large degree, some nutrient concentrations within the roots are directly governed by geochemical processes rather than active nutrient assimilation by the plant. These geochemical processes are influenced, in turn, both by the plant's attempts to ameliorate suboptimal soil conditions and by communities of microorganisms in the rhizosphere (Dassonville and Renault 2002; Ehrenfeld et al. 2005; Jones et al. 2004). Similarities in correlations for Fe/P and Mn/P in belowground tissues in the present study (Table 2) to those found in soils subjected to a range of redox potentials (Shahandeh et al. 2003) imply some degree of continuity between the soil and root tissues that is not present between root and shoot tissues. Such relationships may be elucidated by further studies examining the continuum of nutrient concentrations in rhizosphere pore water, root tissues, belowground stem tissues (e.g., rhizomes), and aerial portions of the plant.

Understanding the underlying mechanisms governing nutrient retention and release in wetlands is crucial for establishing management practices that improve water quality. Although limited in scope, findings from this study indicate enhanced rhizosphere immobilization of P in conjunction with Fe and Mn under flooded conditions, ostensibly via plant-induced rhizosphere oxidation. Generally, this mechanism for nutrient sequestration has not been separated from plant nutrient assimilation (i.e., synthesis of organic phosphorus). Plant senescence not only results in decomposition of organic material but also compromises the potential for rhizosphere oxidation in perennial plants. As the dying tissues comprising the lacunae, which serve as a diffusion conduit from the air to the sediment, become brittle, they may be damaged by wind or flowing water; or water may infiltrate the lacunae. These plant-mediated seasonal changes in redox status and potential effects on water quality merit further investigation.

## 5 Conclusions

Flooding led to increases in belowground tissue concentrations of P, Fe, and Mn in *L. oryzoides*,

whereas no effect on belowground tissue concentrations of N and K were noted. In general, flooding decreased nutrient concentrations in aboveground tissues. However, Mn concentration and uptake in all tissues were progressively higher in response to increased hydroperiod. The differences in accumulation of Fe and Mn in plant tissues are primarily caused by Eh and pH differences in the soil of the various flooding treatments, but it is important to note that these changes may be attenuated or amplified by plant-mediated changes to the rhizosphere. These results may be applied to water quality in agricultural ditches, wherein increasing water retention time in ditches for improvement of water quality may result in short-term increases in plant sequestration of P via formation of rhizosphere plaque; however, as the plant's ability to effectively oxidize the rhizosphere is compromised, either by decreased redox potential or plant senescence, bound P may be rereleased into interstitial water and eventually into surface water. Differences in plant nutrient allocation (aboveground versus belowground) under different hydrologic regimes should be taken into account when considering ditch management practices to increase hydraulic retention time or removal of plant biomass.

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