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FLOOD RESPONSES OF *LEERSIA ORYZOIDES* AND *BACOPA MONNIERI*: IMPLICATIONS FOR WATER QUALITY IN AGRICULTURAL DRAINAGE DITCHES

by

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Flood Responses of *Leersia oryzoides and Bacopa monnieri*: Implications for Water Quality in Agricultural Drainage Ditches

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Abstract

Laboratory and greenhouse experiments were conducted using mesocosms, comparing flood responses of two species common to rural drainage ditches in the lower Mississippi Alluvial Valley. *Leersia oryzoides* and *Bacopa monnieri* were subjected to four treatments: 1. a well-watered, well-drained control, 2. an intermittently flooded treatment, 3. a partially flooded treatment, and 4. a continuously flooded treatment. Over the course of these experiments, soil redox potential (Eh) decreased in flooded treatments, while aerated conditions were maintained in the control. *L. oryzoides* was fairly resilient to flooding, demonstrating increased biomass, and no effect on root depth under any flooding regime. However, rhizome penetration depth and eventually, net photosynthesis was reduced in the continuously flooded treatment. While macronutrient concentrations in aboveground tissues of *L. oryzoides* decreased slightly in response to flooding, belowground concentrations of iron and phosphorus, and belowground and aboveground manganese concentrations increased. *B. monnieri* exhibited decreased root penetration and aboveground concentrations of nitrogen and phosphorus in response to flooding, with greater effects observed under partial and continuously flooded treatments. Soil Eh was significantly lower in flooded treatments of *L. oryzoides* when compared to *B. monnieri*. *L. oryzoides* demonstrated greater overall uptake of phosphate than *B. monnieri* resulting in lower concentrations of phosphate in effluent from mesocosms containing *L. oryzoides*. Several interactions were noted. For instance, in the control soil nitrogen was higher in *L. oryzoides* mesocosms than in *B. monnieri* mesocosms. Additionally, specific conductivity of effluent from *B. monnieri* mesocosms increased in response to flooding, an effect that was not observed in *L. oryzoides* mesocosms.

Preface

This dissertation is presented as five separate but related papers:

Chapter 1: An earlier version of the introduction was published as S.C. Pierce and S.R. Pezeshki. 2009. Vegetation in Agricultural Ditches: Limitations to Establishment, Productivity, and Ecosystem Functioning. In Moore, M.T., and Kroger, R. Eds. Agricultural Ditches: Mitigation Wetlands of the 21st Century. Pp 75-106.

Chapter 2 was published as S.C. Pierce, S.R. Pezeshki, and M.T. Moore. 2007. Ditch Plant Response to Variable Flooding: A Case Study of *Leersia oryzoides* (Rice Cutgrass). Journal of Soil and Water Conservation 62: 216-225.

Chapter 3 was published as S.C. Pierce, S.R. Pezeshki, M.T. Moore, and D. Larsen. 2009. Nutrient response of Water Hyssop to varying degrees of soil saturation. Journal of Plant Nutrition 32: 1687-1701.

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Chapter 5 was published as Pierce, S. R. Pezeshki, D. Larsen, and M.T. Moore. 2009. Hydrology and Species-Specific Effects of *Leersia oryzoides* (Rice cutgrass) and *Bacopa monnieri* (Water Hyssop) on Soil and Water Chemistry. Ecohydrology 2: 279-286.

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Chapter 1

Introduction

Degradation of wetlands and aquatic resources due to alteration in natural flow patterns has been a topic of concern for a variety of researchers, policy makers, and the general public. Although wetlands and meandering streams have gained status as a valuable resource with regard to wildlife habitat and water quality, historically efforts at flood control and arable land for agricultural production have resulted in large tracts of lands being drained and extensive lengths of streams being channelized. Dahl (1990) estimated that as of the 1980s over 50% of wetlands in the contiguous United States had been drained since 1780. Similarly, few lowland streams in arable land remain unchannelized. Shankman (1996) goes so far as to state that almost all of the streams in the flatlands of western Tennessee and northwestern Mississippi have been channelized. One need only view the straight lines and right angles of waterways in aerial photographs of Arkansas, Louisiana and the Bootheel of Missouri to realize that this pattern extends throughout most of the Lower Mississippi Alluvial Valley.

This change in hydrology of meandering streams and overland sheet-flow to deeply incised streams and ditches has broad-ranging ecological ramifications including effects on vegetation and nutrient relations (Shankman, 1996, Franklin et al., 2009). The trend toward channelization and draining of water resources is intertwined with agricultural expansion. Coincidentally, extensive agriculture not only reduces an ecosystem's ability to mitigate for excess nutrients, agricultural practices are currently the greatest source for excess nutrients in surface waters (Birgand et al., 2007). Eutrophication due to agricultural inputs have been implicated in a number of environmental problems, including the now infamous “Dead Zone” in the Gulf of Mexico near the mouth of the Mississippi River (Birgand et al., 2007).

This extensive environmental degradation has led to interest in restoration of portions of degraded waterways to more pristine, or at least ecologically functional systems. The functioning of highly impacted areas, such as drainages in agricultural land, had previously been overlooked as low quality habitat that did not merit study for applications in restoration or mitigation practices. Within the last two decades, however, interest in these systems has grown concurrently with the growing body of research implicating their broad-scale impacts on receiving waters.

Two of the most practical ways to improve water quality by altering agricultural ditches are increasing hydrologic residence time and encouragement of vegetative growth. Research into ditch hydrology in its most basic sense is integral to functioning of the ditch as a water conveyance. It would logically follow that from an engineering perspective, a great deal is known about the general hydrologic functioning of ditches within the Lower Mississippi River Alluvial Valley. Comparatively less is known about pragmatic ways to alter ditch design to improve water quality without significantly impacting water conveyance. Even less is known plant functioning within ditch systems.

Because headwater ditches are eutrophic, hydrologically dynamic systems that are subjected to extensive anthropogenic disturbance, they can be a stressful environment for plant establishment and growth. Seasonal and stochastic alternations between flooded soils and desiccated soils are common, as are alternations between erosion and sedimentation. Ongoing anthropogenic influences include input of plant macronutrients, pesticide application – either direct or as by-spray – mowing, and dredging. This chapter summarizes relevant literature describing the current state of the science, including both plant ecological and physiological considerations. These scientific advances and considerations for hydrologic and vegetative management of ditches are synthesized with respect to broad-scale implications for water quality and habitat enhancement.

Losses of aquatic and wetland habitats associated with draining land for agriculture have fundamentally altered the ecological functioning of agricultural watersheds. As naturally occurring plants are limited to field margins in areas of intensive agriculture, ditches are habitat for aquatic and wetland organisms (Armitage et al., 2003; Freemark et al., 2002; Boutin et al., 2003). The concurrent trends of increased demands for agricultural output in conjunction with increased concern over the environmental ramifications of industrial agriculture mean that farmers and farm conglomerates will face pressure to manage water resources with the same intensity historically given to soil conservation and pest control.

Along with habitat losses resulting from improved drainage, non-point pollution from agricultural runoff is a major problem in many parts of the world (Bazzaz, 2001; Birgand et al., 2007; Day et al., 2003). While vegetated buffer strips have proven effective in reducing the contamination of natural streams and large drainage canals, a growing body of research exists on using aquatic or wetland macrophytes in drainage ditches for the mitigation of agricultural contaminants including macronutrients (Deaver et al., 2005; Kroger et al., 2007b) and pesticides (Hand et al., 2001; Lytle and Lytle, 2002; Moore et al., 2002; Runes et al., 2001).

The term “agricultural ditch” may be used to describe a range of drainage structures ranging from in-field grass waterways that only hold water during flood events to large-scale canals that are effectively channelized rivers (Bouldin et al., 2004; Dabney et al., 2007). The present review will consider ditches that lie between these two extremes – i.e. those that function neither as terrestrial nor riverine systems. Included in this classification are seasonally flooded ditches in which the beds develop hydric soils and support hydrophytic vegetation, even if the surface soil is dry much of the year. The larger end of the spectrum includes drainages with dry season baseflows of approximately one meter that potentially could support rooted vegetation within the ditch bed. Compared with large drainage canals, such ditches have higher water retention time and a greater density of bed vegetation than larger drainage structures (Bouldin et al., 2004). These properties may allow the use of headwater ditches for mitigation of non-point source pollutants such as those found in runoff from row crops, pasture, etc, potentially serving as an important buffer-zone component (Moore et al., 2000).

As agricultural ditches are by definition anthropogenic, management practices are likely to influence landscape features and microhabitat characteristics that limit plant establishment and productivity, as well as higher-order ecosystem properties involved in wildlife diversity and watershed management. Management practices that influence ditch ecosystem functioning include: general land use (e.g. – frequency of crop rotation: Liira et al., 2008; organic versus conventional: Manhoudt et al., 2006) and the subsequent pesticide (de Snoo and van der Poll, 1999) and/or nutrient concentrations (Best et al., 1995; Portielje and Roijackers, 1995; Billeter et al., 2008; Van Strien et al., 1989), ditch cleaning methods (Beltman, 1987; Milsom et al., 2004;Best, 1993; Blomqvist et al., 2006), controlled drainage (Twisk et al., 2003), and number of ditches in a given area (Geertsema et al., 2002; Geersema and Sprangers, 2002). Additionally, a number of well-studied conservation practices, such as use of winter cover crops or buffer strips are likely to have indirect effects on ditch functioning by influencing hydrology or pesticide and nutrient concentrations. Obviously, many of the factors affecting plant establishment and maintenance in ditches would not be expected to demonstrate short term responses to changes in management, as they are due to inherent habitat properties such as climate and parent material for soils. Additionally, there will be lags between management initiation and measured results (e.g. – changes in community composition: Helm et al., 2006). Rather than elucidating regional differences in agricultural watersheds, the present study will focus on abiotic interactions with vegetation in agricultural ditch systems in a broad ecological context.

Although ditches differ from "natural streams" in both physical structure and plant communities, they often share the basic properties of lotic systems (Giller and Malmqvist, 1998): 1. Unidirectional flow; 2. Continuous physical change; 3. Spatial and temporal heterogeneity at all scales; 4. High variability between systems; 5. Organisms are specialized to deal with water flow (i.e. – motility or anchorage). Because ditches are designed to convey water away from cropland, they tend to be more "flashy" in hydrology than unmanaged streams, especially in headwater ditches. These extreme fluctuations in water level in conjunction with proximity to intensive agriculture result in an environment that limits species recruitment, possibly resulting in the observation that small ditches have fewer plant species than larger systems (Bouldin et al., 2004; Davies et al., 2008).

As ditches are an integral part of riverine systems worldwide, a seemingly useful approach would be to apply ecological approaches for riverine and riparian systems to ditch systems (e.g. – River continuum concept, Vannote et al., 1980; Flood-Pulse concept, Junk et al., 1989). While these concepts may be useful for describing energy flow and ecological drivers in some river systems, in agricultural ditches, reduced size of the system and agricultural activity limit their utility. Systems-level analysis of energetics and the impact of biotic interactions fall outside the scope of this review.

The variable hydrology of ditches make the flood-pulse concept (Junk et al., 1989) particularly attractive; however, according to this concept soil nutrients in floodplains are primarily derived from aquatic systems, while the reverse is likely true in agricultural ditches. Rather than attempt to reconcile these differences using the limited available data, we will take a bottom up approach of describing ditch ecosystems using basic ecological concepts, both in terms of the physicochemical factors affecting ditches as habitat, and placing ditches in the context of the agricultural landscape. The objective is an examination of the functioning of ditches as habitat for plant communities, effects on the diversity of vegetation, and the interrelationship of ditch vegetation with its immediate habitat.

Why is plant diversity important?

Generally agricultural ditches are high in primary productivity owing to ample water and nutrients. While more diversity in plant communities can result in greater productivity (Tilman, 2000), the high level of productivity of the few plant species found in ditches can retard the development of complex, diverse plant communities, as fast growing plant species can quickly occupy open habitat, reducing establishment of other species (Herzon and Helenius, 2008; Blomqvist et al., 2006). Ecosystems with fewer species are less robust in the face of catastrophic events such as unseasonable weather or pest outbreaks. Therefore, fewer plant species mean an increased likelihood of ecosystem collapse (Tilman, 2000). There is evidence to suggest that plant diversity in ditches may promote diversity in soil microorganisms or animals (e.g. - Armitage et al., 2003). Diversity in plant communities may also be related to increased removal of non-point source pollution associated with agriculture. It has been suggested that low plant diversity increases nutrient losses to leaching (Engelhardt and Ritchie, 2001; Tilman, 2000), which could impact water quality in agricultural drainages (Boutin et al., 2003). Under seasonal or fluctuating limiting environmental conditions, different species will function optimally during different times of the year (Herzon and Helenius, 2008), potentially buffering nutrient uptake and release pulses related to plant growth and senescence (Kroger et al., 2007).

Plant Colonization and Extinction Dynamics in Agricultural Ditches

Species colonization plays a major role in determining community composition in frequently disturbed or highly fragmented environments (Geertsema et al., 2002; Liira et al., 2008). In the case of agricultural ditches, not only are these factors in play; but, additionally, a number of sub-lethal environmental stressors may alter plant competitive ability in a species-specific manner, potentially resulting in loss of intolerant species within the local community. Not surprisingly, long-term decreases in macrophyte species richness in ditches have been observed in areas of intensive agriculture (Blomqvist et al., 2003; Helm et al, 2003; Kuussaari et al., 2008 as cited by Herzon and Helenius, 2008; Hietala-Koivu et al., 2004). While the majority of long-term surveys published on ditch plant diversity have been centered in the Netherlands and the United Kingdom (see Table 1), the theoretical framework used to explain the observed patterns and trends in diversity allow short-term plant surveys to be placed in a useful context.

Island biogeography theory (MacArthur and Wilson, 1967) was developed to predict species richness for systems in equilibrium. The number of species in isolated ecosystems, such as islands or, in this case, agricultural headwaters, is influenced by both immigration from source populations and localized extinction (Geertsema et al., 2002). Species immigration is determined by the distance from the source population, with greater distance resulting in decreased likelihood of immigration. In the theory as originally proposed, source populations are derived from a mainland or another island. Species extinction is determined by island size, with smaller islands more prone to localized extinctions due to limitations of home range, fewer types of habitat, and increased likelihood of localized disturbance resulting in habitat destruction. Hypothetically, agricultural ditches may be populated from a number of sources including natural wetlands, riparian zones, farm ponds, or within the ditch network itself; whereas localized extinction in smaller headwater ditches can result from greater environmental stochasticity (Davies et al., 2008), extreme physicochemical conditions (Davies et al., 2008; Milsom et al., 2004; Biggs et al., 2007; Bouldin et al., 2004), and higher anthropogenic stress and disturbance (Bouldin et al., 2004).

Over time the balance between immigration and extinction results in an equilibrium number of species in a given area, with larger habitat patches close to population sources having the greatest diversity and small isolated habitat patches having the least. While habitat size in conjunction with isolation has been applied to agricultural systems (e.g. – farm ponds - Oertli et al., 2002), comparisons to ditch watersheds should be interpreted carefully, as island biogeography theory assumes that areas between landforms (e.g. – open water versus islands) are totally hostile to colonization and that landforms are conducive to colonization. There is evidence to suggest, however, that with proper watershed management, available habitat for source populations is a major factor in explaining ditch floral diversity (Geertsema et al., 2002; Boutin et al., 2003). However, due to the high degree of environmental variability and disturbance in agricultural ditches it is unlikely they are reaching any sort of ecological equilibrium or established community (see Mountford, 2006; Geertsema and Springers, 2002). Rather, ditch habitat may serve as marginal or temporary habitat with populations of individual species appearing and disappearing in response to hydrologic or management regimes.

The source-sink relationships of such temporary populations can be interpreted in terms of metapopulation theory (Levins, 1969, Haanski, 1999). A metapopulation is defined as a collection of distinct populations that infrequently exchange genes within the metapopulation via immigration and emigration. Metapopulations develop in fragmented landscapes, with patches of suitable habitat being occupied by a population. While the individual populations are subject to decline or extinction, the overall metapopulation may be stable, dependent upon the species ability to colonize the extirpated population's habitat. The inclusion of metapopulation concepts into the framework of ditch community development is especially important given that periodically vegetation is removed from ditches and ditch banks (Beltman, 1987; Best, 1993; Blomqvist et al., 2006; Milsom et al., 2004), essentially creating areas of open habitat that must be repopulated either from extant vegetation, such as rhizomes, from the seed bank, or from dispersal from adjacent habitat (Geertsema, 2002; Blomqvist et al., 2003). Additionally, seasonal drying of ditches can result in sporadic shifts in available habitat patches from aquatic to wetland to terrestrial potentially extirpating terrestrial or aquatic species (Milsom et al., 2004; Biggs et al., 2007).

Waterways serve as important corridors for plant dispersal (Gurnell et al., 2008), but as this dispersal would tend to be limited to downstream colonization events (Gornall et al., 2002) colonization of remote headwater ditches by water-dispersing plants may likewise be limited. Additionally, due to seasonal drying, portions of ditches may sometimes be disconnected from larger aquatic systems (see Biggs et al., 2007), potentially decreasing this mode of colonization (Leyer, 2006). In agricultural landscapes, the value of linear elements such as ditches as habitat corridors may be limited to animals which are capable of upstream dispersal to remote ditches and emigration during seasonal declines in habitat quality (Liira et al., 2008). Within the agricultural landscape patches of natural habitat (Liira et al., 2008), and, in particular for ditches, riparian areas (Armitage et al., 2002; Boutin et al., 2003) serve as important sources for repopulation of habitat regularly disturbed by agricultural management.

Intensive agriculture generally leads to dominance of ruderal or early successional species (Liira et al., 2008). Such species have quick reproductive cycles and high dispersal distances that allow them to take advantage of regularly disturbed soils (Grime, 1979). Most surveys of ditch plants; however, have been performed in open grassland or pasture in Western Europe where woody vegetation is sparse (Table 1). In large tracts of North America, ditches are often minimally managed and sometimes dominated by woody vegetation, especially near riparian zones. At least one survey found that such

Table 1. Select studies relating to biomass, productivity, or diversity of ditch plants.

Study	Location	Ecosystem/study	Findings
De Snoo and Van der Poll 1999	Netherlands	Multiple	Use of pesticides decreased ditch plant diversity compared with unsprayed crops
Liira et al., 2008	Europe	Multiple	Increased agricultural intensity reduces occurrence of rare species. Ditches and hedgerows alone contribute little to plant diversity.
Milsom et al., 2004	United Kingdom	Survey	Extirpation of aquatic species in response to hydrologic stochasticity was greater than wetland, terrestrial. Frequent mowing reduced species losses, compared to annual mowing. Light dredging on 3 year cycle increased aquatic diversity.
Mountford, 2006	United Kingdom	Survey	Ditch community assemblages differ from existing classifications, likely due to disturbance (annual dredging) limiting development
Manhoudt et al., 2006	Netherlands		Plant species richness is higher on organic and environmentally managed farms than conventional farms.
van Strien et al., 1989	Netherlands	Survey	pH, water table, and N main factors influencing species richness. High water increased richness and "nature value"
Williams et al., 2004	United Kingdon	Survey	Ditches harbored fewest species of all water bodies in agricultural areas. Species composition primarily related to hydrologic factors.
Herzon and Helenius, 2008	Review	Experimental ditches	High nutrient loads promoted changes in plant composition during early succession
Freemark et al., 2002	Ontario, Canada	Survey	Ditches are important habitat for native plant species
Geertsema et al., 2002			Lower density of ditches results in dominance of poorly dispersing species. Species with persistent seed banks more common in younger ditches. Species with transient seed banks common in older more stable ditches.

ditches have improved water quality compared to open ditches (Bouldin et al., 2004); however, whether water quality was improved by the presence of trees or related management and environmental variables is unclear. The large volume of water transpired by trees along with nutrient sequestration means they may have a larger impact on water quality than herbaceous plants both in the ditch bed and banks. Boutin et al. (2003) specifically recommend a buffer of evergreen trees to reduce drift from pre-emergent pesticide into aquatic systems. Taken for granted that trees can improve water quality, establishment of wooded buffer zones takes time and may be complicated by crop herbicide application and climate change. Bouldin et al. (2004) recommend that a combination of grass and forest buffer strips be used in conjunction with some level of ditch management, particularly promotion of stable plant communities in ditches.

A number of regional and watershed-level conservation practices and agricultural management practices are likely to influence both the water quality and the plant communities draining agricultural land; however, as these topics have been discussed elsewhere (Day et al., Birgand et al.; Moore et al 2005; Dabney et al., 2007), the present focus will shift to a discussion of habitat criteria for vegetation establishment in ditches, and the potential for such vegetation to form the basis of a self-regulating ecosystem with minimal management.

Habitat Quality and Local Effects on Establishment and Persistence

Ditches in open fields offer two types of habitat: a terrestrial ditch bank which may harbor facultative wetland plants and upland grasses; and a ditch bed which supports a variety of aquatic and wetland plants, as well as annual facultative species that can colonize more remote ditches during dry periods (Bouldin et al,. 2004). Management for robust communities of ditch banks requires different management approaches than those for ditch beds (Milsom et al., 2004), although in general decreases in pesticide exposure and nutrient concentrations lead to increased diversity (Liira et al., 2008; Van Strien et al., 1989). Bank and bed vegetation are both influenced to varying degrees by duration and frequency of inundation (Milsom et al., 2004; Best et al., 1995). Such plants may be classified by their position relative to the water surface (Beyrouty, 2002). Emergent vegetation displays a growth form similar to terrestrial vegetation, with roots for both anchorage and uptake of nutrients and water, and with the majority of leaf tissue above the water surface. In submergent vegetation, roots serve to anchor plants and share nutrient uptake with stem and leaf tissue that is predominantly subsurface. Floating plants have both leaf tissue at or above the water surface and roots may be either free-floating or anchored. The degree to which nutrients are acquired by roots or leaves is a matter of some debate, and likely depends upon ratios of nutrients in the sediment and water column as well as pH and redox conditions (Konclova, 1990; Chen and Barko, 1988; Beyrouty, 2002; Madsen and Cedergreen, 2002). In general, however, it is safe to say that wetland plants display decreased root development in conditions of excess nutrients, preferentially utilizing assimilated carbon for shoot production, a pattern common among terrestrial plants as well (Beyrouty 2002).

In highly productive habitats, including eutrophic ditches, microhabitat characteristics may be a more important limiting factor for colonization than dispersal characteristics (Tilman, 1982; Blomqvist et al., 2006). Increased productivity, and subsequent increased vegetative cover and shading, can limit colonization of new species by decreasing seedling germination and emergence, as well as decreasing survival of some established species (Blomqvist et al., 2006). Thus as elevated nutrients increase productivity within established vegetation they decrease recruitment of new plants. As mentioned previously, in some areas of Europe ditches and ditch banks may serve as refuge for aquatic and wetland plants. Various methods and frequencies of vegetation removal, such as mowing and harvesting, have resulted in increased diversity, presumably the result of increased light enhancing establishment of new plants. In North America, research on ditch vegetation has focused on its potential for water quality remediation, rather than plant ecology. However, as this biomass is an important source of organic carbon for soil formation, harvesting of ditch vegetation may not lead to long term improvements in water quality, and may in fact limit such ditches as functional ecosystems. The understanding of how plant influences on soil characteristics can in turn affect the plants themselves is limited (Ehrenfeld et al., 2005), though it is clear that plants affect water quality indirectly via soil formation (Blom, 1999; Birgand et al., 2007; Needelman et al., 2007).

Removing aboveground biomass of emergents allows establishment of submergent and floating plants that have greater potential for leaf-water interface, enhancing biofilm development and increasing the likelihood for hydrophobic pesticide adsorption on plant surfaces. Conversely, emergent vegetation can decrease sediment resuspension and nutrient concentrations in the water column when compared to submergents (Horppila and Nurminen, 2001; Madsen et al., 2001, as cited by Bouldin et al., 2004). However, establishment of such plants depends upon a reliable presence of water. Additionally, turbidity and high loadings of nutrients may restrict growth of submerged vegetation (Portielje and Roijackers, 1995; Janse et al., 1998; Bouldin et al., 2004, and citations therein).

As opposed to colonization in mobile organisms, initial plant colonization of a given habitat depends upon some transport vector for adult plants (in the case of vegetative propagation) or, more commonly, for seeds or spores. Generally, in highly productive environments establishment is limited. Germination requirements for individual species are generally more selective than the corresponding tolerance ranges for mature individuals. Once the individuals of the initial population have reached reproductive maturity, localized extinction may be curtailed via extensive vegetative reproduction or formation of persistent seed banks. The environmental sieve model (van der Valk, 1981) incorporates plant functional groups to predict the dependence of wetland plant community composition upon the hydrologic regime. Plants that time establishment and reproductive events around regular environmental variations may experience greater reproductive fitness. In the case of ditches, seasonal drying may limit the species pool. The dominant plant in many headwater ditches in the southeastern United States, *Leersia oryzoides*, is promoted by a summer drawdown similar to what is observed in ditches (Meeks, 1969). While the model proposed by Van der Valk (1981) was developed with regard to wetland hydrology, vegetation within and adjacent to agricultural ditches has also been shown to respond to timing of management practices such as cleaning and dredging, ostensibly because of increased likelihood of germination (Best, 1993, Blomqvist et al., 2006).

Hydrologic Influences

Hydrology, along with the resulting changes in soil character, is the primary determining factor for community composition in wetlands within a given region. The same properties likely hold true for agricultural drainage ditches. In field studies, "hydrology" may be characterized in a number of ways with varying degrees of relevance to plant response, depending upon the frequency and duration of inundation. In areas experiencing regular flooding due to tidal or strong seasonal influence, in systems that have relatively stable water level over time, measuring surface water depth in conjunction with piezometers can yield accurate assessments of hydrologic influence on plant community development and individual plant acclimation. Agricultural ditches and many riparian areas, though responsive to seasonal precipitation, show greater variation in surface hydrology. When direct measures of water level prove impractical, various proxy measures may be used to assess flooding impact, or incorporated into models to estimate surface water hydrology. Within a riparian floodplain, elevation was used to estimate site flooding frequency and compared to plant community composition (Menges and Waller, 1983). Elevational models, used in conjunction with flood-stage data may prove useful in understanding riparian communities, however, given the linear nature and small-scale of ditch systems such models are not likely to be predictive of species assemblages. Another proxy for estimating the impact of flooding on plants is by characterizing the oxidation-reduction potential of the soil (redox potential, Eh). In many respects Eh is superior for estimating effects on plants, as it is indicative of soil demand for oxygen and predominant chemical species in the soil. In a two-year study on multiple wetland species grown in sand, flooding alone had little effect on nutrient concentration, likely due to the low capacity of sand for chemical reduction (Gusewell et al., 2003). While the potential for reduction of a given chemical may be estimated by Eh, the capacity for reduction is dependent upon the amount of electrons accepted by soil oxidants and is determined by labile carbon and other potential energy sources available for microorganisms (Kludze and DeLaune, 1995).

When soil is inundated, oxygen diffusion into the soil is limited, creating an anoxic environment. Organisms then begin using alternate electron acceptors in a step-wise fashion, leading to the chemical reduction of plant nutrients. At circumneutral pH conditions, when Eh drops below +350 mV oxygen can no longer be utilized as an electron acceptor for respiration (Patrick and DeLaune, 1972). When Eh reaches +250 mV nitrate is the predominant electron acceptor, resulting in reduced forms of nitrogen (ammonium, diatomic nitrogen, etc.). Given adequate supply of organic material in the flooded soil, Eh will continue to decrease leading to the progressive reduction of manganese, iron, sulfate, and finally organic compounds as Eh reaches values < -200 mV(Gambrell and Patrick 1978, Gambrell et al. 1991). This depletion of oxygen and the subsequent changes to soil chemistry result in stressful conditions of depleted nitrate and increased toxicity of manganese, iron and sulfate (Pezeshki, 2001). It is important to note that these processes may occur when soil is saturated below the surface even if surface water is not observed, leading to plant flood stress and, potentially, plant acclimation response (Pezeshki 2001, Li et al., 2004). Detrimental effects of flooding on macronutrient uptake have been reported for numerous terrestrial species, primarily crops (Drew, 1991), ostensibly due to decreased root functioning resulting from hypoxic or anoxic conditions in root tissues, as these decreases occurred prior to any changes to soil nutrients (Armstrong and Drew, 2002). Conversely, increases in xylem phosphate concentrations were observed in flooded tomato plants (Else et al., 1995). A number of changes in nutrient relations of wetland plants also occur as a result of soil reduction, both because of changes in the availability of nutrients in soil water and because of root dysfunction resulting from lack of oxygen (Pezeshki, 2001). However, as wetland plants are adapted to ameliorate such conditions, the nutrient responses of these plants to flooding is broad ranging and depends upon species response and soil redox conditions. Plant tissue concentrations of manganese, iron and sulfate can be elevated in response to flooding (Pezeshki, 2002), though partitioning of iron in the plant may limit such increases to only root or stem portions (McKelvin et al., 1987, Chen et al., 2005; Liang et al., 2006; Rubio et al., 1997). The partitioning of iron in flooded plants is important as it is often correlated with phosphorus (McKelvin et al., 1995, Chen et al., 2005; Liang et al., 2006; Rubio et al., 1997).

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

After initial reduction, partial recovery of net photosynthesis (Pn) and stomatal conductance (gs) occur in many species related to the duration and intensity of reduction (Pezeshki 1993; Pezeshki and Anderson 1997; Brown and Pezeshki 2000). However, even with substantial recovery of gas exchange, plant survival and growth may be impacted (Pezeshki and Anderson 1997), which is reflected in reduced plant biomass, particularly belowground biomass (DeLaune et al. 1983; Pezeshki 1991; Kludze and DeLaune 1995) and root penetration depth (Pezeshki 1991). The threshold level of soil reduction (redox potential, Eh in mV) for these responses differs among wetland species, ranging from +300 mV to -200 mV (Pezeshki 1991; Will et al. 1995). The upper level of this threshold corresponds roughly to the onset of oxygen disappearance from the soil, which begins to occur at Eh of +350 mV (DeLaune et al. 1990).

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

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

Because of the limitations to plant gas exchange, growth, and reproduction outlined above, plants found in flood-prone areas have three general coping strategies (Voesenek et al., 2004):

1. Avoidance – life history related;
2. Tolerance - metabolic adaptations to ethanol production and decrease in cytosolic pH, generally considered short-term responses for most plants;
3. Amelioration – conveyance of oxygen to roots from atmosphere and photosynthetic organs.

Voesenek et al. (2004) additionally cited a number of wetland plant adaptations for amelioration of flooded habitat: 1. Fast shoot elongation to the water surface, 2. Longitudinally interconnected gas chambers to facilitate diffusion, 3. A barrier to oxygen loss along the portions of the plant's perimeter, 4. Adventitious rooting, and 5. Underwater photosynthesis and the resulting oxygen production.

Ethylene is generally cited as a signal for aerenchyma development and auxin as a signal for adventitious root development, but a number of root-generated growth factors affect response to flooding both directly and by inhibition and promotion of other growth factors (Jackson, 2002; Voesenek et al., 2004). The specific sensors and signals that initiate amelioration responses and how this signal is propagated is unknown and is a subject of several recent reviews (e.g. – Dat et al., 2004; Visser et al., 2004). Regardless of the biochemical and physiological pathways involved, with the exception of adventitious rooting and underwater photosynthesis, the goal of these morphological changes is to create continuity of the oxygen-rich atmosphere with the regions of the root responsible for nutrient uptake. Generally, the more pronounced the morphological changes, the greater the plant's ability to withstand flooding. Root porosity in particular has been cited as a major factor in conveyance of oxygen to distal root tissues (Armstrong and Beckett, 1987; Reddy et al., 1989). In two *Trifolium* species, for instance, greater root development was observed in the species with the greatest root porosity (Gibberd et al., 1999). Additionally, it is thought that shorter, thicker roots are preferable over long fine roots because of lower resistance to oxygen diffusion (Jeperson et al., 1998; also see review by Armstrong and Drew, 2002). Wetland graminoids (grasses and plants with similar growth habits e.g. – *Typha*) produce two kinds of roots in response to flooding: thick poorly branched soil roots and fine adventitious roots for nutrient uptake (Koncalova, 1990).

One difficulty plants face in reduced soils is not only the lack of oxygen, but the demand for oxygen in the soil. As the porosity of plant tissue increases, so does the potential for diffusion, not only into the plant roots, but from the roots into the surrounding soil (Visser et al., 2000). The loss of oxygen is related not only to porosity, but also the demand for oxygen in the soil and, in some instances, the leaf area available for oxygen uptake (Connell et al., 1999). This diffusion pathway, termed radial oxygen loss (ROL) is common to all plants under a limited set of environmental conditions, even submerged plants (Laskov et al., 2006); however, many wetland monocots such as rice develop a barrier that limits ROL to portions of the roots that are growing or the primary sites of nutrient uptake (Colmer et al., 1998). By limiting oxygen loss to growing root tips, oxygen may be delivered to greater depths in the soil, potentially ameliorating anoxia well below the soil surface (Visser et al., 2000). Additionally some monocots have pressurized gas chambers and flow-through systems that have been hypothesized to force oxygen to submerged tissues (Jackson and Armstrong, 1999; Armstrong and Drew, 2002). The ability to convey oxygen deep within flooded soils may explain why some monocots have demonstrated increased biomass production under flooded versus drained conditions in some studies (Li et al., 2004; Rubio et al., 1995).

The conveyance of oxygen to the roots of flooded plants is dependent upon the plant leaf or stem being in contact with atmospheric oxygen. As a response to submergence, wetland plants may increase apical shoot growth in order to maintain contact with the atmosphere (Voesenek et al., 2004a,b). Not surprisingly, depth of inundation, in addition to its frequency and duration is a species-specific limitation to establishment and reproduction of plants (Blom, 1999; Cassanova and Brock, 2000;

Miller and Zedler 2003; Kirkman and Sharitz, 1993). The ability of plants to convey oxygen to flooded roots is related to their distribution in nature, with plants that have greater porosity or pressurized oxygen flow growing in deeper or longer duration of flooding (Sorrell et al., 2000). Vivian-Smith (1997) created artificial tussocks on a wetland marsh, resulting in varying water depths. The increase in heterogeneity of the habitat was credited with the observed increases in number of species in altered plots.

Ditch management techniques directed at improving water quality or ditch stability such as 2-stage ditches (Figure 1; Powell et al., 2007) or low-grade drainage control structures (Figure 2; Kroger et al., 2008) may have added benefits of increasing habitat heterogeneity, thus improving ditches ecosystem functioning (Benton et al., 2003); however, in headwater ditches such effects may be limited by a high degree of variability in the water level. Short-term, but frequent flood events may simply demonstrate less intense soil reduction than long-term flooding, resulting in a plant response intermediate to drained or prolonged flooding, including a reduction in net photosynthesis and decrease in root growth (Pezeshki et al. 1998; Anderson and Pezeshki, 1999; Pezeshki and Anderson, 1997; Li et al., 2004). As these alternating patterns of wet:dry become more pronounced or prolonged, they may produce a complementary pattern of reduction:oxidation in the soil, which produces its own difficulties for plants. The intensity and duration of flood events in areas of variable hydrology are likely more important for determining plant communities than depth at any one time (Cassonova and Brock, 2000). In aquatic and wetland environments that experience regular drying, the availability of water in the dry season is likely a major factor limiting establishment of wetland vegetation (Cassanova and Brock, 2000;

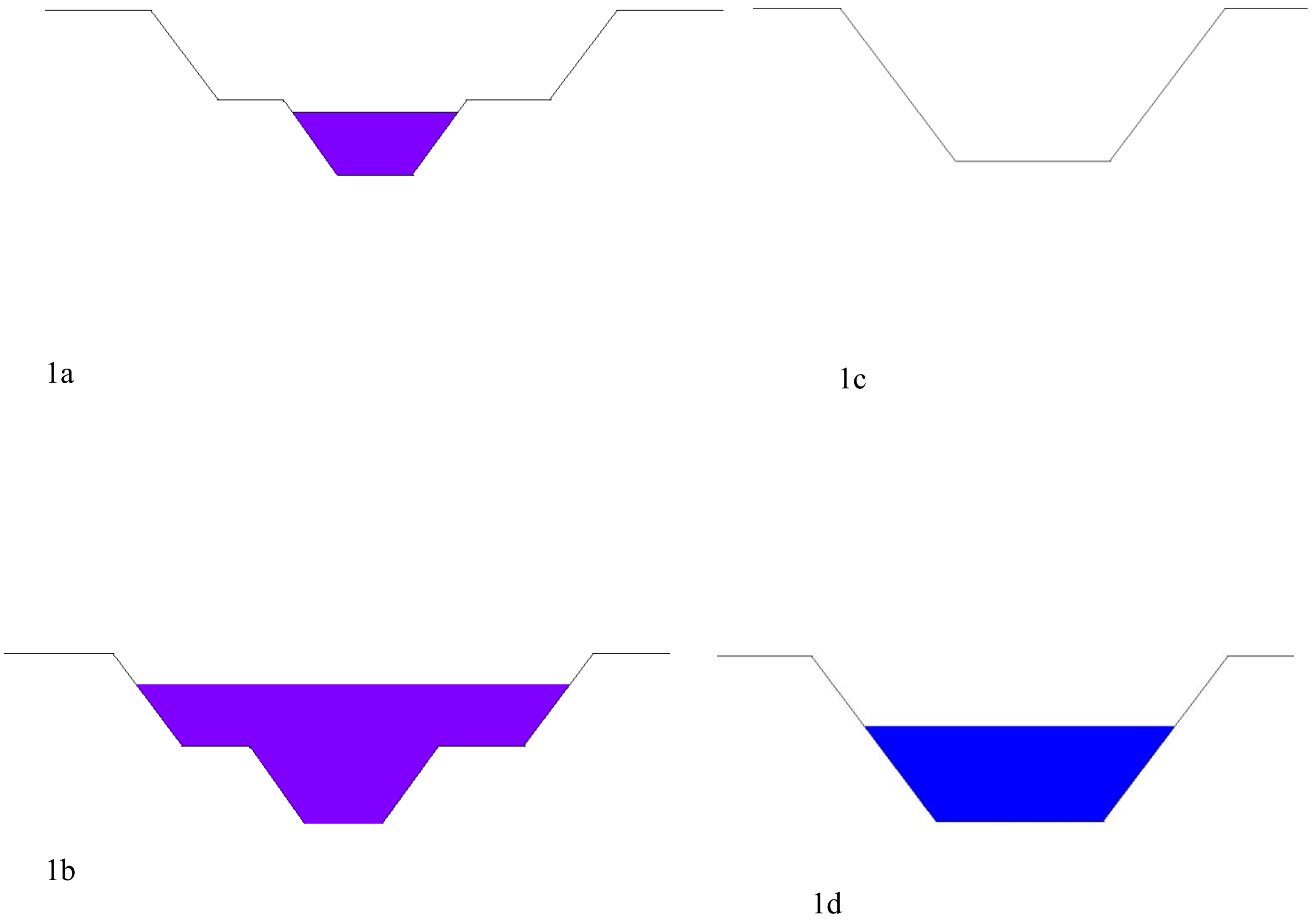

Figure 1: Comparison of traditional trapezoidal ditch cross-section (1c and 1d) to 2-stage ditch cross section (1a and 1 b) at high water and baseflow. Seasonal weather patterns and lack of connectivity to groundwater result in alterations between aquatic, saturated, and dry habitat for plant growth, potentially hampering stable vegetation throughout the growing season. In spite of this high temporal variability, only the ditch bank provides a range of soil moisture that would promote plant diversity. 2-stage ditches potentially offer a more spatially heterogeneous environment, with an aquatic ditch bed, a saturated ditch bench that would allow for emergent vegetation, and a ditch bank comprised of facultative wetland and upland plants. This design would be especially effective if the ditch bed is not subjected to regular drying that would extirpate aquatic vegetation. These suppositions do not take into account potential indirect effects on water level as a result of 2-stage ditches.

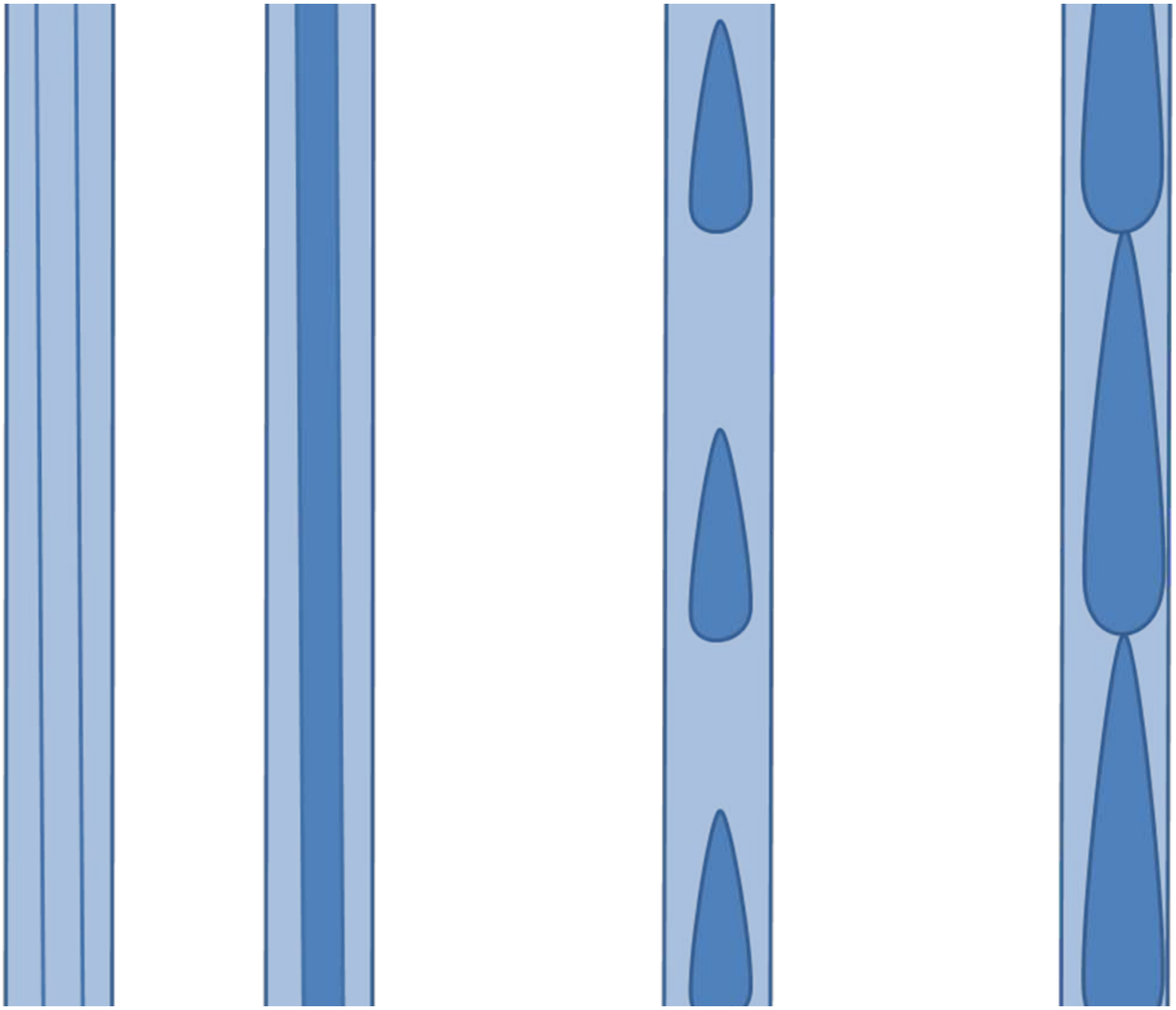

Figure 2: Comparison of standard trapezoidal ditch at low water (a) and high water (b) with ditch modified by low-grade flow control structures at low water (c) and high water (d). The shaded portion represents the presence of surface water. The resulting pool and riffle system would result in a longitudinal gradient of water level, with upper reaches subjected to periodic drying, while lower reaches (i.e. directly upstream of control structures) would maintain standing water and serve as local refugia for aquatic plants during dry periods. This design has the advantage of reducing temporal variation in the presence water, while increasing microhabitat heterogeneity, as well as increasing hydraulic retention time.

Sabbatini and Murpy, 1996), in the most extreme cases species establishment may be limited to annuals or species with short growing seasons that avoid more extreme conditions (Voesenek et al., 2004). Distribution of black willow along streambanks, for example, may be limited by drought (Li et al., 2004; Pezeshki, et al, 1998; Pezeshki et al., 2006).

Because of the importance of water regime on vegetation and the high degree of variability in water depth, frequency, and duration in agricultural ditches, especially headwater ditches where the potential for plant-water interface is the greatest, measures attempting to stabilize fluctuations could benefit the functioning of ditch vegetation. Depending upon the system, low grade weirs (Kroger et al., 2008) may be sufficient to retain small pools of water that support hydrophytic vegetation during dry periods, however in many cases more far-reaching measures utilizing a greater land area may be required to dampen such effects (Evans et al., 2007).

Mechanisms for Plant Functioning in Agricultural Ditches

Autotrophs form the energy basis for ecosystem productivity with photosynthesis being the greatest source of primary productivity worldwide. In terrestrial, freshwater, and estuarine systems plants also form an integral part of the physical habitat. A fact attested to by communities in these systems being described by the dominant vegetation. In some cases, high productivity of only a few plant taxa can yield higher-order diversity in heterotrophic taxa. Estuaries, for example serve as important refuges and breeding grounds for a number of marine organisms, in spite of limited plant diversity. There is limited evidence, however, to suggest that increased plant diversity in ditches can have cascade effects leading to overall increases in ecosystem diversity (Armitage et al., 2002).

Plants are important structural components of both the soil and water in ditch systems. Aboveground portions of the plant serve to increase bed roughness, increasing hydraulic retention time (Bouldin et al., 2004), stabilizing sediment and increasing nutrient and carbon storage in soil (Braskerud, 2001; Sand-Jensen, 1998a,b). Additionally, submerged stems and leaves serve as surfaces for bacteria biofilms and phytoplankton (Brix, 1997; Toet et al., 2003; Wu et al., 2006). The extent to which plants serve as secondary growth surfaces is probably related to likelihood of surface desiccation. Plants also produce shade, lowering soil and water temperatures and potentially reducing photochemical catabolism of organics. Finally, submerged leaves alter water chemistry. While such changes would potentially occur in any plant, leaf gas exchange during submergence is restricted to varying degrees in terrestrial and wetland plants. Submerged aquatics, on the other hand, can alter diurnal fluctuations in water pH by releasing CO_2 at night as a product of respiration and uptake of HCO_3^- from the water column during photosynthesis, as well as altering diurnal dissolved oxygen patterns (Sand-Jensen and Frost-Christensen, 1998).

Roots and stem tissue below or at the soil surface alter the soil and the soil-water interface, potentially reducing erosion. Black willow, for instance, has been shown as an effective plant for erosion control in highly impacted streams, especially when used in conjunction with artificial structures to enhance bank or bed stability (Shields et al., 1995). Plant roots are involved in a constant interface with the soil matrix, creating small scale gradients in soil water potential and altering soil hydraulic conductivity via changes in density and porosity. Roots can also impact soil chemistry, as discussed below, not only through mineral uptake, but also by the release of chemicals into the rhizosphere. With regard to soil structure, the release of organics into soils can fundamentally change soil character as well as alter soil microbial communities. Plants have been reported as releasing from approximately 5% to 40% of the carbon fixed via photosynthesis into the soil directly through the roots (Ehrenfeld et al., 2005; Neumann and Romheld, 2002), increasing the available resource pool for fungal mycelium, which in terrestrial soils may be even more important for terrestrial soil development than the plant roots themselves. Soil anoxia or reduction resulting from inundation may limit fungal colonization of plant roots (but see Weishampel and Bedford, 2006). In hydric soils bacteria are generally thought to play a more prominent role in rhizosphere chemistry and soil formation (Dassonville and Renault, 2002), particularly via regulating soil redox conditions (Weiss et al., 2003).

In addition to being a source of food for heterotrophic organisms, plants may also serve as an environmental sink for contaminants. Fertilizers and pesticides from adjacent or upstream crop fields reach ditches through both surface and subsurface flows. While excess nutrients may limit community diversity and alter productivity, plants, in turn, may remove nutrients from the water column (Read et al., 2008; Deaver et al., 2005; Kroger et al., 2007b) through a number of mechanisms, including direct uptake and metabolism. The amount of nutrients stored in plant tissues is species dependent and influenced by nutrient availability and other environmental factors such as soil flooding (Kao et al., 2003; Kroger et al., 2007b). The degree to which direct plant uptake of nutrients reduces eutrophication depends in large part upon hydraulic retention time and the concentration of nutrients (Peterson and Teal., 1996). As nutrient concentrations begin to exceed uptake capability, the plant-related benefits to water quality show diminishing returns even if hydraulic retention time is sufficient for removal. Very high-loading rates would tend to overload the system. Finally, direct uptake and storage of nutrients by plants is only a temporary fix, as during plant senescence, nutrients may be released into the soil and water column (Kroger et al., 2007b). As such plants may serve only as a seasonal buffer to spikes in nutrient concentrations.

However, as previously mentioned, plants may alter soil and water chemistry not only through nutrient uptake, but also by releasing materials such as oxygen into the soil. Radial oxygen loss from plants with extensive root systems can raise redox potential in the bulk soil, although these effects may be attenuated by a high demand for oxygen in the soil (Barko et al., 1991; Jeperson et al., 1998; Sorrel and Armstong, 1994; Chen and Barko, 1988). Nevertheless, the potential for plant oxidation of flooded soil is an important topic of study not only with regard to effects on dissolved nutrients in soil water, but also effects on aerobic microbe activity (Armstrong, Armstrong and Beckett, 1990 and potential for enabling survival of plants that are not as well adapted to survive reduction (Callaway and King, 1995).

Soils that are anoxic or reduced as a result of inundation may be especially prone to plant mediated changes in chemistry. The reduction of iron and manganese increases their water solubility, as well as that of phosphorus that is bound with the oxidized species of these metals. Oxygen diffusion from the root cortex into the rhizosphere results in their oxidation and precipitation as plaques on the root surface and surrounding soil (Reddy and DeLaune, 2008). Possibly as a result of this plaque formation, plants grown in flooded soils have high belowground tissue phosphorus concentrations (Chen et al., 2005; Liang et al., 2006; Rubio et al., 1997). Additionally, the oxidation of ferrous iron releases protons causing rhizosphere acidification (Kirk and Du, 1997). This mechanism in combination with proton release resulting from ammonium uptake (Neumann and Romheld, 2002) and increased deposition of organic acids by flooded plants (Jones et al., 2004) lead to pH decreases in the rhizosphere. This decrease in pH may lead to increases in the aforementioned minerals in the water column, potentially counteracting the beneficial effects of plant mediated precipitation. The importance of pH versus Eh effects on inundated plants is likely species-specific and dependent upon the capacity of the soil to buffer or enhance this effect.

Research Conspectus

The literature review indicated a deficiency of research on how plants common to ditches respond to changes in hydrology and the potential impacts on water quality. To elucidate the potential impact of variable hydrology on ditch plants, and to quantify the potential effects of hydrology-plant interactions on soil and water chemistry, a series of greenhouse and laboratory studies were conducted examining plant physiological response and soil and water response across a range of soil flooding regimes in two species common to rural drainages in the southeastern United states, *L. oryzoides* and *Bacopa monnieri*. Four hydrology treatments were imposed: well-watered, well drained; intermittently flooded, partially flooded, and continuously flooded. The hypotheses described below and further developed in chapters 2-5 are based on individual species responses to these treatments. Chapter 5 also discusses species-related responses, however the emphasis in that study was the effects of flooding and presence of either *L. oryzoides* or *B. monnieri* on soil and water chemistry.

Leersia oryzoides (Rice cutgrass) is a widely distributed obligate wetland grass, which is common to agricultural ditches in the Mississippi alluvial plain (Bouldin et al., 2004). Although described as a rhizotomous perennial (Darris and Barstow, 2006), it is found in wetland seed banks (Peterson and Baldwin, 2004). Growth may be encouraged by summer drawdown (Meeks, 1969). When grown in water high in nitrogen and phosphorus, *L. oryzoides* increases shoot tissue nutrient concentrations, which, in turn, release higher levels of nitrogen and phosphorus into the water column upon plant senescence (Kroger et al., 2007b). The effects of *L. oryzoides* on mesocosm effluent has been compared to *Juncus effuses*, and *Ludwigia peploides* (Deaver et al., 2005). Effluent from mesocosms with *L. oryzoides* in the study conducted by Deaver et al., (2005) showed higher concentrations of nitrogen than mesocosms planted with other species, whereas *L. oryzoides* plantings decreased phosphorus effluent concentrations compared with unplanted controls.

Bacopa monnieri (Water hyssop) has a pan-tropical distribution and is a common species in wetlands near the Gulf of Mexico, forming a common component of marsh communities in areas that are sporadically subjected to flooding and salt stress during storm events (Visser et al., 1998). Hurricane Camille, which made landfall near the mouth of the Mississippi River in August of 1969, devastated a number of marsh species; however *B. monnieri* was largely unaffected (Chabreck and Palmisano, 1973). *B. monnieri* seeds are not highly viable (Tiwari et al., 2001); the plant requires bare ground to become established (Shah, 1965 from Tiwari 2001). If nutrients are adequate, *B.*

monnieri can quickly colonize bare marsh soils, becoming a major component of the flora (Dalrymple et al., 2003), even in waters subjected to agricultural and industrial pollution (Gupta, 2003). Few published studies examine the basic physiological and morphological responses of this species to common environmental stresses (but see Sinha and Saxena, 2006). The same traits that allow *B. monnieri* to cope with periodic disturbance and pollution make it a potential candidate for introduction into systems that have been highly degraded, such as agricultural ditches.

Overview of Objectives and Hypotheses

Objective 1 (Chapter 2): Characterize productivity and morphological response of *L. oryzoides* to variable flooding

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

1. Decrease in belowground:aboveground biomass ratios along with decrease in root penetration depth. In addition, decrease in rhizome penetration depth would be expected in flooded plants, with this effect being most pronounced in the Continuously Flooded treatment.
2. Increased number of shoots under the Intermittently Flooded treatment.

3. Reduction of root function and belowground carbon sink capacity due to flooding would lead to decreased net carbon assimilation rates.

Objective 2 (Chapter 3): Characterize nutritional, morphological, and productivity response of *B. monnieri* to variable flooding

The objective of this study was to characterize the nutritional, morphological, and biomass allocation of *B. monnieri* undergoing a range of flooding treatments. It was hypothesized that while *B. monnieri* is adapted to survive saturation, such conditions impose a number of constraints on nutrient sequestration including phosphorus (P) and N. Based on previous studies in other species, two generalized responses were predicted: 1) Below-ground: above-ground biomass ratios, and root penetration depth will be decreased in flooded plants, with this effect being most pronounced in the treatments with longer hydroperiods, and 2) Phosphorus and N concentrations will be decreased in above-ground tissues as a result.

Objective 3 (Chapter 4): Characterize macronutrient and redoximorphic metal allocation in *L. oryzoides* under variable flooding

The objective of this study was to characterize the macronutrient and redoximorphic metal allocation in *L. oryzoides* undergoing a range of flooding intensities. It was hypothesized that:

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.

Objective 4 (Chapter 5): Characterize response of soil and water chemistry with regard to plant species and variable flooding

The purpose of this study was to compare the overall response of soil and water chemistry in mesocosms containing either *L. oryzoides* or *B. monnieri* across a range of flooding intensities. It was hypothesized that the deeper rooted *L. oryzoides* would have a greater impact on soil chemistry than *B. monnieri*, resulting in comparatively decreased water nutrient concentrations, but that this effect would be decreased under more intensive flooding due to decreased root elongation.

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Chapter 2

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

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 the remediation process; however, responses of *L. oryzoides* to flooding are undocumented. The objective of this greenhouse study was to characterize responses of *L. oryzoides* to various soil moisture regimes representative of agricultural ditches, including four treatments ranging from well-drained to saturated. Over the eight weeks of the study, plants were subjected to four flooding regimes that included a 1) well-watered, well-drained control; 2) well-watered, well-drained intermittently flooded treatment; 3) partially flooded treatment; and 4) continuously flooded treatment. The Intermittently Flooded treatment was well-watered and well-drained except on days 7, 14, 19, 28, 35, and 45, when pots were flooded to 5 cm (2 inches) above soil surface for a period of 48 hours. In the Partially Flooded treatment, water was maintained at 15 cm (5.9 inches) below soil surface. *L. oryzoides* showed decreases in photosynthesis only when soil redox potential (Eh) dropped below +350 mV. Although flooding reduced belowground:aboveground biomass ratios, overall productivity and root development was unaffected. Results indicated that management practices increasing retention time in agricultural ditches would not reduce productivity in *L. oryzoides* until soil oxygen is depleted for periods of several days to weeks.

Keywords: drainage ditch-*Leersia oryzoides*-primary productivity-variable flooding

Introduction

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

A recent survey of 36 agricultural ditches in the Mississippi and Arkansas deltas found that in small edge-of-field ditches, species richness was less than half that found in larger-sized drainages, with the most prominent species in smaller ditches belonging to the genus *Leersia* (cutgrass) (Bouldin et al., 2004). Anecdotal evidence suggests a majority of *Leersia* found in these ditches is probably *L. oryzoides* [L.] Sw (rice cutgrass), often a major component of wetland seed banks, especially those subject to sedimentation and variation in water level (Galatowitsch and van der Valk, 1996; Le Page and Keddy, 1998; Peterson and Baldwin, 2004). Colonization of ditches by *L oryzoides* may result from a combination of hydrologic regime, dredging, and dispersal ability leading to an environment where it can become quickly established in the absence of major competition.

In areas prone to flooding, water depth and persistence can have major impacts on plant species composition (Blom, 1999; Cassanova and Brock, 2000; Voesenek et al., 2004) and plant productivity (Pezeshki, 2001). Agricultural ditches are one such area. A number of plants found in agricultural ditches are obligate wetland species (Bouldin et al., 2004). However, even plants commonly found in wetland areas may be negatively affected by flooding. Flooding effectively stops diffusion of oxygen into the soil resulting in an anoxic, reduced soil environment (Gambrell et al., 1991). This transition to a reduced root environment results in a number of metabolic and morphological changes that diminish root functions (Pezeshki, 2001; Gibbs and Greenway, 2003; Greenway and Gibbs, 2003).

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

After initial reduction, partial recovery of net photosynthesis (Pn) and stomatal conductance (gs) occur in many species related to the duration and intensity of reduction (Pezeshki, 1993; Pezeshki and Anderson, 1997; Brown and Pezeshki, 2000). However, even with substantial recovery of gas exchange, plant survival and growth may be impacted (Pezeshki and Anderson, 1997), which is reflected in reduced plant biomass, particularly belowground biomass (DeLaune et al., 1983; Pezeshki, 1991; Kludze and DeLaune, 1995) and root penetration depth (Pezeshki, 1991). The threshold level of soil reduction (redox potential, Eh in mV) for these responses differs among wetland species, ranging from +300 mV to -200 mV (Pezeshki, 1991; Will et al., 1995). The upper level of this threshold corresponds roughly to the onset of oxygen disappearance from the soil, which begins to occur at Eh of +350 mV (DeLaune et al., 1990).

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

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

Like many grasses, *L. oryzoides* utilizes an extensive system of belowground rhizomes and aboveground stolons for vegetative propagation, and potentially carbohydrate storage (Darris and Bartow, 2006). While it may be expected that *L. oryzoides* would show a similar pattern in aboveground stem production to *P. arunidacea*, currently little information is available regarding how flooding affects belowground rhizome development in this species, or grasses in general. Although given the trend for plants to show an increase in the ratio of aboveground:belowground growth in response to flooding (Pezeshki, 2001), a pattern of decreased rhizome soil penetration, with increased biomass allocation to aboveground stems would be expected.

The purpose of this study was to examine responses of *L. oryzoides* to four water regimes common to drainage ditches: a well-watered, well-drained control, an intermittently flooded treatment, a partially flooded treatment, and a continuously flooded treatment. We hypothesized that carbon assimilation and allocation will be affected by flooding regimes, with a general shift toward reduction in belowground:aboveground ratios of production. We predicted three generalized responses to these flooding treatments: 1) belowground: aboveground biomass ratios, root penetration depth, and rhizome penetration depth would be decreased in flooded plants, with this effect being most pronounced in the Continuously Flooded treatment; 2) number of shoots would increase under the Intermittently Flooded treatment; and 3) reduction of root function and belowground carbon sink capacity due to flooding would lead to decreased net carbon assimilation rates.

Methods and Materials

Plants were collected from wild populations found in wetland cells at the Jamie L. Whitten Plant Materials Center in Coffeeville, Mississippi, and grown under natural light in the Life Sciences Greenhouse at the University of Memphis. Plants were grown in pots 60 cm (23.6 inches) high constructed of 15 cm 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_p horizon of the Waverly Silt Loam Series (Soil Conservation Service 1989). Although the high sand content of the sand/soil mixture used in this study is not representative of agricultural ditches in the region, the hydraulic conductivity of such soils is so low that a well-drained control was essentially impossible given the time scale of the study.

After placement in PVC pipes, plants were well-watered and well-drained for a period of three weeks prior to treatment initiation. During this time, plants were fertilized weekly with 500 mL of 20-20-20 Peter’s fertilizer mixed with tap water at 1.25g/L. The study was terminated 56 days after treatment initiation.

Soil Moisture Treatments. A completely randomized design was employed, examining stress response of *L. oryzoides* across four soil moisture regimes. Each treatment was replicated 12 times, with individual plants being treated as replicates. After treatment initiation, plants were watered daily with approximately two liters of a nutrient solution containing a maximum of 12 mg/L 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).

Treatments included a 1) well-watered, well-drained control; 2) well-watered, well-drained intermittently flooded treatment; 3) partially flooded treatment; and 4) continuously flooded treatment. Water-level was manipulated by placing pots in polyethylene bags and raising or lowering the level of the bag to the appropriate distance from the top of the soil. Control plants were well-watered and allowed to drain freely. The Intermittently Flooded treatment was well-watered and well-drained except on days 7, 14, 19, 28, 35, and 45, when pots were flooded to 5 cm above soil surface for a period of 48 hours. In the Partially Flooded treatment, water was 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. In the Continuously Flooded treatment, water was 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 hours (Figure 1). Standing water in the three flooded treatments was refreshed with nutrient solution once a week prior to the 48 hour intermittent flood. This design was intended to mimic variable hydrologic conditions on both ditch slope and trough as represented by the Partially Flooded and Continuously Flooded treatments, respectively.

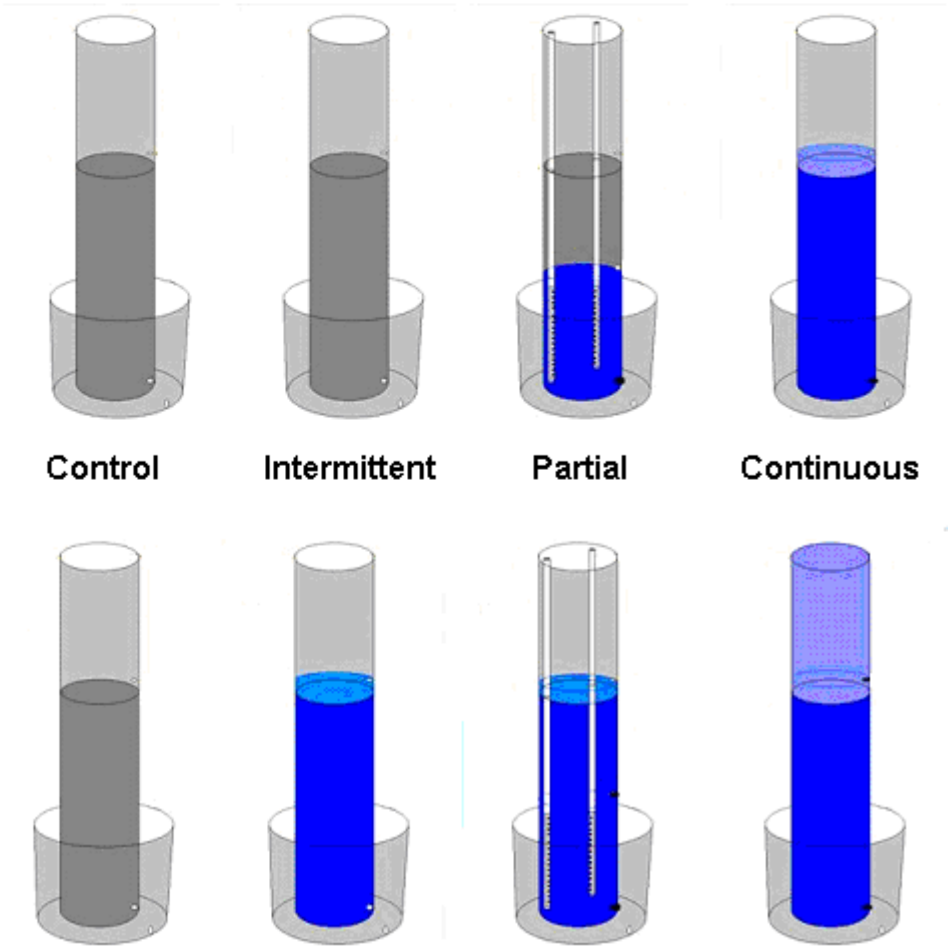

Figure 1. (A) Water level during the main portion of the study. (B) The same treatments during the 48-hour pulse flooding phase of the experiment, where water-level is raised to 5 cm above soil surface level in the 48-hour and partially flooded treatment, and to 20 cm above soil surface level in the saturated treatment.

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 cm 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.

Plant Photosynthetic Responses. Measurements of plant photosynthetic responses were made prior to treatment initiation and on days 8, 20, and 29. All photosynthetic measurements were conducted between the hours of 9:00 a.m. and 2:00 p.m. local time. Measurements of net photosynthesis (P_n) and stomatal conductance (gs) were obtained on the same eight plants per treatment, one measurement per plant, on each sampling day using a portable infrared gas analyzer (CIRAS1, PP Systems, Haverville, MA). By the third week after treatment initiation, shoots included recumbent stolons hanging outside of the pot; however, gas exchange measurements were confined to the tallest upright shoots using leaves on the second or third node from the tip. On day 30, chlorophyll fluorescence was measured using an OS-100 Modulated Fluorometer (Opti-Sciences, Tynsboro, MA). Specifically, efficiency of excitation capture of open Photosystem II (Fv/Fm) was measured following guidelines of Maxwell and Johnson (2000).

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

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

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

Statistical Analyses. Soil Eh was analyzed for four flooding treatments across five sample dates at 10 cm and 30 cm sample depths using the general linear model for ANOVA. Leaf gas-exchange measures (Pn and gs) were analyzed using a repeated measures MANOVA (Hotelling's Trace) with four levels of soil moisture. Chlorophyll fluorescence (Fv/Fm) and all final measures of plant growth were analyzed using the general linear model for ANOVA. Because of non-normality, number of shoots was log transformed prior to data analyses. All statistical analyses were made using SPSS 14.0.

Results

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

Soil Eh. Prior to treatment initiation all treatments were well aerated; mean soil Eh across all treatments were +549 mV +/- 76 and +573 mV +/- 29 mV at 10 cm and 30 cm, respectively. Immediately following treatment initiation, flooded treatments showed a significant decrease in soil Eh ($p = 0.006$). In general, soil Eh continued to decrease in flooded treatments throughout the study. By day 30 soil Eh was near or below the critical oxygen threshold at 10 cm depth and 30 cm depth across all flooded treatments (Figure 2). With the exception of the Continuously Flooded treatment, which had mean soil Eh values near or below +350 mV after day nine of the study (Table 1), Eh differences between treatments prior to day 30 may be largely disregarded, as even large changes in soil Eh have little effect on soil chemistry or wetland plant response if oxygen is readily available (Pezeshki, 2001).

An overall treatment effect was demonstrated across sampling depths and sampling times (Table 2). However, there was a significant interaction for sampling date and treatment, as well as depth and treatment for soil Eh. Further analysis showed the former interaction to be a general decline in soil Eh for all flooded treatments, while the control maintained a mean Eh of +580 mV +/- 69 mV at 10 cm depth and +556 mV +/- 93 at 30 cm depth throughout the study (Table 1). The latter interaction resulted from treatment differences between 10 cm sample depth and 30 cm sample depths. While Eh was generally lower at 30 cm than 10 cm in all treatments, these differences were not

Table 1. Summary of results, giving mean values and standard deviation for each sampling date, as well as treatment effects for each parameter.

Treatments

		Control	Intermittent		Partial		Continuous	
Measures	Day	mean +/- s.d.	mean +/- s.d.	effect	mean +/- s.d.	effect	mean +/- s.d.	effect
Pn (umol m-2 s-1)	0	15.5 +/- 1.6	15.1 +/- 1.2	0	15.3 +/- 1.8	0	15.5 +/- 2.0	0
gs (mmol m-2 s-1)	0	208.2 +/- 42.0	212.1 +/- 29.1	0	222.2 +/- 41.6	0	216.8 +/- 39.1	0
Eh (mV) 10 cm	0	571.6 +/- 76.0	560.5 +/- 77.6	0	562.5 +/- 67.9	0	497.9 +/- 75.8	0
30 cm	0	572.9 +/- 37.8	576.3 +/- 19.6	0	576.9 +/- 38.9	0	569.4 +/- 20.3	0
Eh (mV) 10 cm	1	556.0 +/- 77.8	435.6 +/- 142.3	0	501.2 +/- 120.1	0	455.5 +/- 130.5	0
30 cm	1	568.1 +/- 54.8	402.4 +/- 126.2	0	465.1+/- 109.4	0	434.4 +/- 137.7	0
Pn (umol m-2 s-1)	8	14.2 +/- 2.9	14.0 +/- 2.3	0	12.4 +/- 2.0	0	14.6 +/- 2.8	0
gs (mmol m-2 s-1)	8	258.0 +/- 67.4	262.2 +/- 43.4	0	236.5 +/- 46.2	0	253.8 +/- 51.6	0
Eh (mV) 10 cm	9	589.6 +/- 67.9	554.4 +/- 139.0	0	348.3 +/- 101.0	0	272.1 +/- 208.6	0
30 cm	9	579.7 +/- 69.6	442.0 +/- 159.8	0	250.3 +/- 165.2	0	207.0 +/- 210.5	
Eh (mV) 10 cm	16	588.7 +/- 69.1	532.4 +/- 86.3	0	557.0 +/- 94.8	0	329.3 +/- 130.0	0
30 cm	16	528.3 +/- 157.5	379 +/- 184.9	0	260.1 +/- 103.4	-	262.6 +/- 267.6	0
Pn (umol m-2 s-1)	20	7.4 +/- 3.5	7.6 +/- 3.2	0	8.4 +/- 2.6	0	7.0 +/- 1.3	0
gs (mmol m-2 s-1)	20	139.6 +/- 62.7	141.6 +/- 52.2	0	155.4 +/- 51.4	0	141.2 +/- 48.9	0
Pn (umol m-2 s-1)	29	9.7 +/- 1.3	7.1 +/- 1.0	-	6.5 +/- 2.2	-	7.3 +/- 1.8	-
gs (mmol m-2 s-1)	29	198.3 +/- 25.0	149.8 +/- 23.5	0	133.8 +/- 38.3	-	160.5 +/- 43.6	0

Table 1 (continued). Summary of results, giving mean values and standard deviation for each sampling date, as well as treatment effects for each parameter.

Treatments

		Control	Intermittent		Partial		Continuous	
Measures	Day	mean +/- s.d.	mean +/- s.d.	effect	mean +/- s.d.	effect	mean +/- s.d.	effect
Eh (mV) 10 cm	30	599.8 +/- 63.6	235.7 +/- 66.6	-	212.6 +/- 129.2	-	272.3 +/- 56.8	-
30 cm	30	522.8 +/- 84.4	136.1 +/- 157.2	-	142.0 +/- 205.3	-	96.3 +/- 112.9	-
Fv/Fm *Day 30*	30	0.748 +/- 0.081	0.737 +/- 0.033	0	0.752 +/- 0.061	0	0.723 +/- 0.069	0
above mass (g)	56	33.8 +/- 13.0	37.4 +/- 8.8	0	35.9 +/- 7.8	0	47.2 +/- 12.3	+
below mass (g)	56	24.8 +/- 8.6	22.1 +/- 6.3	0	20.4 +/- 8.3	0	20.4 +/- 5.5	0
number of shoots	56	48.3 +/- 18.8	53.7 +/- 20.8	0*	48.2 +/- 15.3	0*	65.1 +/- 11.2	0*
shoot length (cm)	56	142.1 +/- 24.3	154.9 +/- 22.1	0	147.9 +/- 23.2	0	143.1 +/- 21.6	0
root depth (cm)	56	47.3 +/- 6.5	46.7 +/- 4.9	0	45.5 +/- 6.6	0	45.8 +/- 4.3	0
rhizome depth (cm)	56	44.8 +/- 6.2	41.7 +/- 6.9	0	43.8 +/- 4.5	0	36.3 +/- 5.5	-

Table 2. ANOVA table showing the main effects and interactions on Eh.

Source	df	F	Sig.
Flooding treatment	3	46.19	0
depth of sample	1	19.347	0
sampling day	4	43.279	0
flooding treatment * depth	3	1.115	0.343
flooding treatment * sampling day	12	7.16	0
sampling depth * sampling day	4	4.412	0.002
flooding treatment * sampling depth * sampling day	12	0.819	0.631
Error	260		
Total	300		
Corrected Total	299		

Table 3. Relative growth rate for *Leersia oryzoides* under various soil moisture regimes: Control; Intermittent Flooding, Partial Flooding, and Continuous Flooding.

	MASS (Δ g^{-1} g^{-1} week)		LENGTH (Δ cm cm^{-1} $week^{-1}$)	
	Aboveground	Belowground	Shoot	Root
Control	0.207	0.281	0.093	-0.018
Intermittent	0.226	0.268	0.104	-0.019
Partial	0.221	0.257	0.098	-0.023
Continuous	0.255	0.259	0.095	-0.022

biologically relevant as soils were either aerated at both depths or anoxic at both depths. Additionally, due to the lack of precision associated with soil redox measures, and the fact that only single electrodes were used within each replicate, any generalizations of these data should be used with caution.

Plant Gas Exchange. A significant time x treatment interaction was found for Pn and gs ($p = 0.015$, $F = 1.953$). Thus, individual sample days were analyzed using the General Linear Model MANOVA, with days 1, 8, and 20 showing no significant treatment effect (Table 1). Only on day 29 did gas exchange show any response to flooding ($p = 0.04$, $F = 5.666$). Pairwise comparisons made using Tukey's HSD found that Pn values for all flooded treatments differed significantly from the control, while flooded treatments did not differ significantly from each other (Figure 3). With regard to gs (Figure 4), a treatment effect was observed ($p=0.015$, $F = 4.117$), with only Partially Flooded plants showing a significant response ($p = 0.011$), while Intermittently Flooded plants showed a marginal response ($p = 0.076$). Because gas exchange measures such as gs and Pn reveal short term stress effects related to carbon fixation, these data suggest that flooding would negatively affect productivity only during times of moderate to severe soil reduction ($Eh < +350$ mV). No difference was found in Fv/Fm on day 30 (mean $Fv/Fm = 0.7402$, $p = 0.695$), implying rate-limiting mechanisms of Photosystem II were not affected by flooding conditions imposed in this study.

Interestingly, although Pn was eventually decreased in all flooded treatments, apparently in response to soil anoxia, the Continuously Flooded treatment showed no decrease in Pn on days 8 and 20, in spite of having mean Eh values at 10 cm of 272 +/-

208 mV on day 9 and 329 +/- 130 mV on day 16 (Table 1). While the large variance in Eh and lack of specific soil data make any generalizations of soil chemistry difficult, it is possible that factors other than oxygen depletion, such as reduction of nitrate, are responsible for the observed decreased in Pn.

Plant Morphology. There were no differences in shoot length among treatments (p = 0.459; Table 1). A significant treatment effect on number of shoots was found in response to flooding showing a general effect of flooding on shoot number (p = 0.043, F = 2.959), with the Continuously Flooded treatment having the most shoots, followed by Intermittently Flooded, Control, and Partially Flooded (see Table 1). The Continuously Flooded treatment had an average of 17 more shoots per plant; however, a post-hoc analysis comparing the Continuously Flooded treatment to the Control found only marginal differences (p = 0.056).

Rhizome penetration depth showed a significant treatment effect (p = 0.021, F = 3.715; Table 1), with Continuously Flooded plants having significantly shallower rhizomes than Control plants (p = 0.017) and marginally shallower rhizomes than Partially Flooded plants (p = 0.057). Root penetration depth was not affected by flooding (p = 0.858; Table 1). A comparison of root depth and rhizome penetration depth is shown in Figure 5. Because rhizomes are belowground stems, it is interesting to note that the Continuously Flooded treatment, which showed decreased belowground stem development also showed the greatest number of aboveground stems, indicating a trend towards aboveground development even under only moderate soil reduction.

A significant treatment effect ($p < 0.001$) was observed in root:shoot biomass ratios demonstrating a typical wetland plant response to flooding by allocating carbon preferentially aboveground rather than belowground ($F = 7.86$, $p < 0.001$). Tukey's HSD showed no differences in aboveground:belowground ratios among flooded treatments, while all differed significantly from controls (Intermittently Flooded: $p = 0.041$, Partially Flooded: $p < 0.001$, Continuously Flooded: $p = 0.017$). Further analyses revealed treatment differences lie in increased aboveground mass for flooded treatments ($p = 0.024$, $F = 3.501$), rather than reduced belowground mass, with only Continuously Flooded plants differing significantly from Control ($p = 0.022$; Figure 6, Table 1). Therefore, although reduction of soil Eh caused a measurable decrease in Pn, this decrease was not reflected in total productivity. Specifically, although the soil in Continuously Flooded treatment was moderately reduced throughout much of the study, and Pn decreased near the end of the study, aboveground biomass was 23% greater in the Continuously Flooded treatment than in the Control.

Statistical analysis of relative growth rate (Δ g^{-1} g^{-1} week, Δ cm cm^{-1} $week^{-1}$) yielded values comparable to the analysis of measured values (cm, g). Mean root penetration depth actually decreased over the course of the experiment for all treatments. Calculated mean values for relative growth rate of belowground and aboveground biomass, as well as root penetration depth and shoot length are recorded in Table 3.

Discussion

Photosynthesis in *L. oryzoides* decreased under flooded conditions when soil was reduced below the critical oxygen threshold (Eh = +350 mV; Table 1, Figures 2, 3, and

4). However, mechanisms for this decrease may differ depending upon whether flooding is continuous, periodic or partial. Although all flooded treatments eventually showed decreases in Pn (Table 1, Figure 2), plants in the Continuously Flooded treatment showed no difference in gs (Figure 3), implying that photosynthetic rates may be limited by non-stomatal (metabolic) factors in this species when repeatedly exposed to extended periods of flooding. This non-stomatal limitation is not reflected in PSII activity, as demonstrated by Fv/Fm data (Table 1). Because Fv/Fm represents the capacity of Photosystem II, rather than actual energy absorbed, it is indicative of photochemical processes that are regulated over a longer time period than gas exchange measures such as Pn (Maxwell and Johnson, 2000). Comparisons of Fv/Fm data to gas exchange data indicated that while soil Eh below the critical oxygen threshold results in a short-term stress response, damage to the photosynthetic apparatus for this species is negligible under moderate soil reduction (Table 1).

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

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

Root penetration depth was unaffected by flooding (Figure 5). In agricultural areas erosion has become a major environmental problem linked not only to localized ecosystem changes, but potentially to downstream turbidity and eutrophication (Lal, 1998). Vegetation establishment has been recommended as an effective method for reducing erosion in agricultural areas (Lovell and Sullivan, 2006), and adjacent riparian areas (Shields et al., 1995). The fact that moderate soil reduction had no effect on root penetration and biomass in *L. oryzoides*, when taken in consideration with the dense, fast growing colonies formed by this species (Darris and Bartow, 2006), suggest that it may serve a valuable role in stabilizing soils in and adjacent to agricultural ditches.

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

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

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

Summary and Conclusions

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

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

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Table 1. Summary of results, giving mean values and standard deviation for each sampling date, as well as treatment effects for each parameter. For treatment effects, + indicates an increase compared with control, - indicates a decrease compared with control, and 0 indicates no significant difference between treatment and control. Treatment effects for Eh values were not considered significant on the basis of statistical analysis alone. Rather, their significance was determined on the basis of probable changes in bioavailable oxygen or other pertinent soil chemistry parameters as estimated by soil Eh. Although an overall treatment effect on number of shoots was observed, pairwise comparisons showed no statistical differences between individual treatments at $\alpha = 0.05$.

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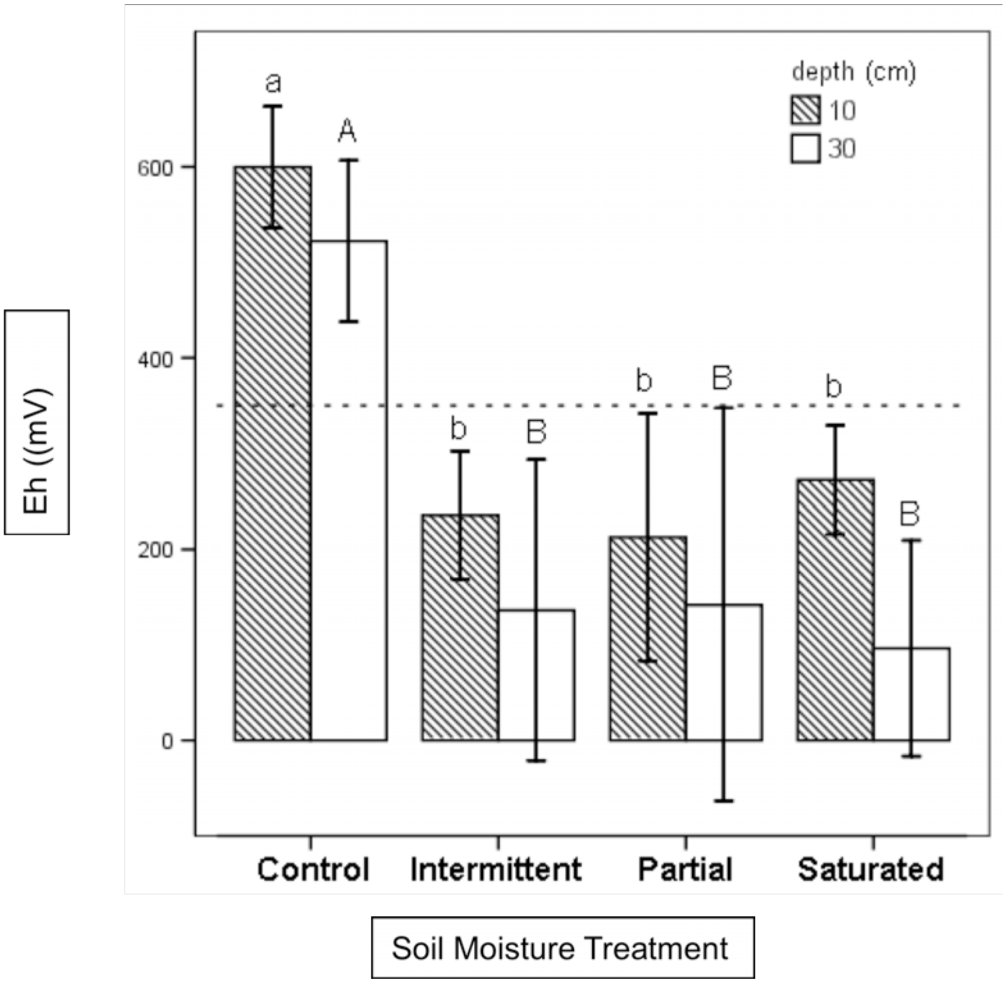
Figure 3. Mean (± s.d.) net photosynthesis (Pn) of *L. oryzoides* across various soil moisture regimes on day 29, showing a significant difference between the Control and all flooded treatments. Significant differences are indicated by different letters.

Figure 4. Mean (± s.d.) stomatal conductance (gs) of *L. oryzoides* across various soil moisture regimes on day 29, demonstrating a difference between the Control and the Partially Flooded treatment. Significant differences are indicated by different letters.

Figure 5. Mean (± s.d.) soil penetration depth of longest root and longest rhizome in *L. oryzoides* across various moisture regimes, demonstrating a flood response for the rhizomes under Continuous Flooding. No comparable response was seen in roots. Significant differences in rhizome penetration across soil moisture treatments are indicated by different letters.

Figure 6. Mean (± s.d.) biomass of *L. oryzoides* across various moisture regimes, demonstrating a flood response for aboveground biomass under Continuous Flooding. No comparable response was seen in belowground biomass. Significant differences for each biomass component across treatments are indicated by different letters.



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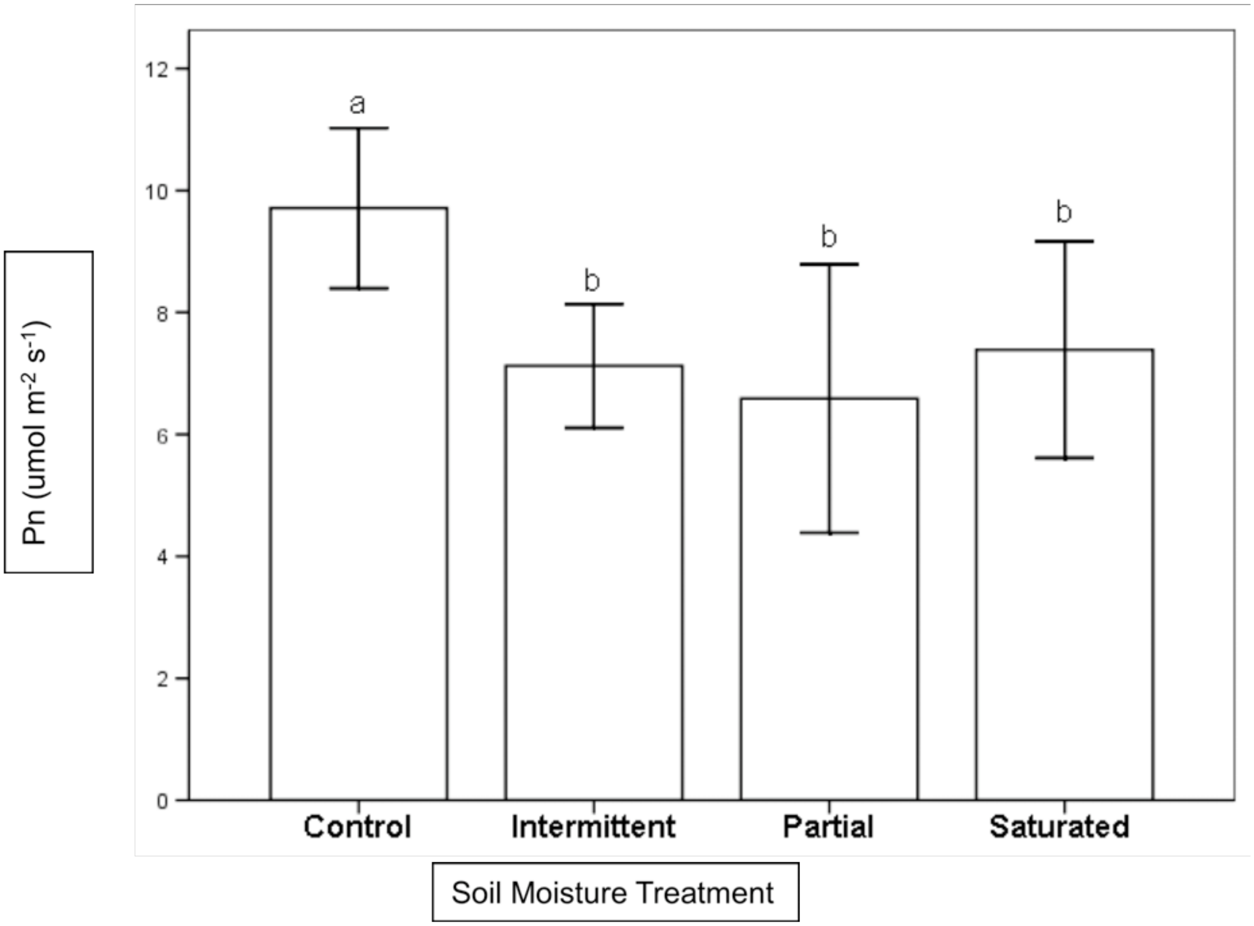

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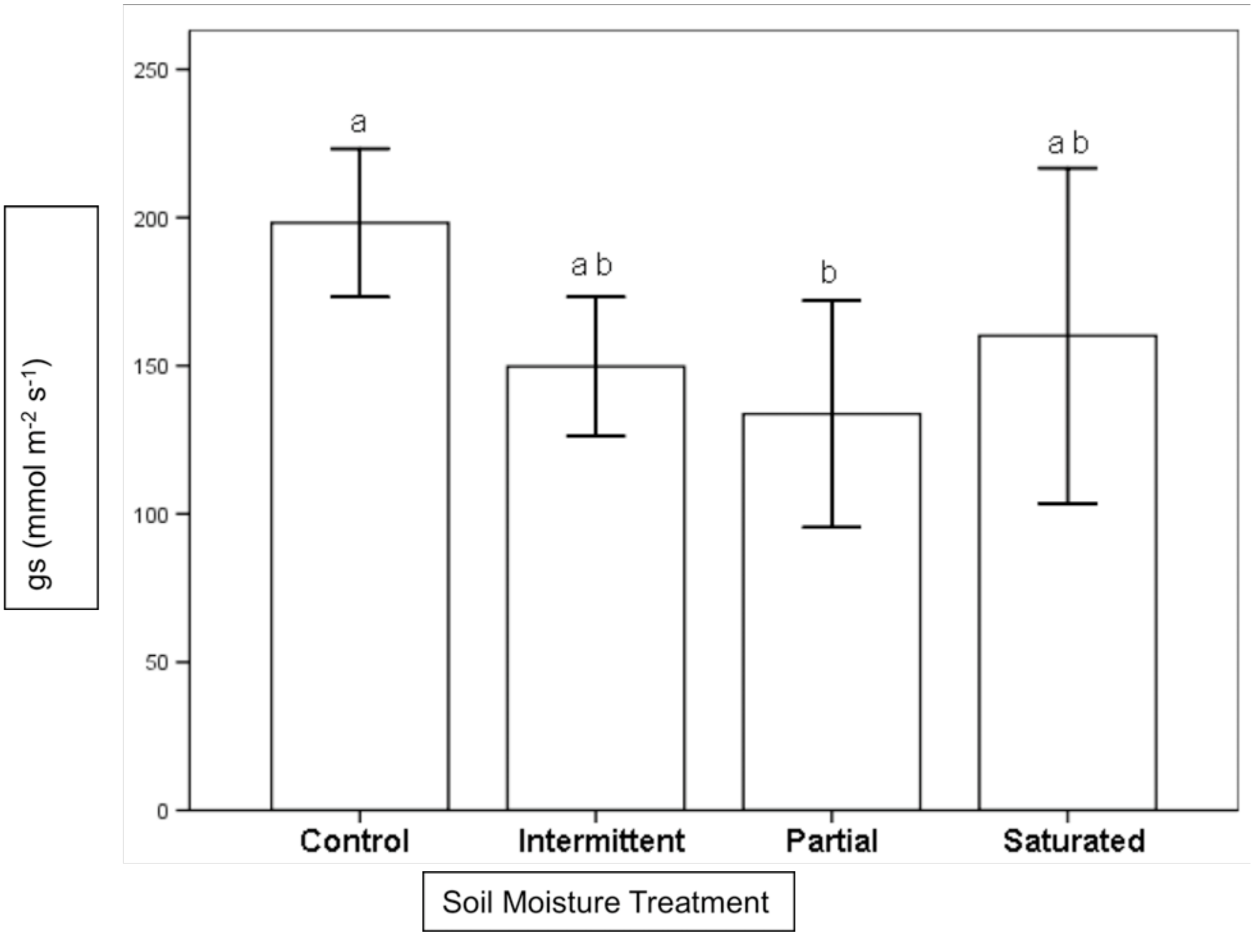

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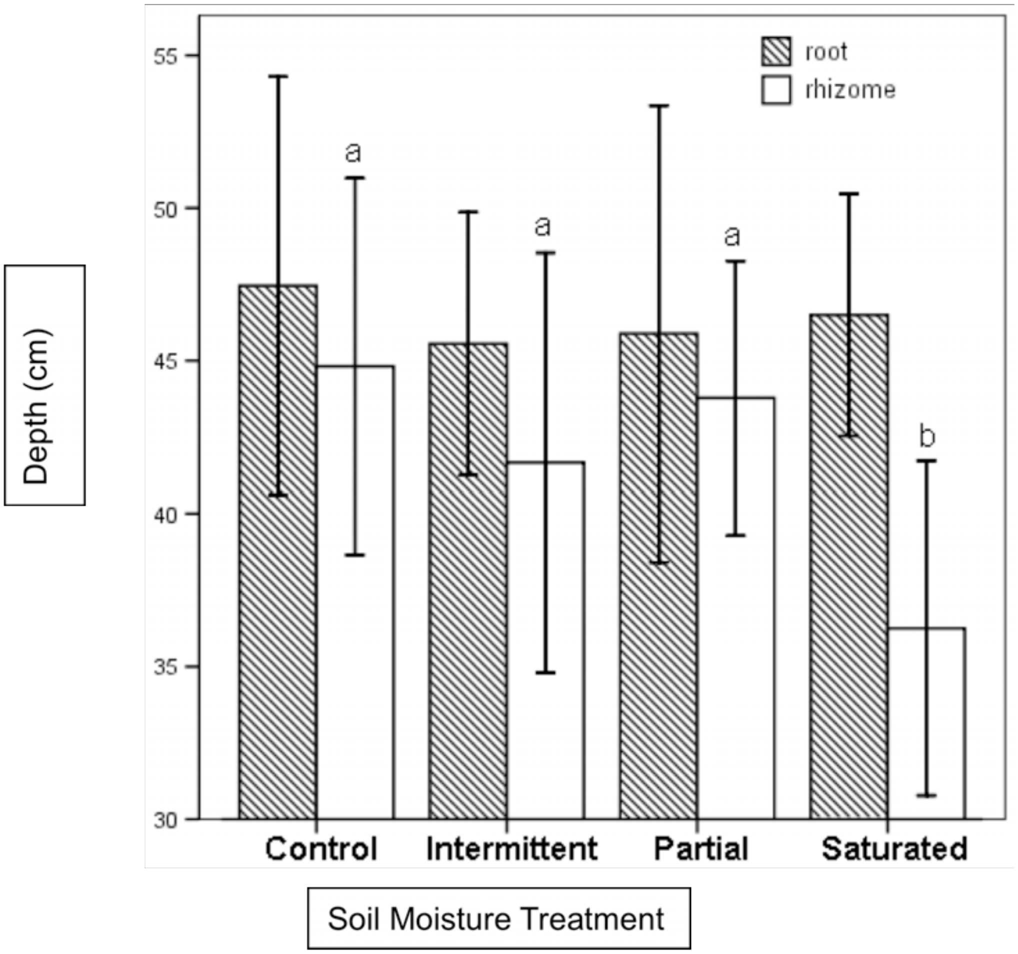

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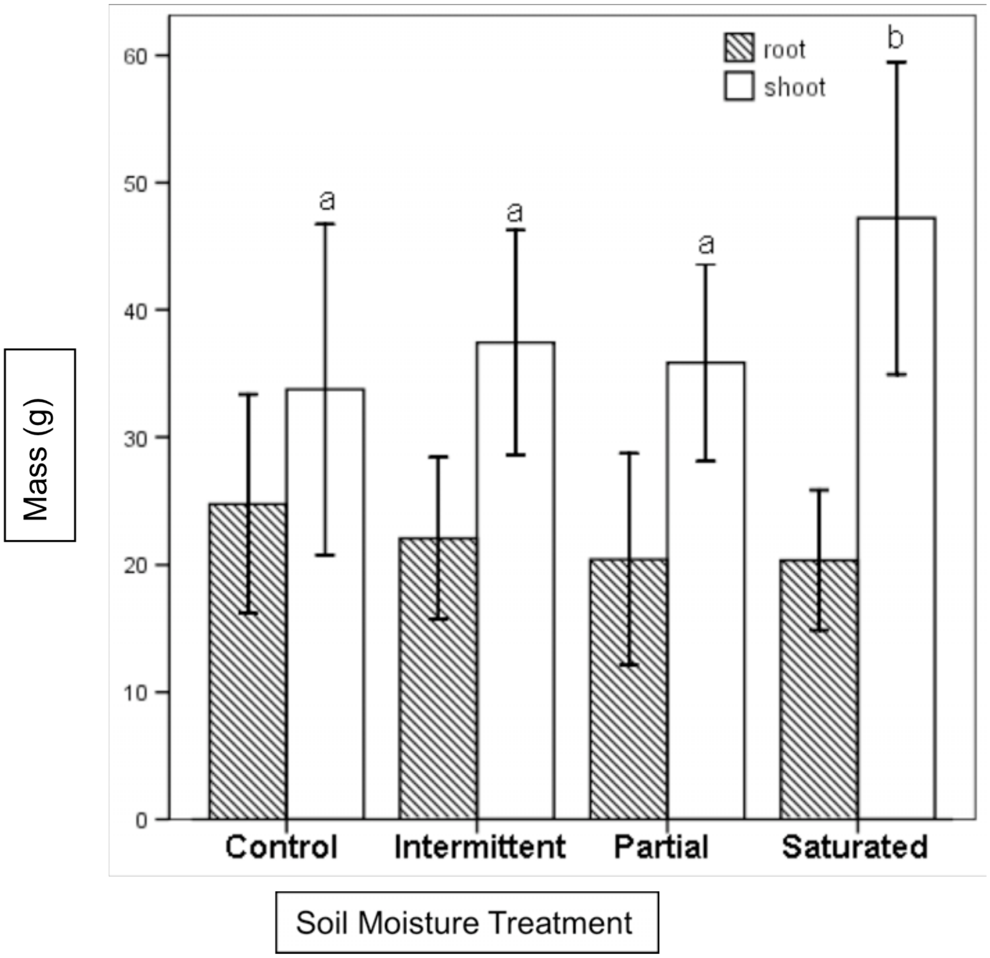

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Chapter 3: Nutrient Response of *Bacopa monnieri* (Water Hyssop) to Varying Degrees of Soil Saturation

ABSTRACT

Tissue concentrations of N and P were measured in *Bacopa monnieri* subjected to four progressive levels of flooding: well-drained Control, Intermittently Flooded, Partially Flooded, and Continuously Flooded. Soil redox potential (Eh), measured at two levels in the mesocosms decreased under flooding. Flooding increased biomass and decreased root growth and Nitrogen (N) and Phosphorus (P) concentrations in shoots, with the decreases being most pronounced in the Partially Flooded and Continuously Flooded treatments. The decreased uptake of N and P under flooding underscores the need to better understand how wetland plants function in nutrient-rich environments subjected to variable flooding. Additionally, the apparent decreased translocation of N and P from the root to the shoot in flooding conditions may be indicative of an overall decrease in mineral transport, which would have implications for the design and management of remediation systems.

Keywords: nitrogen, phosphorus, nutrient uptake, environmental stresses, water quality

INTRODUCTION

When the land surface is inundated with stagnant water, oxygen diffusion into the soil is limited, resulting in a series of step-wise chemical reductions. Trends in oxidation/reduction reactions may be estimated by soil redox potential (Eh), with lower

Eh designating more reducing conditions. Under standard conditions (25˚C and pH = 7) bioavailable oxygen is depleted at Eh = +350 mV. Soil microorganisms then utilize alternate electron acceptors, such as nitrate, manganese, and iron, resulting in further soil reduction (Ponnamperuma, 1984; Gambrell and Patrick, 1978). These changes can decrease the bioavailability of some plant nutrients, such as Nitrogen (N), while increasing others such as iron to detrimental levels (Farmer et al., 2005).

Terrestrial plants subjected to soil reduction must undergo a number of metabolic and morphological changes to acclimate to anoxia and the associated toxins. Initially, metabolic responses in the root system result in diminished root function (Gibbs and Greenway, 2003; Greenway and Gibbs, 2003), followed by decreased leaf gas exchange (Pezeshki 2001). The combination of decreased carbon assimilation and a short-term increase in respiration, often compounded by productions of toxins in reduced soils, results in mortality of flood-sensitive plants. Flood-insensitive plants respond to root anoxia by forming semi-continuous gas conduits in the cortex (lacunae), allowing diffusion of oxygen from the shoot into the rhizosphere (as reviewed by Armstrong and Drew, 2002; Pezeshki, 2001; Evans 2003), somewhat ameliorating the effects of flooding. Under persistent reducing conditions, however, even wetland plants may show long-term stress responses, such as decreased growth and nutrient uptake, and damage to photosynthetic systems (Pezeshki, 2001).

Bacopa monnieri L. has a pan-tropical distribution and is a common species in wetlands near the Gulf of Mexico, forming a common component of marsh communities in areas that are sporadically subjected to flooding and salt stress during storm events (Visser et al., 1998). Hurricane Camille, which made landfall near the mouth of the

Mississippi River in August of 1969, devastated a number of marsh species; however *B. monnieri* was largely unaffected (Chabreck and Palmisano, 1973). *B. monnieri* seeds are not highly viable (Tiwari et al., 2001); the plant requires bare ground to become established (Shah, 1965 from Tiwari 2001). If nutrients are adequate, *B. monnieri* can quickly colonize bare marsh soils, becoming a major component of the flora (Dalrymple et al., 2003), even in waters subjected to agricultural and industrial pollution (Gupta, 2003).

B. monnieri is considered a metal hyperaccumulator, sequestering copper and zinc in fairly high concentrations (Owens et al., 1989), as well as demonstrating potential for remediation of mercury (Sinha et al., 1996), cadmium and chromium (Rai et al., 1995; Shukla and Rai, 2007) and manganese (Sinha, 1999). Use of *B. monnieri* as a memory enhancer in traditional Indian medicine has resulted in a number of studies investigating potential active ingredients related to improved cognitive function (e.g., Deepak and Amit, 2004; Nathan et al., 2004), which has in turn sparked interest in methods for laboratory production of *B. monnieri* (Tiwari et al., 2001; Shrivastava and Rajani, 1999).

Few published studies examine the basic physiological and morphological responses of this species to common environmental stresses (but see Sinha and Saxena, 2006). The same traits that allow *B. monnieri* to cope with periodic disturbance and pollution make it a potential candidate for introduction into wetland systems that have been highly degraded, or into constructed wetland systems designed to remove pollutants. This species is an especially attractive candidate for phytoremediation and wastewater treatment, as it is already widespread throughout developing nations in tropical and subtropical regions, where the extended growing season makes such technologies practical.

We hypothesized that while *B. monnieri* is adapted to survive saturation, such conditions impose a number of constraints on nutrient sequestration including Phosphorus (P) and N. The objective of this study is to examine the physiological responses of *B. monnieri* to different hydrologic regimes as an initial effort to gauge its efficacy for remediation in wetlands.

Based on previous studies in other species, two generalized responses were predicted: 1) Below-ground:above-ground biomass ratios, and root penetration depth will be decreased in flooded plants, with this effect being most pronounced in the treatments with longer hydroperiods; and 2) P and N concentrations will be decreased in above-ground tissues as a result.

METHODS

Experimental design followed procedures previously described in Pierce et al., (2007). Plants were collected from wild populations found in wetland cells at the Jamie L. Whitten Plant Materials Center in Coffeeville, Mississippi, and grown under natural light in the Life Sciences Greenhouse at the University of Memphis. Plants were grown in mesocosms 60 cm high constructed of 15 cm 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 Ap horizon of the Waverly Silt Loam Series (Soil Conservation Service, 1989). Although the high sand content of the sand/soil mixture used in this study is not representative of the soils most commonly associated with *B. monnieri*, the hydraulic conductivity of such soils is so low that achieving a well-drained control was essentially impossible given the hydroperiods under study.

After placement in PVC pipes, plants were watered and drained for a period of three weeks prior to initiation of treatments. During this time, plants were fertilized weekly with 500 mL of 20-20-20 Peter's Professional® fertilizer (N content: 3.94% ammonical (NH_4^+) N, 6.05% nitrate (NO_3^-) N, 10.01% urea N) mixed with tap water at 1.25g/L. The pH of mesocosm effluent was measured periodically throughout the study (mean pH = 7.6 +/- 0.33). The study was terminated 56 days after treatment initiation.

Soil Moisture Treatments

Water-level was manipulated by placing mesocosms in polyethylene bags and raising or lowering the level of the bag to the appropriate distance from the top of the soil. Individual treatments were:

1. A well-watered Control allowed to drain freely. In the Control mesocosm effluent was recycled daily.
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 hours.
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 mesocosms were flooded with an additional 15 cm of water for 48 hours (Figure 1). Each treatment was replicated 12 times in a completely randomized design, with individual plants being treated as replicates. Any excess water was held in overflow buckets and used to maintain water conditions as described above. Once a week prior to the 48-hour intermittent flooding event, standing water in the three flooded treatments was drained overnight and all treatments were refreshed with seven liters of nutrient solution. The nutrient solution contained 12 mg/L ammonium nitrate and 5 mg/L sodium phosphate. These concentrations fall within the range of concentrations expected for surface flows downstream of agriculture and primary wastewater facilities (Kleinman et al., 2007; Schmidt et al., 2007; Bouldin et al., 2004; Peterson and Teal, 1996;).

Soil Redox Potential

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 cm 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.

Tissue Nutrient Analysis

Ten randomly-chosen plants per treatment were analyzed for nutrient analysis. Plants were divided into above-ground and below-ground portions, air dried for two weeks, then weighed and ground in a Wiley Mill and passed through a 1 mm diameter mesh. Samples were then frozen until further processing. Subsamples of approximately

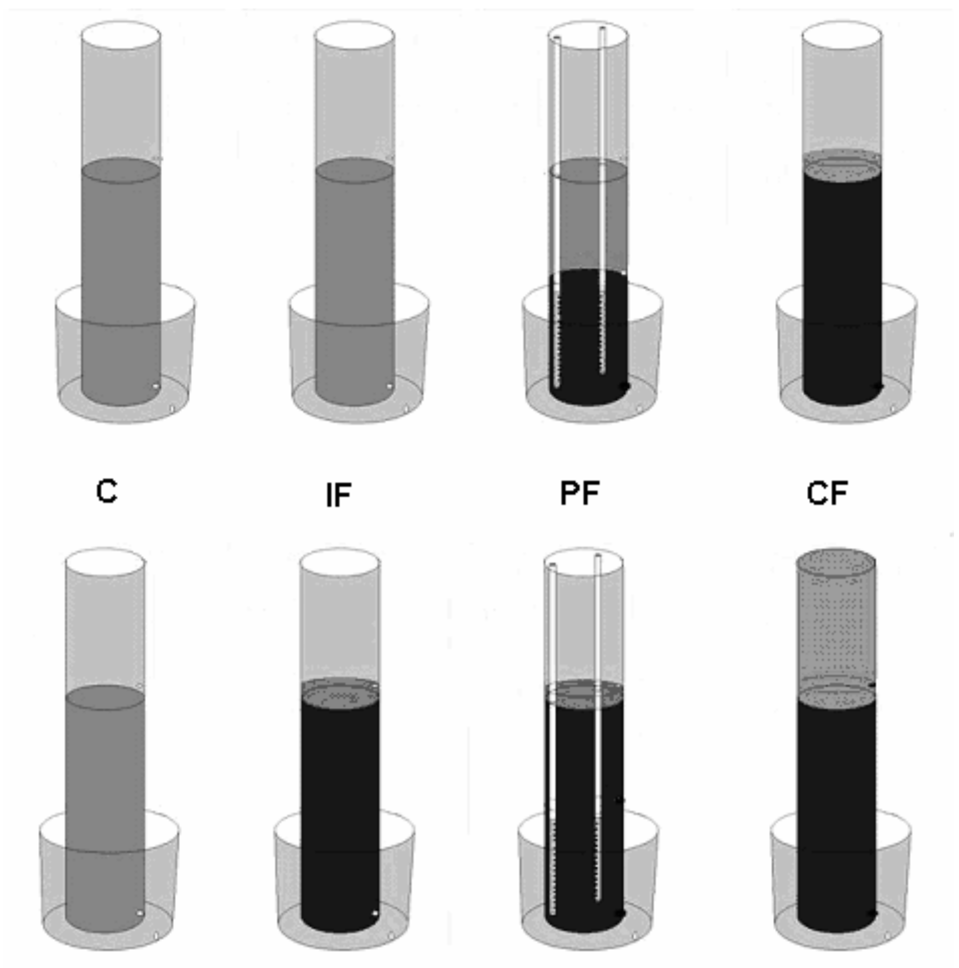

Figure 1. The top row of illustrations show water level during the main portion of the study. The bottom row of illustrations shows the same treatments during the 48-hour pulse flooding phase of the experiment, where water-level is raised to 5 cm above soil surface level in the 48-hour and partially flooded treatment, and to 20 cm above soil surface level in the saturated treatment (from Pierce et al., 2007).

0.2 g were digested according to Quikchem Method 10-107-06-2-E (detection limit 0.018 mg/L) for Total Kjeldahl Nitrogen as described by Wendt (1997) and QuikChem Method 10-115-01-1-C (detection limit 0.015 mg/L) for total phosphorus (Lachat Instruments, 1995). The Kjeldahl digestion process stabilizes ammonium nitrogen and converts organic nitrogen into ammonium. Nitrate is not converted into ammonium. The phosphate digestion process converts organic and inorganic phosphorus to orthophosphate. Ammonium and orthophosphate were subsequently quantified by colorimetry using Lachat Instruments Quickchem FIA+ 8000 series Colorimeter.

Plant Growth and Nutrient Uptake Estimates

Immediately prior to treatment initiation, 10 plants were harvested and shoot and root lengths were measured. Plants were separated into above-ground and below-ground portions and dried in a glasshouse for two weeks, then weighed to determine biomass allocation. N and P were measured in eight randomly-chosen plants to determine initial nutrient concentrations. Initial nutrient allocation was estimated as the product of the respective biomass and concentration for each plant. The same procedures were utilized at experiment termination to compare differences in growth and nutrient allocation before and after flooding. The initial nutrient allocation values were subtracted from the final nutrient allocation values to calculate total plant nutrient uptake.

Data Analysis

Unless otherwise indicated, statistical analyses used the general linear model for ANOVA using SPSS 14, with four levels of water regime as independent fixed factors. Pairwise comparisons were made with Tukey's HSD. Eh was analyzed using a factorial time x treatment MANOVA, with Eh values at 10 cm depth and 30 cm depth analyzed as correlated dependent variables. Due to correlation between N and P concentrations, these factors were incorporated into a single MANOVA for initial analysis, followed by univariate analysis as described above. Although N and P concentrations in the shoots were also correlated with shoot biomass, it was analyzed separately due to a larger sample size.

RESULTS

Soil Redox Potential (Eh)

Initially, Eh values were all in the range expected for aerated soils with mean at Eh = 550 +/- 73 and 585 mV +/- 56 at 10 cm and 30 cm depths, respectively. The Control treatment remained aerated throughout the study, whereas soil Eh in flooded treatments declined in response to flooding (seen as a Time X Treatment interaction, Table 1). Although soil Eh dropped in response to flooding, this response was somewhat attenuated at 30 cm depth with values stabilizing near the critical oxygen threshold (Eh = +350 mV). The Continuously Flooded treatment was fairly stable at 10 cm depth, with mean Eh values slightly higher than at 30 cm. Intermittently Flooded and Partially Flooded treatments had more variable soil Eh at 10 cm than 30 cm depth. By day 30, all flooded treatments were anoxic at 30 cm depth and Partially Flooded and Continuously Flooded treatments were anoxic at 10 cm depth (Figures 2, 3).

Plant Morphology and Biomass

Mean shoot length varied from 102.6 +/- 11.34 cm in the Intermittently Flooded treatment to 89.6 +/- 11.1 cm in the Continuously Flooded treatment, but no significant differences were observed among treatments ($p = 0.099$; $F = 2.22$, $N = 48$).

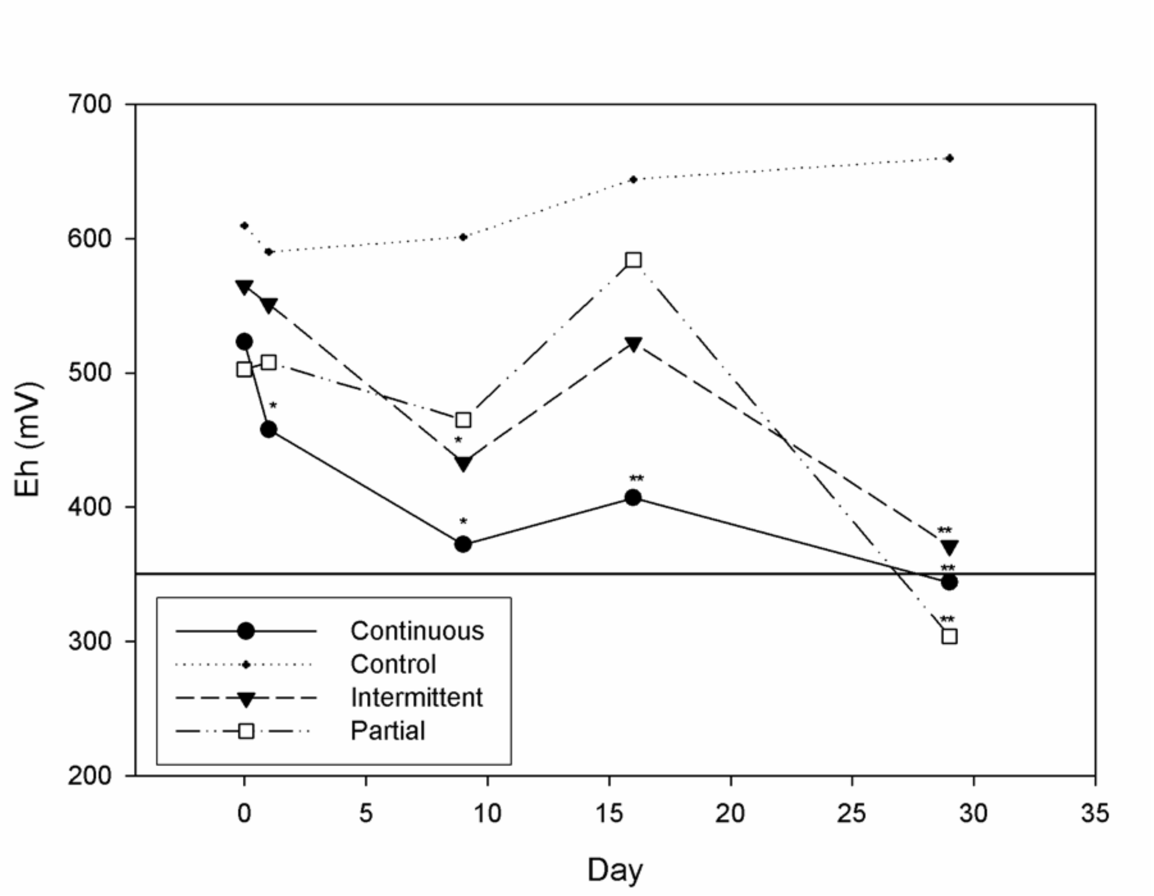

Figure 2. Soil Eh at 10 cm depth, demonstrating the variability in Eh near the soil surface. By day 30 flooded treatments were approaching Eh values that indicated soil hypoxia/anoxia. The reference line at Eh = +350 mV indicates the threshold for aerobic respiration. One asterisk indicates significant difference from the Control for a give sample date ($p < 0.05$); while two asterisks indicates a highly significant difference ($p < 0.01$).

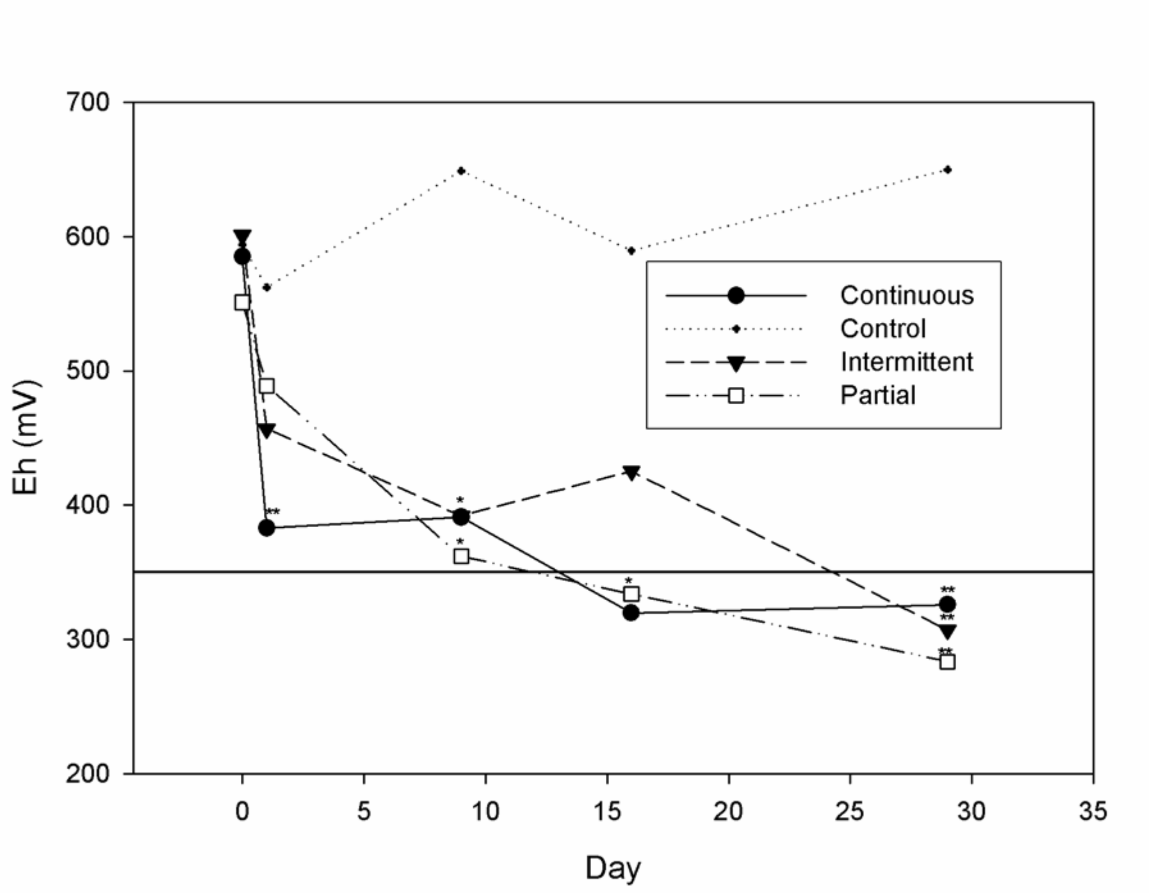

Figure 3. Soil Eh at 30 cm depth, demonstrating immediate decreases in Eh at depth, with soil reduction being somewhat attenuated over time. The reference line at Eh = +350 mV indicates the threshold for aerobic respiration. One asterisk indicates significant difference from the Control for a give sample date ($p < 0.05$); while two asterisks indicates a highly significant difference ($p < 0.01$).

Root penetration depth decreased in the Partially Flooded and Continuously Flooded treatment with final penetration depth values lower than Control ($p = 0.001$ and $p < 0.001$, respectively) and Intermittently Flooded ($p = 0.006$ and $p = 0.001$, respectively; Figure 4).

Neither root biomass nor shoot biomass was significantly affected by flooding. Although root biomass increased over the duration of the study from an initial mean estimate of 1.46 +/- 2.41 (g) to a final mean value of 2.78 +/- 1.51 (g) across treatments, this difference was marginal in comparison to shoot biomass. Shoot biomass increased significantly over the duration of the study ($p < 0.001$, $F = 2.738$), with an initial mean value of 6.10 +/- 2.91 (g), compared to a final mean value of 34.2 +/- 8.9 (g).

Plant nutrients

Mean root nutrient concentrations for N and P were 8.08 +/- 4.2 mg/g and 2.6 +/- 1.4 mg/g, respectively. They were unaffected by flooding treatments, owing to high variance within treatments. Shoot concentrations of N and P were negatively correlated with shoot mass (Pearson correlation: $r^2 = -0.366$, $N = 40$, $p = 0.02$ and $r^2 = -0.365$, $N = 40$, $p = 0.021$). Additionally, shoot concentrations of P and N were correlated with each other (Pearson correlation: $r^2 = 0.612$, $p < 0.001$, $N = 40$).

Analyzing these two variables with MANOVA resulted in a highly significant treatment effect (Hotellings trace $p = 0.006$, $F = 3.31$, $N = 40$). Shoot concentrations of N and P showed decreases in response to flooding ($p = 0.006$, $F = 4.908$ and $p = 0.005$, $F = 5.133$, respectively). Pair-wise comparisons showed that all flooded treatments differed from the Control, with the exception of shoot P in the Intermittently Flooded treatment, which was only marginally lower ($p= 0.055$; Figure 5). Nutrient concentrations were

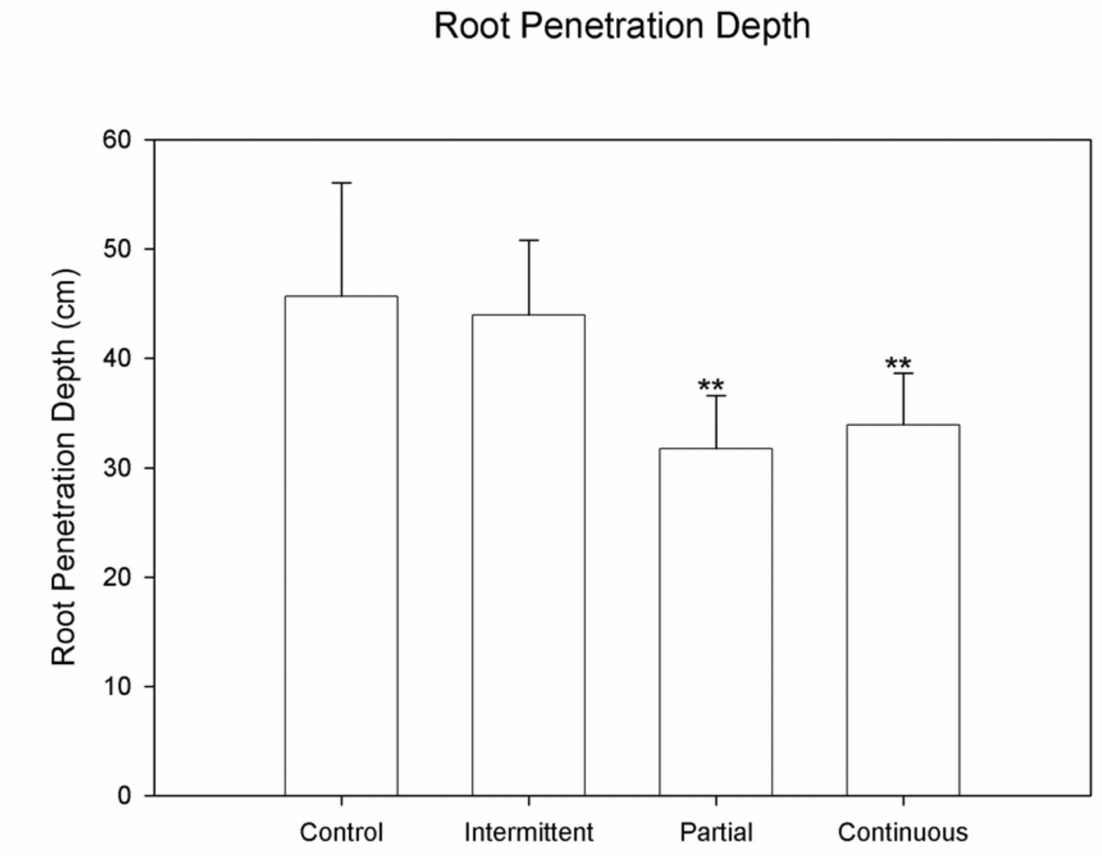

Figure 4. Root Penetration depth decreased in *Bacopa monnieri* in response to Partially Flooded and Continuously Flooded treatments, in which a stagnant water level was maintained through the majority of the study. In contrast, under the Intermittently Flooded treatment, where flooding was only short-term, root penetration was unaffected. One asterisk indicates significant difference from the Control for a give sample date ($p < 0.05$); while two asterisks indicates a highly significant difference ($p < 0.01$). Error bars are one standard deviation.

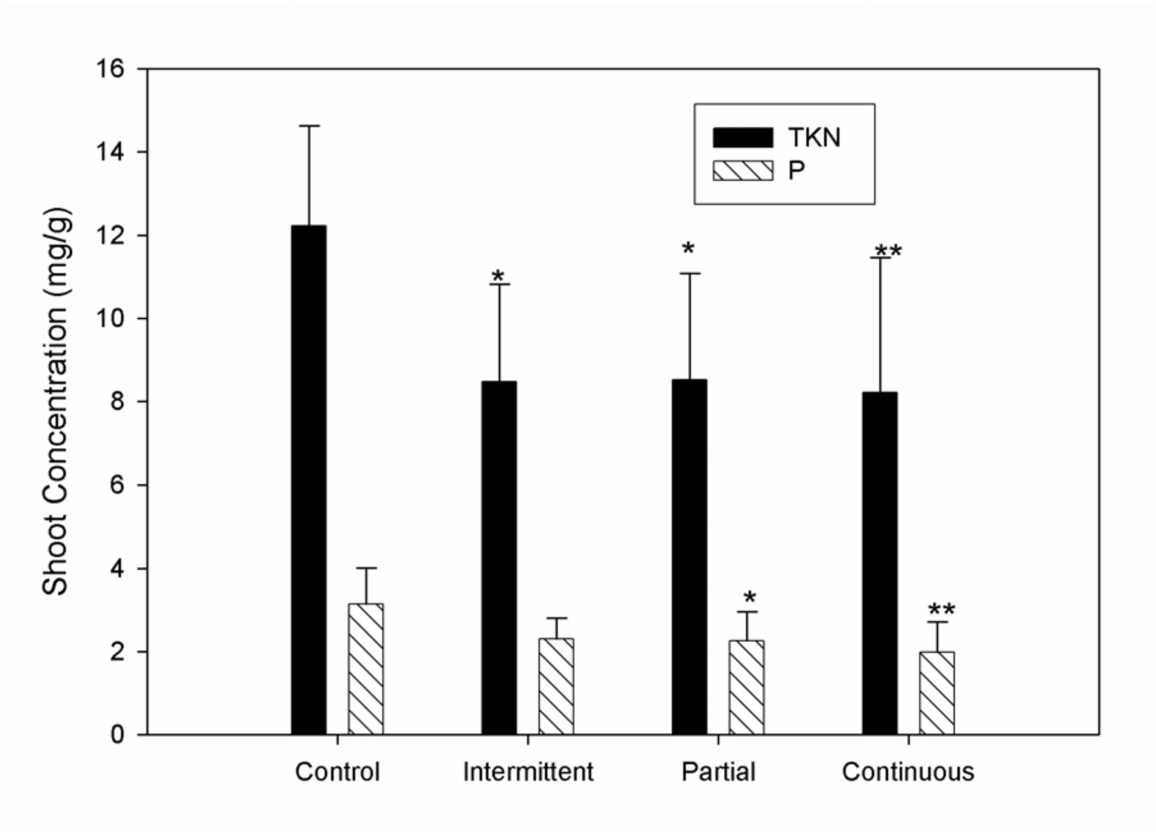

Figure 5. Shoot Concentrations of TKN and P in *Bacopa monnieri*. Although tissue concentration of TKN decreased in all flooded treatments, P concentration was not significantly reduced in the Intermittently Flooded treatment, suggesting a relationship between root length and shoot P concentration. One asterisk indicates significant difference from the Control for a give sample date ($p < 0.05$); while two asterisks indicates a highly significant difference ($p < 0.01$). Error bars are one standard deviation.

correlated to soil Eh to various degrees. For example, shoot N was correlated with soil Eh at 30 cm depth ($r^2 = 0.365$, $p = 0.065$, $N = 25$), whereas shoot P was highly correlated with soil Eh at both 10 cm and 30 cm depths ($r^2 = 0.461$, $p = 0.018$, $N = 26$ and $r^2 = 0.529$, $p = 0.007$, $N = 25$, respectively).

Uptake of N and P demonstrated a significant treatment effect ($F = 2.960$, $p = 0.050$ and $F = 4.248$, $p = 0.015$, respectively). Although all flooded treatments demonstrated lower uptake than the Control (Table 2), only in Continuously Flooded treatment was this trend statistically significant, with $p = 0.043$ for N and $p = 0.015$ for P. These results indicate that although Intermittently Flooded and Partially Flooded conditions influenced N and P concentrations to various degrees, total uptake was only affected by the more reducing conditions in the Continuously Flooded treatment.

DISCUSSION

Control plants demonstrated moderately lower biomass than flooded treatments, a result not uncommon for wetland plants (Farmer et al. 2005; Li et al., 2004, Rubio et al., 1995). Shoot concentrations of N and P were higher in Control than in flooded plants, with mass and nutrient concentrations being negatively correlated. This relationship may be partially explained by increases in shoot mass as a sink for nutrients without a concomitant increase in root uptake capacity (Pezeshki, 2001). However, the trend toward lower plant nutrient uptake in flooded treatments suggests that flooding either decreased the availability of these two nutrients in the rhizosphere, or reduced plant uptake capacity.

Root penetration depth was affected by flooding, likely due to anoxia or reducing conditions in flooded soils. Changes in root penetration due to flooding are often related

Table 1. ANOVA table showing the main effects and interactions on Eh. Because of inherent variability in the methods used to measure Eh, a p = 0.051 for Treatment X Time interaction was considered significant for the purposes of further statistical evaluation**.**

Effect	F	Hypothesis df	Error df	Sig.	Observed Power(a)
Treatment	**12.940**	**6.000**	**208.000**	**.000**	**1.000**
Time	**5.604**	**8.000**	**208.000**	**.000**	**1.000**
Treatment X Time	**1.566**	**24.000**	**208.000**	**.051**	**.962**

Table 2. Nutrient uptake estimates of TKN and P for the eight week study, calculated by subtracting final nutrient content from initial nutrient content. While plant biomass increased throughout the study, nutrient concentration decreased, resulting in a net loss of nutrients in the continuously flooded treatment. Negative values in the Continuous treatment, representing net losses in TKN and P, may be due to partial root senescence resulting from prolonged anoxia.

Treatment	Mean N uptake (mg)	standard dev.	Mean P uptake (mg)	standard dev.
Control	**111.4**	**119.4**	**27.3**	**18.0**
Intermittent	**15.5**	**51.6**	**6.8**	**16.3**
Partial	**40.4**	**70.0**	**14.8**	**23.6**
Continuous	**-12.5**	**86.1**	**-6.78**	**14.1**

to the number of fine roots and root tips, the areas of major interface for nutrient exchange. Decreases in these absorption centers would be most pronounced for nutrients with low solubility, such as P, whereas effects on N would be probably be minimal for *B. monnieri* (Fang et al., 2007).

Previous studies in *Quercus* species have suggested that decreased nutrient uptake due to flooding could be related to decreased soil availability or reduced metabolic function (Pezeshki et al., 1999; Delaune et al., 1998). More recent work with *Lepidium latifolium*, broad-leaf pepperweed, has demonstrated that although nutrient concentrations in roots may show no change or increase under flooding, shoot concentrations decrease, ostensibly the result of decreased apical transport as transpiration is decreased (Chen et al., 2005). Such studies generally use single leaf gas-exchange measures for comparing treatment effects on net photosynthesis and transpiration. Unpublished data on gas exchange suggests that metabolic or transpiration limitations may be related to nutrient uptake in *B. monnieri*; however, leaf morphological differences associated with flooding in this species make such inferences equivocal.

Values for soil redox potential indicated anoxic conditions developed in the bulk soil by midway through the study and persisted through the remainder of the study in the Partially Flooded and Continuously Flooded treatments. Soil reduction may have lowered N availability via a general shift in microbial activity resulting in a concurrent shift in Nitrogen transformations from NO_3^-, the nitrogen species preferred by terrestrial plants, to less available forms such as NH_4^+or diatomic nitrogen (N_2). While *B. monnieri* acquires NH_4^+ and NO_3^- when both are present, NH_4^+ uptake, and thus total N uptake, is reduced in the absence of NO_3^- (Fang et al., 2007). In soils, as opposed to nutrient solutions such as that used by Fang et al. (2007), such effects are likely to be compounded by the cationic attraction of NH_4^+ to negatively charged soil micelles. In the present study, the possibility of reduced N uptake resulting from decreased concentrations of NO_3^- is supported by low concentrations of NO_3^- in effluent from flooded mesocosms (<1 mg/L, unpublished data).

Phosphorus is relatively insensitive to redox changes, but availability to plants is often inversely related to redox potential. Soil reduction dissociates insoluble metal-phosphate complexes, often resulting in an increase in plant-available Phosphorus. (Vepraskas and Faulkner, 2001; Gambrell and Patrick, 1978). Therefore, although decreases in plant tissue concentrations of N may be directly related to changes in plant availability, Eh is unlikely to directly increase the availability of P. pH is known to affect plant available P, with alkaline conditions being generally unfavorable for P uptake. pH values measured from mesocosm effluent were greater than pH = 7.2, indicating conditions of low plant available P. However, as these pH conditions were measured in effluent, rather than derived from rhizosphere soil samples, they are probably not accurate representations of the root environment. This disparity may be especially true for *B. monnieri*, as it exudes substantial amounts of protons into the rhizosphere, resulting in rhizosphere acidification (Fang et al., 2007). The manner in which such root exudates affect soil environments is complex, often involving feedback between soil microorganisms and chemical conditions; however, if decreases in pH of the bulk soil result from proton exudation into the rhizosphere, the solubility of metals as well as PO_4^- would be expected to increase. It is unclear to what degree rhizosphere acidification in *B. monnieri* is related to its ability to sequester metals.

Future studies examining nutrient relations in wetland plants exposed to soil reduction must simultaneously examine multiple mechanisms in order to adequately explain plant response. These mechanisms include changes in assimilation rates due to either soil nutrient availability (e.g. NO_3^- versus NH_4^+) or plant metabolic limitations to nutrient uptake, changes in the root:soil interface, and changes in translocation of nutrients to shoots. The decreased concentrations of N and P in flooded treatments may be indicative of a general trend toward decreased shoot translocation of minerals in this species, and should be considered for further study, particularly with regard to industrial applications where metal uptake is a key factor. Further understanding of nutrient relations in *B. monnieri* may elucidate how this plant functions as a metal hyperaccumulator, as well as management practices to increase its efficacy in soil and water amelioration, in particular under dynamic hydrologic conditions commonly found in agricultural ditches and urban stormwater runoff.

CONCLUSIONS

Hydrology in managed wetlands is important not only in relation to hydraulic retention time and soil reduction, but also indirect effects on plant physiology. Flooding decreased shoot tissue concentrations of N and P in *B. monnieri* to varying degrees, while increasing shoot biomass. Increased biomass only partially explained flooding effects on nutrient concentrations, as calculated values for nutrient uptake were decreased only in Continuous Flooding. Decreased N availability resulting directly from soil reduction in flooded treatments may have affected N uptake, whereas changes in root morphology likely played a part in tissue nutrient concentration differences. This study suggests that although *B. monnieri* is commonly found in aquatic environments, the resulting soil reduction can result in decreased mineral uptake. In environments where plant uptake of nutrients represents a significant portion of the overall mineral immobilization, the need for increased hydraulic retention time should be weighed against the potential stresses on emergent plants. In cases where *B. monnieri* is used for metal phytoremediation of terrestrial sites, inadequate drainage may result in decreased efficacy.

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Chapter 4: Macronutrient (N, P, K) and Redoximorphic Metal (Fe, Mn) Allocation in *Leersia oryzoides* (Rice Cutgrass) Grown under Different Flood Regimes

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, well-drained control; a continuously saturated treatment; a 48-hour pulse-flood treatment; and a partially flooded treatment in which water-level was maintained at 15 cm below the soil surface and flooded to the soil surface for 48 hours 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.

Keywords waterlogging, agricultural drainages, environmental pollution, Iron, Phosphorus, wetlands

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 (0.4-2.0 N m^{-2} d^{-1}) plants can account for a substantial percentage of nitrogen (N) removal (Peterson and Teal 1996). Under flooded conditions oxygen may diffuse from wetland plants 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 (Jiang et al.,2007; Kröger et al. 2007; Meulemann and Beltman 1993; Needelman et al. 2007a; Needelman et al. 2007b; Sharpley et al. 2007; Strock et al. 2007; Thiebault 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; Kröger et al. 2008; Needelman et al. 2007a) 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 (Needleman et al. 2007). 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 (Eh < +350 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 (Richardson and Vepraskas 2001; Szilas et al. 1998), 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, Mississippi, and grown under natural light in the Life Sciences Greenhouse at the University of Memphis. Plants were grown in pots 60 cm high constructed of 15 cm 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_p 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.

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 three 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 1.25g/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 two liters 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 hours.
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 hours. 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 seven liters of nutrient solution once a week prior to the 48 hour intermittent flood. This design was intended to mimic variable hydrologic conditions on both ditch slope and trough as represented by the Partially Flooded and Continuously Flooded treatments, respectively. 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 cm 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 divided into aboveground and belowground portions, air dried for two weeks, then ground in a Wiley Mill and passed through a 1mm diameter mesh. Samples were then 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 hours at 500 °C then oxidized with 10mL H_2O_2 (35-37%), acidified with 4 mL HCl (to pH <1) and brought to 100mL volume with deionized water. Samples were heated for 6 hours at 70 °C then filtered with a 45 μm nitrocellulose filter. 127 mg CsCl (1000 ppm Cs^+) was added to reduce ionization interference in the analysis of potassium (K). Samples were analyzed using a Varian 220 220 atomic absorption spectrometer.

2.4 Statistical Analyses

Unless otherwise indicated, statistical analyses used the general linear model for ANOVA in SPSS 14, with the four levels of water treatment as independent fixed factors. Eh was analyzed as a repeated measures MANOVA, with Eh values at 15 cm depth and 30 cm depth analyzed as correlated dependent variables. Plant nutrient data 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 was analyzed using smaller n-values than desired. Because smaller n-values greatly increase the likelihood of a Type II error, the α level was adjusted accordingly to $\alpha = 0.1$ to examine potential Time x Treatment interactions (see Table 1). All other analyses used the standard $\alpha = 0.05$ value.

3 Results

Soil Eh data are summarized in Figures 1a and 1b. Initially, Eh values were all in the range expected for aerated soils (mean Eh averaged for 10 cm and 30 cm = 562 mV +/- 58). The Control treatment remained aerated throughout the study. By day nine the Eh had dropped below the critical oxygen threshold for anoxia (Eh = 350mV) 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 x 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 +/- 0.28 standard deviation. A time X 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

Table 1. MANOVA table for Eh

Effect		Hotelling's Trace	F	Hypothesis df	Error df	p-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

decrease in pH from 7.86 +/- 0.276 on day 30 to 7.48 +/- 0.313 on day 45 ($p = 0.04$, $F = 5.42$).

Belowground tissue concentrations of Fe increased progressively in response to flooding, with a four-fold increase in the Continuously Flooded treatment as compared to the Control (Figure 2). Belowground tissue concentrations of Manganese (Mn) more than doubled in response to Continuous Flooding (Figure 3). With respect to belowground tissue concentrations of Mn and Fe, only the Intermittently Flooded treatment did not differ from the Control (Figures 2 and 3). Belowground tissue P concentration was significantly increased in response to flooding (Fig 5, $p < 0.0005$)

There was no overall treatment effect on tissue N concentration ($p = 0.086$; see Figure 4). P decreased in 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 (Figure 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; Figure 6). Although tissue Fe concentration did not change in response to flooding in aboveground tissues, Mn concentrations were higher in all flooded treatments (Figures 2 and 3).

Correlational analyses revealed a number of significant relationships in nutrient concentration (Table 2). Belowground concentrations of K and Mn were predictive 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. 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. Previous studies have found similar results for Fe and Mn, although P response varied (Chen et al. 2005; DeLaune et al. 1999; Farmer et al. 2005; Liang et al. 2006; Rubio et al. 1997). 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 NH_4^+ would alter NH_4^+:NO_3^- uptake ratios in plant roots. Incorporation of NH_4^+ into plant tissues is accompanied by the release of 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 NH_4^+ nitrogen uptake (Thompson et al. 1997) can cause increase P solubility (Logan et al. 2002). 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 (Figure 4).

The increase in aboveground tissue Mn concentration 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 hierarchical 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 sub-optimal 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

Table 2. Correlation Summary

A. Pearson Correlation (r^2) N=28		Belowground Tissue Concentration					Aboveground Tissue Concentration				
		N	P	K	Fe	Mn	N	P	K	Fe	Mn
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	0.5957	0.5303	-0.0939	0.0031	-0.2753	0.0208	0.7078
	K	0.1410	-0.0756		-0.2662	-0.0885	0.3019	-0.0203	0.5148	-0.0500	-0.3679
	Fe	-0.0043	0.5957	-0.2662		0.9070	-0.2972	-0.2181	-0.3611	0.1602	0.6449
	Mn	0.0893	0.5303	-0.0885	0.9070		-0.1512	-0.3484	-0.3587	0.1851	0.4642
Aboveground Tissue Concentration	N	0.1367	-0.0939	0.3019	-0.2972	-0.1512		0.5614	0.0928	-0.4710	-0.2995
	P	-0.1780	0.0031	-0.0203	-0.2181	-0.3484	0.5614		0.2799	-0.4360	0.0449
	K	-0.0320	-0.2753	0.5148	-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	0.7078	-0.3679	0.6449	0.4642	-0.2995	0.0449	-0.1168	0.0111	

B. Sig. (1-tailed, p - value)		N	P	K	Fe	Mn	N	P	K	Fe	Mn
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	0.0004	0.0019	0.3172	0.4939	0.0781	0.4582	0.0000
	K	0.2371	0.3511		0.0854	0.3271	0.0592	0.4591	0.0025	0.4003	0.0271
	Fe	0.4913	0.0004	0.0854		0.0000	0.0623	0.1324	0.0295	0.2078	0.0001
	Mn	0.3256	0.0019	0.3271	0.0000		0.2212	0.0346	0.0304	0.1728	0.0064
Aboveground Tissue Concentration	N	0.2440	0.3172	0.0592	0.0623	0.2212		0.0009	0.3192	0.0057	0.0608
	P	0.1824	0.4939	0.4591	0.1324	0.0346	0.0009		0.0746	0.0102	0.4103
	K	0.4358	0.0781	0.0025	0.0295	0.0304	0.3192	0.0746		0.4112	0.2770
	Fe	0.4563	0.4582	0.4003	0.2078	0.1728	0.0057	0.0102	0.4112		0.4777
	Mn	0.2609	0.0000	0.0271	0.0001	0.0064	0.0608	0.4103	0.2770	0.4777	

decomposition of organic material, but also compromises the potential for rhizosphere oxidation in perennial plants. As the dying tissue 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 in all tissues was 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 re-released 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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Table 1. MANOVA (Hotelling's Trace) table for soil Eh, analyzing Eh at 10 cm depth and 30 cm depth as correlated dependent variables. Because small sample size limited statistical power, $\alpha = 0.1$ was considered sufficient to justify further analysis based on a time x treatment interaction. All subsequent analyses used the standard $\alpha = 0.05$.

Table 2. Correlation summary of nutrient concentrations in both aboveground and belowground tissues. Statistically significant values are highlighted in yellow. a. Pearson correlation values (r^2). b. Corresponding p values. p-values of zero 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.

Figure 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.

Figure 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 +/- one standard deviation.

Figure 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 +/- one standard deviation.

Figure 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.

Figure 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.

Figure 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.

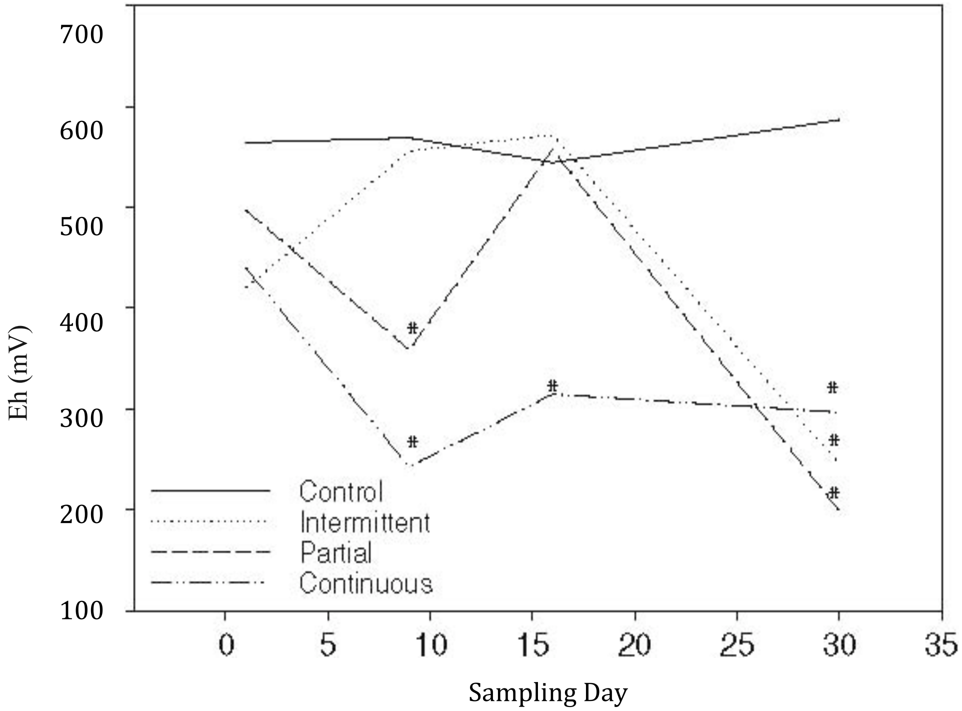

Figure 1a. Soil Eh at 10 cm depth.

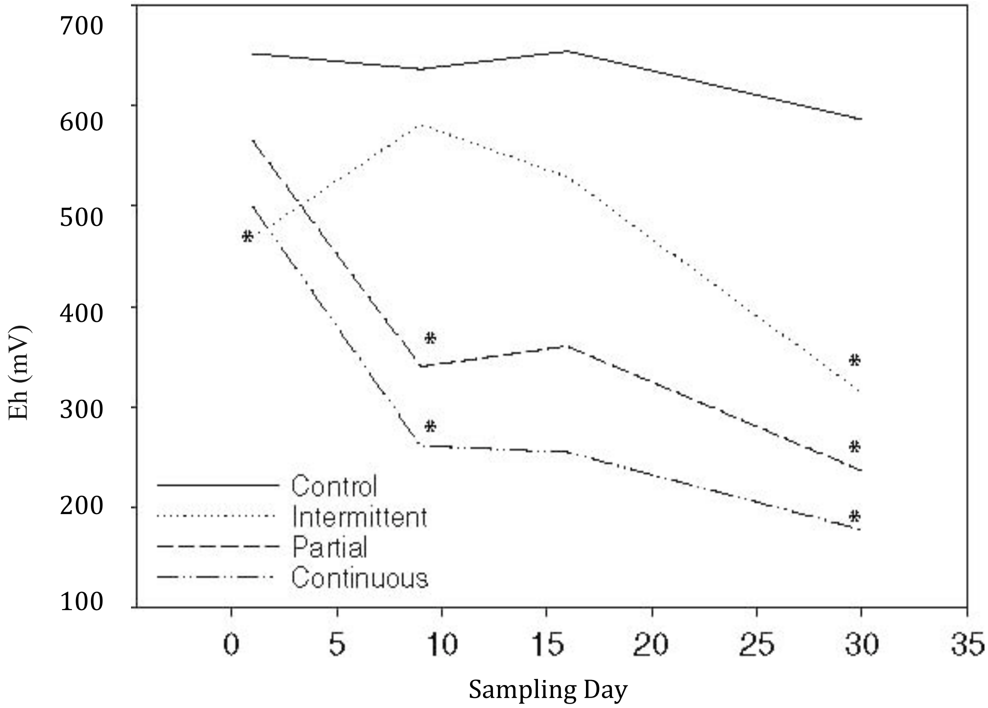

Figure 1b. Soil Eh at 30 cm depth.

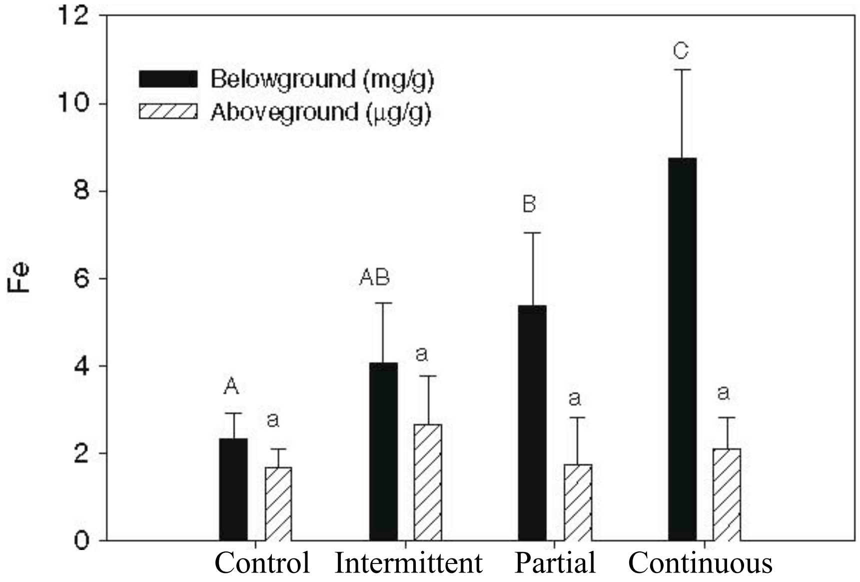

Figure 2. Fe concentration in belowground tissues demonstrated progressive increases with intensity of flooding, whereas aboveground tissues demonstrated no detectable response.

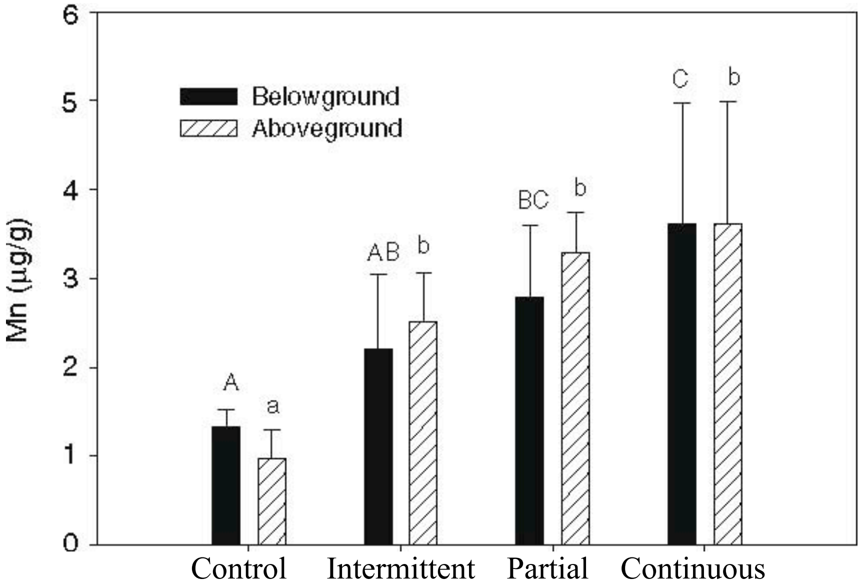

Figure 3. Mn concentration demonstrated a progressive increase in response to flooding in both belowground and aboveground tissues.

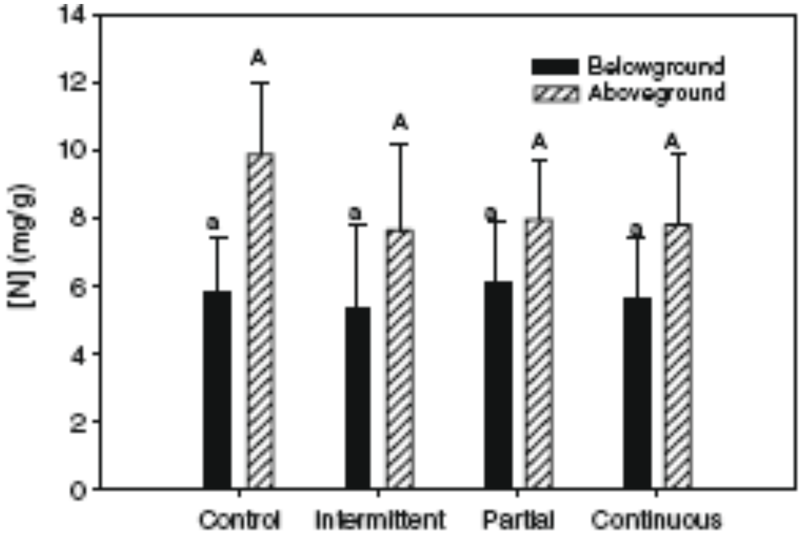

Figure 4. N concentration demonstrated no detectable response to flooding.

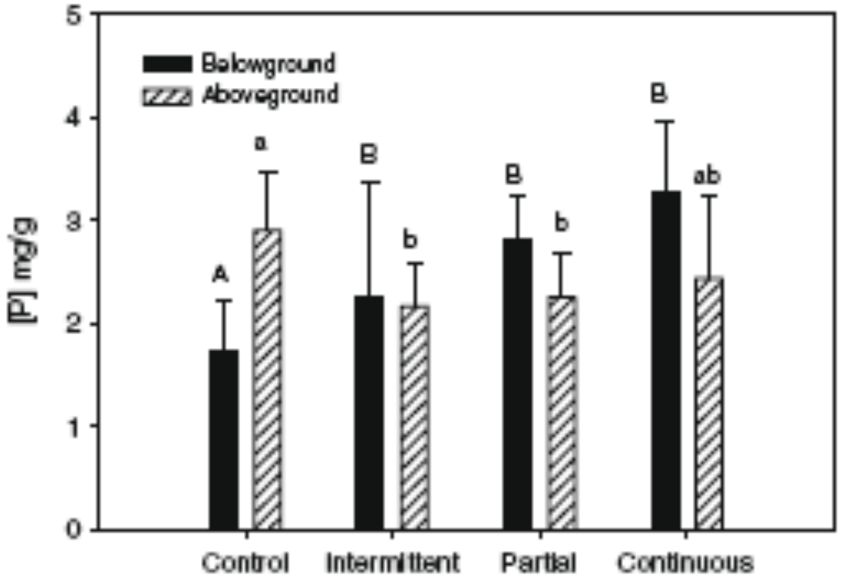

Figure 5. P concentration was significantly higher in below-ground tissues of flooded plants, as compared to the control, whereas aboveground concentrations were decreased under intermittent and partial flooding.

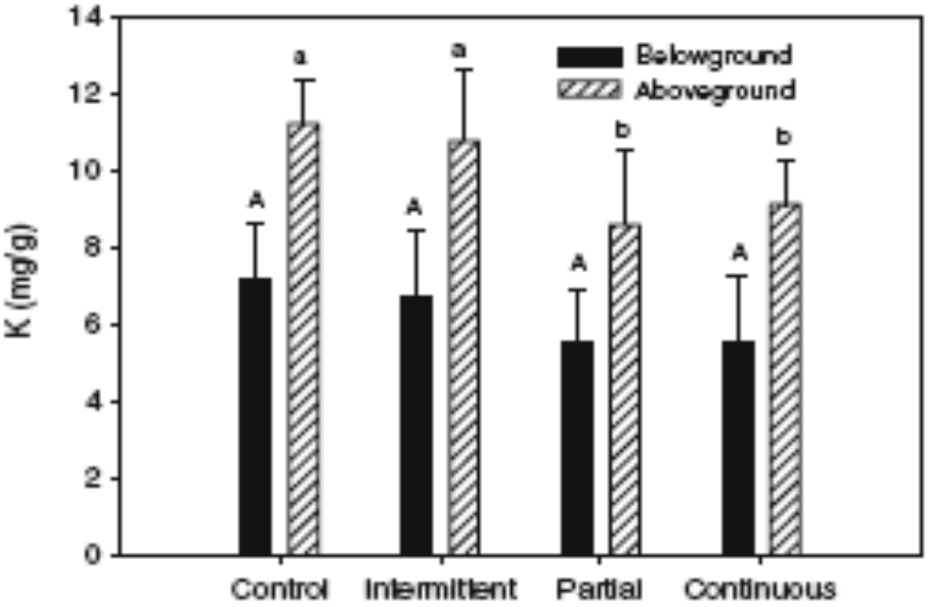

Figure 6. K concentration was significantly lower in aboveground tissues of flooded plants as compared to the control, whereas belowground tissues demonstrated no detectable response.

Chapter 5: Hydrology and Species-Specific Effects of *Bacopa monnieri* and *Leersia oryzoides* on Soil and Water Chemistry

ABSTRACT

In an eight week greenhouse experiment, *Bacopa monnieri* (Water Hyssop) and *Leersia oryzoides* (Rice Cutgrass) were compared for nutrient assimilation as well as soil and water chemistry under variable flooding regimes using a nutrient solution rich in nitrogen (N) and phosphorus (P). Soil redox potential decreased in flooded treatments; however, mesocosms containing *B. monnieri* remained aerobic for much of the study, while flooded mesocosms containing *L. oryzoides* became moderately reduced. Soils containing *L. oryzoides* were higher in nitrogen. Generally, effluent concentrations of PO_4^{3-} were higher in *B. monnieri* mesocosms. Plant concentrations of N and P were affected differently by flooding; flooding led to decreased shoot N and P concentrations in *B. monnieri*, whereas increasing root P concentrations in *L. oryzoides*. *B. monnieri* immobilization of N and P was significantly less in belowground tissues than *L. oryzoides*. P immobilization in *L.* oryzoides generally increased in response to flooding, while *B. monnieri* showed no detectable response. Results indicated that species-specific flood responses in plant nutrient status are due to differing interactions of *B. monnieri* and *L. oryzoides* with the soil environment. Additionally, *L. oryzoides* demonstrated greater P uptake than *B. monnieri* across treatments, resulting in decreased concentrations of PO_4^{-3} in effluent. Although N was also affected by flooding and species, generalizations on N allocation within the system are difficult to describe due to the

changes in species of N in response to oxidation-reduction gradients and biotic assimilation.

KEY WORDS agricultural ditches; wetland plants; phytoremediation; surface water quality; eutrophication; *Leersia oryzoides*; *Bacopa monnieri*

INTRODUCTION

It has been widely accepted that wetlands can improve water quality and that reversing the trend toward water-quality degradation associated with wetland losses will require a multi-faceted approach, including changes in farm management practices to decrease non-point pollution in agricultural areas (Day et al., 2003). In an effort to establish "environmentally friendly" guidelines for farmers, three government agencies, the United States Department of Agriculture Natural Resource Conservation Service (USDA NRCS), USDA Agricultural Research Service (USDA ARS), and the National Association of Conservation Districts (NACD), are involved in an ongoing process to develop best management practices to decrease water pollution (as cited by Cooper et al., 2004). Recent research suggests that the presence of vegetation in agricultural ditches could significantly impact water quality (Cooper et al., 2004; Kröger et al., 2007a)

Several studies in constructed wetlands have demonstrated that vegetation substantially reduces nutrient-load by a number of potential mechanisms (see Cronk and Fennessy, 2001). These mechanisms include physical responses, such as decreased erosion, decreased sediment suspension (Braskerud, 2001), and increased surface areas for microbial growth (Brix, 1997). Plants also alter soil chemistry. Specifically, plant roots exude a number of substances that alter pH and microbial growth (Jones et al., 2004). 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). Finally, plants may reduce nutrient load through direct assimilation and immobilization. Although in constructed wetlands designed for high nutrient loads plants account for only a small fraction of nutrient removal, in low-load systems (0.4-2.0 N m^{-2} d^{-1}) plants can account for a substantial percentage of N removal (Peterson and Teal, 1996).

In agricultural ditches seasonal and short-term fluctuations in water level can lead to plant stress either via water deficits or through soil reduction resulting from flooding. This hydrologic variability has also been demonstrated to affect both phosphorus and nitrogen concentrations (Kröger et al., 2008; Kröger et al., 2007b; Needelman et al., 2007); however, species-specific plant responses to hydrologic variability and the subsequent changes in soil and water chemistry remain largely understudied, even though it is well established that flood-related stress affects wetland plant productivity and nutrient allocation (Pezeshki et al., 2001). Previous research (Deaver et al., 2005) demonstrated that *Leersia oryzoides* can reduce effluent phosphorus concentrations, even when compared to other plants common in agricultural ditches. The present study compares nutrient allocation in two wetland plant species *L. oryzoides*, rice cutgrass, and *Bacopa monnieri*, water hyssop, to a range of water regimes ranging from drained to continuously flooded. We hypothesized that the deeper rooted *L. oryzoides* would have a greater impact on soil chemistry than *B. monnieri*, resulting in comparatively decreased water nutrient concentrations, but that this effect would be decreased under more intensive flooding due to decreased root elongation.

METHODOLOGY

Experimental design

Plants were collected from wild populations found in wetland cells at the Jamie L. Whitten Plant Materials Center in Coffeeville, Mississippi, and grown under natural light in the Life Sciences Greenhouse at the University of Memphis. Plants were grown in pots 60 cm high constructed of 15 cm 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_p horizon of the Waverly Silt Loam Series (Soil Conservation Service 1989). Although the high sand content of the sand/soil mixture used in this study is not representative of agricultural ditches in the region, the hydraulic conductivity of such soils is so low that a well-drained control was essentially impossible given the hydroperiods under study. After placement in PVC pipes, plants were well-watered and well-drained for a period of three weeks prior to treatment initiation. During this time, plants were fertilized weekly with 500 mL of 20-20-20 Peter's fertilizer mixed with tap water at 1.25 g/L. More information about the geochemistry of the municipal water source is available in Larsen et al. (2003). The study was terminated 56 days after treatment initiation.

Soil moisture treatments

Flooding treatments followed procedures previously described in Pierce et al. (2007). A completely randomized design was employed, examining stress response of *L. oryzoides* and *B. monnieri* across four soil moisture regimes. Each treatment was replicated 12 times, with individual plants being treated as replicates. After treatment initiation, plants were watered daily with approximately two liters of a nutrient solution containing a maximum of 12 mg/L 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).

Treatments included a 1) well-watered, well-drained control; 2) well-watered, well-drained intermittently flooded treatment; 3) partially flooded treatment; and 4) continuously flooded treatment. Water-level was manipulated by placing pots in polyethylene bags and raising or lowering the level of the bag to the appropriate distance from the top of the soil. Other details included:

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 (2 inches) above soil surface for a period of 48 hours.
3. A Partially Flooded treatment with water maintained at 15 cm (5.9 inches) below soil surface. Water level within the soil was checked periodically using an internal gauge constructed from 1.9 cm (0.75 inch) perforated PVC pipe.

4. A Continuously Flooded treatment with water maintained at 5 cm above the soil surface.
5. On days 7, 14, 19, 28, 35, and 45, Partially Flooded and Continuously Flooded pots were flooded an additional 15 cm (5.9 inches) of water for 48 hours.

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 seven liters of nutrient solution once a week prior to the 48-hour intermittent flood. This design was intended to mimic variable hydrologic conditions on both ditch slope and trough as represented by the Partially Flooded and Continuously Flooded treatments, respectively.

Soil redox potential (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 cm (3.9 inches) and 30 cm (11.8 inches) 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.

Water analysis

Because of variations in evaporation rates, and occasional tears in the polyethylene bags, water for sampling was not consistently available for each replicate throughout the course of the study. Thus, individual experimental units could not be reliably measured over time. Consequently, water samples were collected from experimental units chosen on the basis of water availability in either the flooding apparatus (for Intermittently Flooded, Partially Flooded, and Continuously Flooded) or the overflow container (for the Control and Open Container). Samples were taken two days after initiation of pulse-flooding on days 16, 30, and 45.

The water pH and specific conductance were measured for samples collected on day 30 and day 45 using a pH Testr 2 double junction (Oaktron Instruments) and YSI 30 SCT, respectively. Raw samples were filtered using 0.47-µm nitrocellulose filter, a portion of the sample was used to measure ammonium concentration, while the remainder of the sample was stored in the dark at 10° C to await anion testing. Ammonium concentrations were measured using a HACH DR/2000 direct reading spectrophotometer utilizing the low range test'n tube Hach method 10023 (range: 0.02 to 2.5 mg/L, standard deviation: +/- 0.03 mg/L). Reactive orthophosphate concentrations were individually analyzed with a HACH DR/2000 direct reading spectrophotometer using the PhosVer 3 Hach method 8048 (range: 0 to 2.5 mg/L, standard deviation: +/- 0.02 mg/L). Concentrations of Fl^-, Cl^-, NO_2^-, Br^-, NO_3^-, and SO_4^{2-} were all measured using a Dionex DX-120 Ion Chromatograph. Concentrations of Fl^-, NO_2^-, and Br^-, were all near or below detection limits and are not included. Internal standards were used to calculate reproducibility for Cl^-, NO_3^-, and SO_4^{2-}. Concentrations, standard deviations, and % reproducibility were as follows: Cl^- standard: 2.62 +/- 0.35 mg/L (13.3% error); $NO3^-$ standard: 10.16 +/- 0.098 mg/L (0.961% error); SO_4^{2-} standard 14.6 +/- 0.67 mg/L (4.61% error).

Plant and soil nutrient analysis

Six soil cores per treatment were taken to a depth of 30 cm using a 3 cm diameter soil probe. Each core was divided into a 0-15 cm depth sample and a 15-30 cm depth sample. Samples were air dried for two weeks and pulverized using a mortar and pestle, any root tissue greater than 1 mm in diameter was removed. Subsamples of approximately 0.1 g were prepared using Kjeldahl digestion and analyzed for N and P as described below.

Ten randomly chosen plants per treatment were analyzed for tissue nutrient analysis. Plants were divided into aboveground and belowground portions, air dried for two weeks, and weighed. Samples were then ground in a Wiley Mill until they passed through a #40 mesh screen. Subsamples of approximately 0.2 g were prepared using Kjeldahl digestion and analyzed for N and P as described below.

Plant and soil samples were digested according to Quikchem Method 10-107-06-2-E (detection limit 0.018 mg/L) for Total Kjeldahl Nitrogen (TKN) as described by Wendt (1997) and QuikChem Method 10-115-01-1-C (detection limit 0.015 mg/L) for total phosphorus (Lachat Instruments, 1995). Reproducibility for nitrogen and phosphorus using these methods are approximately 5% (Horneck and Miller, 1998; Lachat Instruments, 1995). The Kjeldahl digestion process stabilizes ammonium nitrogen and converts organic nitrogen into ammonium. Nitrate is not converted into ammonium. The phosphate digestion process converts organic and inorganic phosphorus to orthophosphate. Ammonium and orthophosphate were subsequently quantified by colorimetry using Lachat Instruments QuickC hem FIA+ 8000 series Colorimeter.

Data analysis

Unless otherwise indicated, statistical analyses used the general linear model for ANOVA using SPSS 14 (SPSS, Inc., 2005), with two species and four levels of water regime as independent factors. Eh was analyzed as a repeated measures MANOVA, with Eh values at 10cm depth and 30cm depth analyzed as correlated dependent variables. Final soil nutrient concentrations were analyzed with a factorial MANOVA (water treatment x time), with concentrations at 15 cm depth and 30 cm depth as correlated dependent variables. Water chemistry measures were individually analyzed with factorial ANOVA tests (water treatment x time). Plant nutrient data were evaluated with MANOVA, with aboveground values and belowground values as correlated dependent variables. Plant nutrient immobilization was calculated by multiplying the respective nutrient concentrations by the biomass of the same plant. Aboveground and belowground immobilizations were analyzed in a multivariate context as previously described.

RESULTS AND DISCUSSION

Results

Plants and Soil. Prior to the initiation of the soil moisture treatment, the soil was aerated in all mesocosms at both 10 cm and 30 cm depth (Eh = 460 +/- 180 mV and 420 +/- 210 mV, respectively). However, following treatment initiation, flooded treatments showed a decline in Eh within 24 hours, while drained mesocosms remained aerated. Flooded mesocosms containing *L. oryzoides* were moderately reduced at 10 cm depth and 30 cm depth by day 15, while mesocosms containing *B. monnieri* remained aerated at 10 cm depth until day 30. *B. monnieri* demonstrated consistently higher Eh at both depths, as indicated in Figures 1a and 1b ($F = 8.39$, $p < 0.0005$).

L. oryzoides produced greater total biomass compared to *B. monnieri*, but no treatment effect was observed (Table 1). Root: shoot biomass ratios displayed a significant species x treatment interaction. Root: shoot ratios in *B. monnieri* was unaffected by flooding treatments ($F = 1.078$, $p < 0.368$), whereas flooding decreased root: shoot ratios in *L. oryzoides* ($F = 6.82$, $p < 0.0005$). All flooded treatments displayed significantly lower root: shoot ratios than the drained treatment ($p < 0.05$). Root: shoot biomass ratios in *L. oryzoides* were nearly an order of magnitude higher than in *B. monnieri* (Table 1, for all pair-wise comparisons $p < 0.0005$).

Final soil nitrogen concentrations (mean at 0-15 cm = 0.149 +/- 0.016 mg/g, mean at 15-30 cm = 0.152 +/- 0.022 mg/g) did not significantly differ from those of initial concentrations (mean at 0-15 cm = 0.149 +/- 0.015 mg/g, mean at 15-30 cm = 0.163 +/- 0.022 mg/g). Nitrogen concentration at 15-30 cm decreased in response to flooding, an effect most pronounced in *L. oryzoides* (Figure 2). Both shallow and deeper samples in mesocosms containing *L. oryzoides* had higher nitrogen than those containing *B. monnieri* ($F = 6.46$, $p = 0.003$); however, these differences were negated by flooding. Final soil phosphate concentration (mean at 0-15 cm = 0.0178 +/- 0.050, mean at 15-30 cm = 0.153 +/- 0.029) did not significantly differ from those of initial concentration (mean at 0-15 cm = 0.0176 +/- 0.013, mean at 15-30 cm = 0.144 +/- 0.034). Overall, no treatment effect or species effect was observed on soil phosphate concentrations.

In *L. oryzoides*, P immobilization was enhanced in response to flooding ($F = 2.96$, $p = 0.013$), but no differences were observed among individual treatments. Although mean P concentrations of *B. monnieri* decreased in response to flooding, this trend was not statistically significant. *L. oryzoides* immobilized more P in both aboveground and belowground tissues than *B. monnieri* and immobilized more N in belowground tissues. Total plant N showed no response to flooding in either species (Table 1).

Water Chemistry. Specific conductance increased over time, with a mean value of 130 µS on day 30 increasing to 168 µS by day 45. This increase was intensified by flooding ($F = 8.36$, $p < 0.0005$). Effluent from mesocosms containing *L. oryzoides* were higher in conductivity than those containing *B. monnieri* ($F = 18.4$, $p < 0.0005$; Figure 3). Effluent pH was neutral to slightly alkaline across treatments (pH = 7.81 +/- 0.46). Generally pH for *B. monnieri* effluent was higher than *L. oryzoides* in more intensely flooded treatments, whereas it was lower in effluent from drained and intermittently flooded treatments. Rice cutgrass pH declined in Continuous flooding from 7.86 +/- 0.28 to 7.48 +/- 0.31 ($F = 5.42$, $p = 0.040$). Effluent pH in *B. monnieri* increased in the Partially Flooded treatment from 7.70 +/- 0.23 to 8.72 +/- 0.58 ($F = 17.85$, $p = 0.002$).

SO_4^{2-} concentrations ranged from approximately 3 to 6 mg/L. There were no species differences in drained treatments, but effluent SO_4^{2-} was generally higher in *L. oryzoides* than *B. monnieri* in flooded treatments ($F = 18.3$, $p < 0.0005$; Figure 4). Cl^- concentrations ranged from approximately1 to 6 mg/L and increased in flooded treatment of both species, resulting in higher concentrations compared to controls ($F = 24.1$, $p < 0.0005$; Figure 5). Cl^- concentrations were higher in *L. oryzoides* across all flooded treatments ($F = 3.23$, $p = 0.043$).

Phosphate concentration demonstrated a treatment effect in *L. oryzoides* ($F = 4.277$; $p = 0.009$) with Partially Flooded values initially higher than other treatments. Initially, effluent phosphate concentrations were higher in *B. monnieri* than in *L. oryzoides* across all treatments. Phosphate concentrations in effluent from flooded mesocosms declined to values similar to *L. oryzoides*, while phosphate concentrations from Drained mesocosms with *B. monnieri* were more than triple those of *L. oryzoides* (F = 4.277, p =.009). Effluent from drained mesocosms of *B. monnieri* was higher in PO_4^{3-} than flooded effluent from mesocosms (F =9.321, p =0.001; Table 2).

B. monnieri effluent had higher NO_3^- under drained conditions than did *L. oryzoides* (F = 17.81, p <0.0005). Additionally, flooded *B. monnieri* demonstrated lower NO_3^- concentrations than drained mesocosms on day 45 (F = 23.8, p <0.0005). Ammonium concentrations did not differ between species treatments. An increase was observed in continuously flooded *B. monnieri* mesocosms (p = 0.033) on day 30, but not thereafter.

Discussion

Flooded soils generally display decreased Eh as seen in the present study. As oxygen availability declines (at ~ Eh = 350 mV), soil microorganisms use alternate electron acceptors in a stepwise fashion with decreasing Eh (e.g. – Eh ≈ 250: NO_3^- and Eh ≈ 100: Fe (III); Mitch and Gosselink, 2001). The weekly draining of water from all treatments during refreshment of the nutrient solution introduced air into the interstitial soil pores and may have somewhat attenuated this trend toward soil reduction. However, any oxidation occurring during this period would likely last less than a day and would be counteracted by the flooding treatments applied approximately 10 hours after initiation of draining. A study examining soil redox response in *Spartina alternaflora* mesocosms utilizing high resolution time series data found that alternating flooding: draining resulted in periodic Eh fluctuations of 75 mV (Catallo, 1999). The trend toward oxidation that occurred during draining was reversed within a few hours of flooding.

Because the anoxic environment can be stressful for plants, many wetland plants have internal aeration ducts (lacunae) to convey oxygen into the rhizosphere (Reddy et al., 1998). Species differences in influencing soil Eh may reflect a greater ability in *B. monnieri* for rhizosphere oxidation. Both root and shoot tissues appeared highly porous compared to *L. oryzoides*. Alternately, these differences may be the result of *L. oryzoides* indirectly promoting soil reduction either through rhizosphere exudation of organic carbon (Ehrenfeld et al., 2005; Neuman and Römhel, 2002), or through facilitation of microbial growth (Jones et al., 2004). Roots of *L. oryzoides*, for example, have been found to harbor populations of Fe–oxidizing bacteria, which have been implicated in redox reactions in the soil (Emerson et al., 1999).

Because there was no unplanted treatment (bare soil) in the present study it is unclear which of these mechanisms is most likely for the observed species differences in soil Eh. Existing research would imply that plants generally increase Eh in flooded soils (Chen and Barko, 1988; McKee et al., 1988; Wright and Otte, 1999). Due to the high sand content of the soil used in this study, intense reduction would not be expected in unplanted soil (DeLaune et al. 1990, Pezeshki and DeLaune 1990). In addition to changes in Eh, flooding can also affect the pH of soils. A series of chemical transformations occurring in response to reducing conditions in the soil often causes acidification, processes that are exacerbated by rhizosphere acidification resulting from plant interactions with reduced forms of nitrogen and iron (Neuman and Römhel, 2002; Begg et al., 1994).

The decreased nitrogen concentrations measured in the soils of Continuously Flooded mesocosms containing *L. oryzoides* were somewhat counterintuitive. It would be expected that soil reduction would increase NH_4^+: NO_3^- ratios, and as the method for determination of nitrogen concentration in the present study does not include NO_3^-, nitrogen values would subsequently increase. However, such conditions may also enhance ammonification of organic nitrogen and denitrification, leading to the production of gaseous or volatile forms of nitrogen that would diffuse into the atmosphere (see Mitch and Gosselink, 2000).

Higher concentrations of NO_3^- and PO_4^{3-} in effluent from *B. monnieri* mesocosms compared with *L. oryzoides* mesocosms are partially due to increased plant immobilization in belowground tissues of *L. oryzoides* resulting primarily from greater biomass in the latter. Previous studies have demonstrated that *L. oryzoides* may act as a phosphorus source (Kröger et al., 2007) or a sink (Deaver et al., 2005). With regard to nitrogen, *L. oryzoides* appears to be more limited in its nutrient removal capability. Whereas two common ditch plants, *Juncus effuses* and *Ludwigia peploides*, decreased N in effluent water in a mesocosms experiment (measured as NO_3^- and NH_4^+) when compared to controls, *L. oryzoides* did not (Deaver et al., 2005). In the present study, differences in effluent NO_3^- may be indicative of nitrogen species transformation from NO_3^- to reduced forms, as *B. monnieri* remained above the threshold for this transformation (i.e. Eh ≈250) through day 30, whereas soils planted with *L. oryzoides* approached this threshold within nine days of flooding.

Twisk et al. (2003) found that flooding lowered effluent Cl- at very high concentrations (>200 mg/L). For the present study, increases in effluent Cl^- resulting from flooding may be related to plant uptake of NO_3^-. Bar-Tal (1999) observed that uptake of NO_3^- and Cl^- may be regulated to maintain a steady state value for $NO_3^- + Cl^-$. As plants in drained treatments are likely to use NO_3^- as the predominant form of nitrogen, there may be the added benefit of increased Cl^- uptake; however, low reproducibility of Cl- standards in the present study (i.e. – error +/- 13.3%) preclude further interpretation.

When iron-rich soil, such as the soil used in this study, is flooded for an extended time, the reduction of Fe (IIII) to soluble Fe (II) results in the dissolution of iron-phosphate complexes in the soil, yielding soluble PO_4^{3-} (Szilas et al., 1998). While water concentrations of PO_4^{3-} would generally be expected to increase in response to flood-induced soil reduction, no such response was observed. Concentrations of PO_4^{3-} in *B. monnieri* were actually lower in all flooded treatments than controls. Previous studies suggest that the diffusion of oxygen to the rhizosphere via lacunae may lead to the oxidation of Fe (II) and subsequent co-precipitation of phosphorus along the root epidermis and cortex (Chen et al., 2005; Liang et al., 2006). This process likely explains higher levels of phosphorus in belowground tissues of *L. oryzoides*. The mechanisms explaining phosphorus differences in effluent water from *B. monnieri* are not so straightforward, but may involve subtle rhizosphere pH differences between treatments influencing predominant forms of P (Fang et al., 2007).

CONCLUSIONS

Whereas previous research has demonstrated that *L. oryzoides* may reduce effluent phosphorus concentrations under saturated conditions, the present study shows that immobilization of phosphorus in belowground tissues is related to intensity of soil reduction during flooding. The increased uptake of PO_4^{3-} and NO_3^- from *L. oryzoides* effluent compared with *B. monnieri* effluent was mitigated after 45 days of flooding. These results imply that *L. oryzoides* may be more effective than shallow-rooted semi-aquatic species such as *B. monnieri* for remediation of agricultural water when exposed to periodic drawdown allowing for soil oxidation. Mesocosms with *B. monnieri*, on the other hand showed decreases in effluent macronutrients only after continuous flooding. Agricultural management practices employing controlled drainage for enhanced water quality may promote dominance of semi-aquatic species such as *B. monnieri*. The species-specific differences in soil redox potential, pH, and conductivity imply that if hydrological alterations do affect species composition, they may be accompanied by plant-mediated differences in soil and water chemistry.

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Table 1. Biomass, root:shoot ratio and total plant immobilization of phosphorus and nitrogen.

			Total Plant Immobilization (g)			
			TP		TKN	
	Biomass	R:S	Below-ground	Above-ground	Below-ground	Above-ground
Leersia						
Drained	58.5 +/-18.4	0.807+/-0.039	41.4+/-15.8	98.7+/-16.0	141+/-63.5	341+/-79.0
Intermittent Flooding	59.5 +/-14.6	0.59 +/-0.041	58.4+/-29.5	81.5+/-25.5	139+/-10.2	289+/-120
Partial Flooding	56.3 +/-14.7	0.57 +/-0.039	59.3+/-26.7	81.8+/-22.2	123+/-49.6	294+/-102
Continuous Flooding	67.6 +/-17.4	0.43 +/-0.41	62.4+/-16.8	111+/-41.5	110+/-49.3	356+/-136
Bacopa						
Drained	58.5 +/-18.4	0.096 +/-0.039	10.8+/-8.05	85.5+/-27.8	23.2+/-10.1	339+/-120
Intermittent Flooding	40.5+/-8.75	0.076 +/-0.039	6.25+/-4.30	87.5+/-34.2	19.3+/-9.4	324+/-153
Partial Flooding	39.2+/-16.5	0.071+/-0.41	7.04+/-5.46	76.8+/-20.9	25.2+/-21.6	291+/-73.6
Continuous Flooding	39.3 +/-7.34	0.075 +/-0.039	4.62+/-1.63	68.6+/-25.8	17.3+/-9.21	270+/-93.9

Table 2. Summary table of measured nitrate, ammonium and reactive phosphate in mesocosm effluent. "Day" indicates number of days from treatment initiation. Bold numbers indicate significant difference from Drained treatment. "Reference water" refers to water that was poured directly into overflow containers without passing through the mesocosms.

	Effluent Concentrations (mg/L)							
	NH_4^+		NO_3^-			PO_4^{-3}		
	Day		Day			Day		
	30	45	16	30	45	16	30	45
Reference water	1.92+/-0.82	0.62+/-0.16	5.05	9.48+/-0.619	5.51+/-0.73	3.00	5.35+/-0.87	3.29+/-0.57
Leersia								
Drained	0.12+/-0.09	0.14+/-0.06	1.19+/-0.89	0.91+/-1.25	0.67+/-0.45	0.46+/-0.42	0.19+/-0.16	0.76+/-0.58
Intermittent Flooding	0.17+/-0.16	0.06+/-0.10	1.72+/-2.66	0.98+/-2.09	.60+/-0.65	0.41+/-0.29	0.53+/-0.61	0.21+/-0.28
Partial Flooding	0.22+/-0.28	0.07+/-0.11	0.93+/-1.00	2.87+/-3.38	1.87+/-1.78	2.01+/-1.66	1.33+/-1.02	0.45+/-0.59
Continuous Flooding	0.29+/-0.42	0.03+/-0.04	1.67+/-3.52	2.48+/-3.10	0.28+/-041	0.52+/-0.63	0.75+/-1.55	.075+/-0.02
Bacopa								
Drained	0.16+/-0.04	0.18+/-0.08	1.67+/-1.77	2.77+/-0.55	2.32+/-0.46	1.82+/-2.18	1.13+/-0.55	2.03+/-1.37
Intermittent Flooding	0.09+/-0.76	0.07+/-0.07	0.84+/-0.80	1.15+/-1.19	0.72+/-0.58	2.61+/-2.47	0.73+/-0.47	0.34+/-0.27
Partial Flooding	0.04+/-0.04	0.10+/-0.21	1.41+/-1.86	1.56+/-0.78	.059+/-0.10	3.03+/-1.92	1.65+/-0.96	0.10+/-0.01
Continuous Flooding	0.49+/-0.51	0.06+/-0.08	0.80+/-0.58	3.02+/-2.74	0.35+/-0.77	1.68+/-2.60	1.05+/-0.88	0.22+/-0.16

Figures 1A & B. Soil redox potential (Eh) on day 30 at 10 cm depth and 30 cm depth, respectively. The reference line indicates the approximate Eh at which oxygen is depleted. Species differences within a flooding treatment are indicated by an asterisk ($p < 0.05$). Differing upper-case letters indicate a significant difference from drained conditions for *L. oryzoides*, while differing lower-case letters indicate a significant difference from drained conditions for *B. monnieri* ($p < 0.05$). If no letters are present, no effect of flooding treatments was observed. Error bars indicate +/- 1 standard deviation.

Figure 2. Soil N concentration at 15-30 cm depth on day 56, demonstrating a flood related decline in soil N for mesocosms containing *Leersia oryzoides*. Differing upper-case letters indicate a significant difference from drained conditions for *L. oryzoides*, while differing lower-case letters indicate a significant difference from drained conditions for *B. monnieri* ($p < 0.05$). If no letters are present, no effect of flooding treatments was observed. Error bars indicate +/- 1 standard deviation.

Figure 3. Mean effluent specific conductance. The reference line is the mean specific conductance of reference water that was not exposed to mesocosms. Differing upper-case letters indicate a significant difference from drained conditions for *L. oryzoides*, while differing lower-case letters indicate a significant difference from drained conditions for *B. monnieri* ($p < 0.05$). If no letters are present, no effect of flooding treatments was observed. Error bars indicate +/- 1 standard deviation.

Figure 4. Mean effluent sulfate concentration. The reference line is the mean concentration of sulfate in reference water that was not exposed to mesocosms. Differing upper-case letters indicate a significant difference from drained conditions for *L. oryzoides*, while differing lower-case letters indicate a significant difference from drained conditions for *B. monnieri* ($p < 0.05$). If no letters are present, no effect of flooding treatments was observed. Error bars indicate +/- 1 standard deviation.

Figure 5. Mean chloride concentration. The reference line is the mean concentration of chloride in reference water that was not exposed to mesocosms. Differing upper-case letters indicate a significant difference from drained conditions for *L. oryzoides*, while differing lower-case letters indicate a significant difference from drained conditions for *B. monnieri* ($p < 0.05$). If no letters are present, no effect of flooding treatments was observed. Error bars indicate +/- 1 standard deviation.

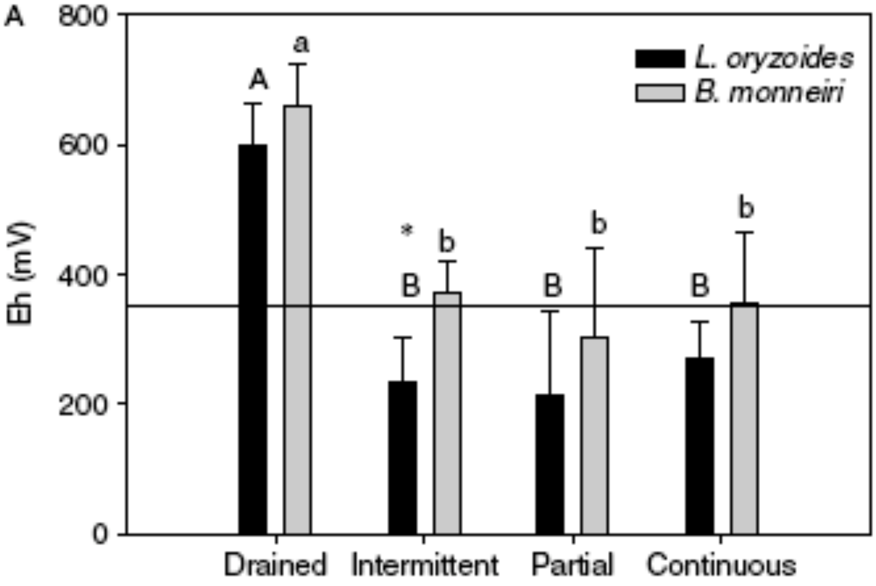

Figure 1 A. Eh at 10 cm depth day 30.

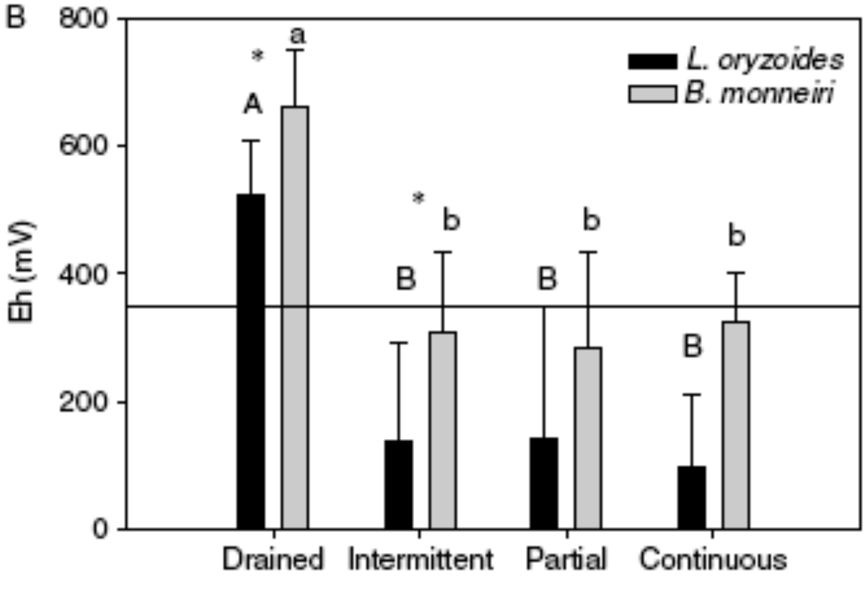

Figure 1 B. Eh at 30 cm depth day 30.

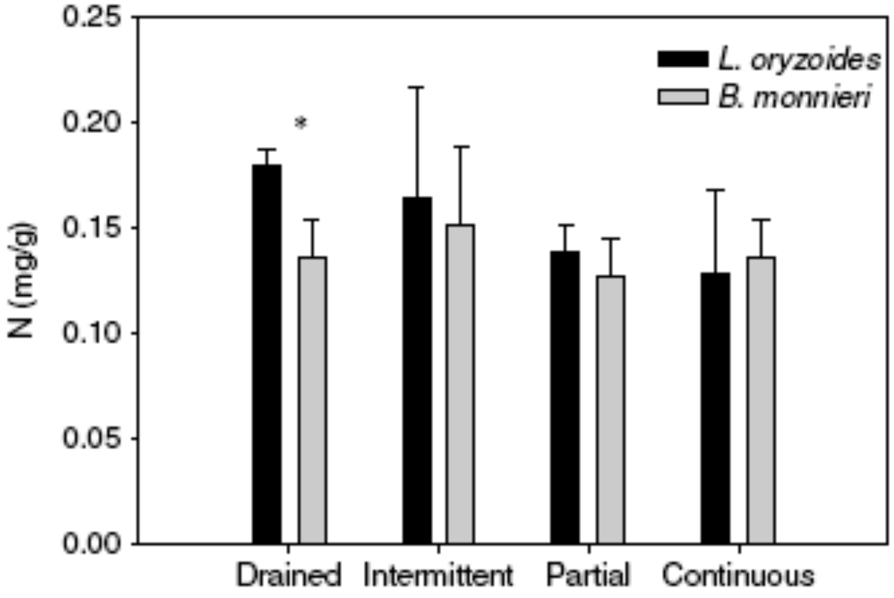

Figure 2. Soil N 15-30 cm depth.

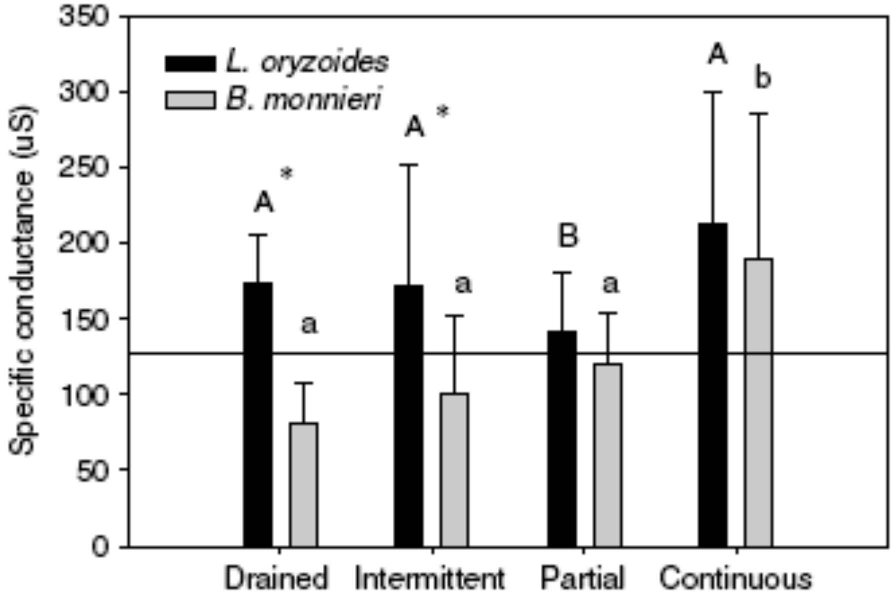

Figure 3. Specific conductance

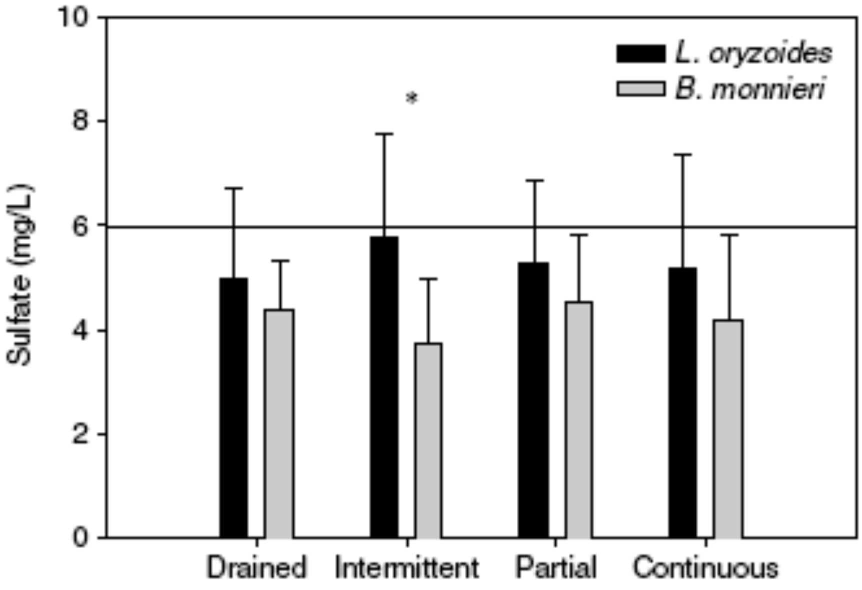

Figure 4. Sulfate concentration

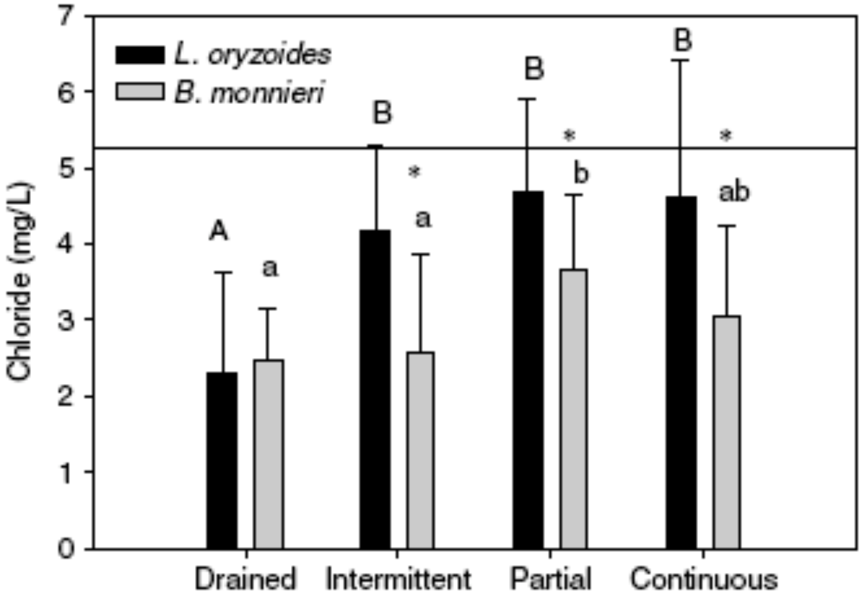

Figure 5. Chloride Concentration

Chapter 6

Conclusions

A series of laboratory and greenhouse experiments compared both plant and system responses of mesocosms undergoing a range of flooding treatments and planted with either *Bacopa monnieri* or *Leersia oryzoides*. The conclusions from these experiments are synthesized below.

Photosynthesis in *L. oryzoides* decreased under flooded conditions when soil was reduced below the critical oxygen threshold (Eh = +350 mV). However, mechanisms for this decrease may differ depending upon whether flooding is continuous, periodic or partial. Although all flooded treatments eventually showed decreases in net production (Pn), plants in the Continuously Flooded treatment showed no difference in gs, implying that photosynthetic rates may be limited by non-stomatal factors in this species when repeatedly exposed to extended periods of flooding. This non-stomatal limitation is not reflected in Photosystem II (PSII) activity, as demonstrated by efficiency of excitation capture of open Photosystem II (Fv/Fm) data. Because Fv/Fm represents the capacity of PSII, rather than actual energy absorbed, it is indicative of photochemical processes that are regulated over a longer time period than gas exchange measures such as Pn. Comparisons of Fv/Fm data to gas exchange data indicated that while soil Eh below the critical oxygen threshold results in a short-term stress response, damage to the photosynthetic apparatus for this species is negligible under moderate soil reduction.

Belowground biomass in *L. oryzoides* was unaffected by the moderate soil reduction in this study, while aboveground biomass was significantly greater in

Continuously Flooded plants than Control plants. Root penetration depth was unaffected by flooding. The fact that moderate soil reduction had no effect on root penetration and biomass in *L. oryzoides*, when taken in consideration with the dense, fast growing colonies formed by this species, suggests that it may serve a valuable role in stabilizing soils in and adjacent to agricultural ditches.

Although root penetration depth was unaffected by flooding, rhizome penetration depth was decreased in Continuously Flooded plants. In other words, belowground stems were not penetrating or elongating into the anoxic zone. It is well established that root penetration depth can differ between flooded and non-flooded plants; however, differences in depth of rhizome penetration have not been previously reported. The observed difference in rhizome penetration depth due to flooding has two potential implications with regard to the ecology of *L. oryzoides* . First, continuous flooding of *L. oryzoides* may reduce belowground carbon storage capacity. Our data fail to support this notion, as belowground biomass was unaffected by flooding. Alternately, the rhizome may be functioning primarily for vegetative propagation rather than for storage, with rhizomes preferentially growing toward more favorable conditions when the mother plant is stressed. Such a pattern of rapid vegetative spread would be advantageous for an early successional species in an environment where much of the ground is bare. Further study of this species is needed to determine what portion of the belowground biomass is composed of root tissue versus rhizomatous tissue under different water regimes and the role that rhizomes play in storage and/or processing of the carbohydrate pool, as well as the functioning of clonal integration under variable flooding.

B. monnieri plants in the Control treatment demonstrated moderately lower biomass than flooded treatments, a result not uncommon for wetland plants. Shoot concentrations of N and P were higher in Control than in flooded *B. monnieri* plants, with mass and nutrient concentrations being negatively correlated. This relationship may be partially explained by increases in shoot mass as a sink for nutrients without a concomitant increase in root uptake capacity. However, the trend toward lower plant nutrient uptake in flooded treatments suggests that flooding either decreased the availability of these two nutrients in the rhizosphere, or reduced plant uptake capacity. Root penetration depth of *B. monnieri* was affected by flooding, likely due to anoxia or reducing conditions in flooded soils. Changes in root penetration due to flooding are often related to the number of fine roots and root tips, the areas of major interface for nutrient exchange. Decreases in these absorption centers would be most pronounced for nutrients with low solubility, such as P, whereas effects on N would probably be minimal for *B. monnieri*.

Values for soil redox potential indicated that anoxic conditions developed in the bulk soil by midway through the study and persisted through the remainder of the study in the Partially Flooded and Continuously Flooded treatments. Soil reduction may have lowered N availability via a general shift in microbial activity resulting in a concurrent shift in Nitrogen transformations from NO_3^-, the nitrogen species preferred by terrestrial plants, to less available forms such as NH_4^+or diatomic nitrogen (N_2). While *B. monnieri* acquires NH_4^+ and NO_3^- when both are present, NH_4^+ uptake -and thus total N uptake- is decreased in the absence of NO_3^-. In soils, as opposed to nutrient solutions, these effects are likely to be compounded by the cationic attraction of NH_4^+ to negatively-charged soil micelles. In the present study, the possibility of decreased N uptake resulting from lower concentrations of NO_3^- is supported by low concentrations of NO_3^- in effluent from flooded mesocosms (Chapter 4).

Phosphorus is relatively insensitive to redox changes, but availability to plants is often inversely related to redox potential. Soil reduction dissociates insoluble metal-phosphate complexes, often resulting in an increase in plant-available P. Therefore, although decreases in plant tissue concentrations of N may be directly related to changes in plant availability, decreased Eh is unlikely to directly increase the availability of P without intermediary mechanisms. pH is known to affect plant-available P, with alkaline conditions being generally unfavorable for P uptake. pH values measured from mesocosm effluent were greater than pH = 7.2, indicating conditions of low plant available P. However, as these pH conditions were measured in effluent, rather than derived from rhizosphere soil samples, they are probably not accurate representations of the root environment. This disparity may be especially true for *B. monnieri*, as it exudes substantial amounts of protons into the rhizosphere, resulting in rhizosphere acidification. The manner in which such root exudates affect soil environments is complex, often involving feedback between soil microorganisms and chemical conditions; however, if decreases in pH of the bulk soil result from proton exudation into the rhizosphere, the solubility of metals as well as PO_4^- would be expected to increase.

Because the anoxic environment can be stressful for plants, many wetland plants have internal aeration ducts (lacunae) to convey oxygen into the rhizosphere. Species differences in influencing soil Eh may reflect a greater ability in *B. monnieri* for rhizosphere oxidation. Both root and shoot tissues appeared highly porous compared to

L. oryzoides. Alternately, these differences may be the result of *L. oryzoides* indirectly promoting soil reduction either through rhizosphere exudation of organic carbon, or through facilitation of microbial growth. Roots of *L. oryzoides*, for example, have been found to harbor populations of Fe–oxidizing bacteria, which have been implicated in redox reactions in the soil.

Because there was no unplanted treatment (bare soil) in the present study it is unclear whether mechanisms leading to soil reduction or soil amelioration via radial oxygen loss are most likely for the observed species differences in soil Eh. Existing research would imply that plants generally increase Eh in flooded soils. Due to the high sand content of the soil used in this study, intense reduction would not be expected in unplanted soil. In addition to changes in Eh, flooding can also affect the pH of soils. A series of chemical transformations occurring in response to reducing conditions in the soil often causes acidification, processes that are exacerbated by rhizosphere acidification resulting from plant interactions with reduced forms of nitrogen and iron.

The decreased nitrogen concentrations measured in the soils of Continuously Flooded mesocosms containing *L. oryzoides* were somewhat counterintuitive. It would be expected that soil reduction would increase NH_4^+: NO_3^- ratios, and as the method for determination of nitrogen concentration in the present study does not include NO_3^-, nitrogen values would subsequently increase. However, such conditions may also enhance ammonification of organic nitrogen and denitrification, leading to the production of gaseous or volatile forms of nitrogen that would diffuse into the atmosphere.

Higher concentrations of NO_3^- and PO_4^{3-} in effluent from *B. monnieri* mesocosms compared with *L. oryzoides* mesocosms are partially due to increased plant immobilization in belowground tissues of *L. oryzoides* resulting primarily from greater biomass in the latter. Previous studies have demonstrated that *L. oryzoides* may act as a phosphorus source or a sink. With regard to nitrogen, *L. oryzoides* appears to be more limited in its nutrient removal capability. In the present study, differences in effluent NO_3^- may be indicative of nitrogen species transformation from NO_3^- to reduced forms, as soils planted with *B. monnieri* remained above the threshold for this transformation (i.e. Eh ≈250) through day 30, whereas soils planted with *L. oryzoides* approached this threshold within nine days of flooding.

When iron-rich soil, such as the soil used in this study, is flooded for an extended time, the reduction of Fe (III) to soluble Fe (II) results in the dissolution of iron-phosphate complexes in the soil, yielding soluble PO_4^{3-}. While water concentrations of PO_4^{3-} would generally be expected to increase in response to flood-induced soil reduction, no such response was observed. Concentrations of PO_4^{3-} in *B. monnieri* were actually lower in all flooded treatments than controls. Previous studies suggest that the diffusion of oxygen to the rhizosphere via lacunae may lead to the oxidation of Fe (II) and subsequent co-precipitation of phosphorus along the root epidermis and cortex. This process likely explains higher levels of phosphorus in belowground tissues of *L. oryzoides*. The mechanisms explaining phosphorus differences in effluent water from *B. monnieri* are not so straightforward, but may involve subtle rhizosphere pH differences between treatments influencing predominant forms of P.

The synthesis of effects on the general chemical environment resulting from hydrologic character and plant species, contributes incremental advances in the understanding of the physiological responses of two common wetland plant species to a range of flooding treatments. Specifically, information on belowground versus aboveground nutrient allocation in response to flooding is rare, especially in studies where important soil chemistry parameters such as Eh are quantified.

Additionally, these experiments provided evidence for a mechanistic basis for *L. oryzoides* regulating seasonal phosphorus release in intermittently dry agricultural drainage ditches. *L. oryzoides* provided a novel response to flooding in that under continuous flooding, even though root biomass or penetration depth was unaffected, penetration of rhizomes into the soil was decreased. To the author's knowledge, this is the first record of rhizome penetration depth being affected by soil flooding. The most valuable advances provided by these studies, however, lay in the interpretation of the interactions between the species planted in the mesocosms and the flooding treatments. Specifically, the different effects of Eh, pH, and specific conductivity indicated that the overall character of the soil and water is subject to plant-mediated interactions with hydrology.

Both plant species interacted with their immediate environment to fundamentally change the soil and water chemistry. Furthermore, the flooding treatments and plant species demonstrated statistically-significant interactions. This interactive response indicates that the different acclimation strategies of plants to flooding-induced changes in soil and water chemistry may lead to either a positive or negative feedback response depending upon the plant's acclimation response, soil oxygen demand, and which chemical properties are being considered. Although previous research has demonstrated species-specific effects on soil and/or water, this is the only experimental study to the author's knowledge that demonstrates such interactive effects on a number of soil and water properties. Consideration of such interactions has been largely overlooked in efforts to manage aquatic resources in favor of more empirical approaches.

This line of study is especially important when placed in the context of small drainage ditches in agricultural areas. These ditches are considered a major conveyance of non-point source pollution, and a number of management practices are under consideration for reducing pollution into receiving streams. Because many of these management practices involve promoting vegetative growth along with altering flow patterns and relative water levels, a basic understanding of the environmental physiology of predominant plant species in these ditches is essential for science-based policy decisions. Furthermore, consideration of plant-environment feedback responses have not been previously considered, although this study indicates that such responses may result in profound changes in the immediate environment of the plant, which, in turn may affect system-level processes.

Future Research Directions

Plant environmental physiology may be broadly defined as the physiological response of plants to their environment, and has historically focused on a whole-plant response to limiting resources or environmental stress, including physical, chemical, and biotic factors. These plant responses are then placed in the context of habitat. Because plants are sessile organisms, initially determining the habitats they utilize is a relatively straightforward process. Research in environmental physiology in the 20th century relied upon advances in both basic physiology and ecology, while, in turn providing concise explanations for the response of a plant in a given setting. One natural extension of this line of research employs molecular techniques to discriminate how a given biochemical pathway in a plant allows for acclimation in one species, while another species lacking that biochemical pathway is unable to acclimate.

Research bridging molecular biology and environmental response has resulted in remarkable advances demonstrating that different species may have a similar physiological response to a given stress, whereas the biochemical pathways governing those responses may differ markedly. Given the myriad stresses in agricultural drainage ditches and the ever-increasing variety of chemical inputs into agricultural land, research into understanding the molecular pathways that result in stress or benefit to a given plant species is likely to extend beyond the traditional borders of weed management and crop productivity.

Another example of refinement of the whole-plant approach that has yielded a greater understanding of how plants cope with variable resources relates to increased precision in studies examining the allocation of such resources, primarily carbon and macronutrients. For example, if *L. oryzoides* increases belowground:aboveground ratios of phosphorus in reduced soils, is that phosphorus located primarily in fine roots or rhizomes? These studies may be especially telling when they incorporate temporal information into the analysis, as many plants are known to allocate carbon and nitrogen seasonally.

While these studies represent an extension of the classical environmental physiological approach, they also provide a basis for understanding how plants may elucidate systems-level responses. It is well-known, for example, that leaf-litter is a driving force in the soil chemistry and ecology of deciduous forests. It is somewhat paradoxical that comparatively little is known regarding how roots affect the soil environment. In particular, how root amelioration of an unfavorable rhizosphere affects the bulk soil and the extent of these acclimation responses to whole-plant functioning is open to debate. To some extent, the classical whole-plant approach to environmental physiology limits the understanding of how plants are actually functioning in their environment.

This statement is based on two suppositions: 1. A single plant may experience optimal conditions in one portion of the root system, canopy, etc., while another portion experiences suboptimal conditions. 2. Because plants demonstrate modular growth, individual portions of the plant, may demonstrate a localized response to increase overall fitness. This localized response, may or may not result in an overall acclimation response. While interesting from a basic science perspective, this line of research may additionally be important in understanding how different levels of subsurface saturation interact with plants to affect predominant chemical transformations in the rhizosphere, which, in turn may have systems-level impacts.

Although agricultural drainage ditches likely comprise more linear stream distance in lowlands of the Lower Mississippi Alluvial Valley than naturally formed, non-impacted streams, until recently the science of and management of these ditches has only been cursory. Recently, the U.S. Department of Agriculture's National Resources Conservation Service (NRCS) has adopted a broad set of best management practices (BMPs) for drainage ditches in the state of California. Similar BMPs are currently under consideration in both Mississippi and Arkansas. These BMPs only set forth basic guidelines for work in ditches including recommendations for erosion control and vegetation establishment. The guidelines for vegetation establishment, however, generally follow procedures developed for grassed buffer strips, and do not take into account saturation or inundation of the soil.

As part of a larger effort the Mississippi River Basin Healthy Watershed Initiative has allocated $320 million to NRCS offices in 11 states for use in watershed-level measures aimed at improving water quality in agricultural drainages. This initiative will provide a unique opportunity to study the successes and failures of efforts to improve water quality in agricultural areas, including the primary intercept for agricultural run-off represented by drainage ditches. It is hoped that examination of the functioning of ditch vegetation at multiple spatial and temporal levels will remove some of the guess work associated with in-field practices, and transition management from personal judgment based on anecdotes to general principles based on science.