Effect of Phosphorus Concentration on Growth of *Muhlenbergia capillaris* in Flooded and Non-Flooded Conditions[1]

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Abstract

Research was conducted to evaluate the effect of phosphorus (P) concentration in irrigation water on growth of a southeastern U.S. native grass *Muhlenbergia capillaris* in flooded and non-flooded conditions. Plants of *Muhlenbergia capillaris* (Lam.) Trin. (gulf muhly grass) growing in 3.8 liter (1 gal) containers in 85:15 sand:peat were flooded to the substrate surface for 0 (non-flooded) or 3 days (flooded). Between flooding events, plants were drained for 6 d with no additional irrigation. The flood-drain process was repeated five times. Non-flooded plants were hand watered as needed. Plants were irrigated (non-flooded) or flooded with one of several tap water solutions, each with a different P concentration ranging from 0 to 0.8 mg·liter^{-1} (ppm) P (hereafter referred to as P irrigation rate). The experiment was repeated once (total two runs). Shoot dry weight (SDW) root dry weight (RDW) were higher in non-flooded plants than in flooded plants in both runs. Shoot dry weight increased linearly with an increasing P irrigation rate, while RDW changed cubically with increasing P irrigation rates in run 1 (no effect on either in run 2). Phosphorus concentration in leachate increased with increasing P irrigation rate in run 1 but not in run 2. Phosphorus concentration in leachate was usually higher in flooded plants than in non-flooded plants in both runs. All plants maintained root and shoot growth when flooded suggesting *M. capillaris* would be appropriate native species for rain gardens or bioretention areas. Phosphorus concentrations in leachate were lower than what was applied indicating P was removed via plant uptake or substrate adsorption or both.

Index words: water, rain garden, bioretention, inundation, native, sustainable, landscape.

Species used in this study: *Muhlenbergia capillaris* (Lam.) Trin. (gulf muhly grass).

Significance to the Nursery Industry

Rain gardens and bioretention areas have become an accepted landscape practice in commercial and residential developments used to remediate stormwater runoff. Information is needed for nursery producers regarding native plants that may be effectively used to remove nutrient loads in stormwater runoffs in these catchment systems. Results of this research indicate that the native grass, *Muhlenbergia capillaris* is an appropriate plant species for use in rain gardens. Additionally, rain gardens or bioretention areas utilizing a sand:peat (85:15 by vol) substrate and planted with *M. capillaris* are effective for remediating at least some of the P in stormwater runoff.

Introduction

Phosphorus (P) is now one of the most ubiquitous forms of water quality impairment in the developed world and thus is receiving increased research attention (17). Nutrients not taken up by plants move through the soil to surface waters or subsurface waters. Movement of small amounts of P through the soil profile can have adverse effects on water quality and cause excessive enrichment of surface soils (15). Phosphorus enrichment of the soil surface layer can result in increased P losses from soil to surface waters when soil particles move to storm drains or are lost as a result of surface erosion (15, 16). The movement of P from soil into surface runoff and subsurface flow during a rain event can result in storm P loads often twice that of corresponding non-storm loads (4, 21). The primary loss of P from soil occurs through erosion and runoff of particulate P from lands affected by agricultural and urban activities (17, 18). These areas are typically where high soil P or P application via mineral fertilizer or manure coincide with high runoff or erosion potential (17). Repeated cycles of soil saturation followed by infiltration and then reflooding can also result in significant P release from soils (13). Soil sediment characteristics are important since sediments act as either sources of or sinks for nutrients within the water column (13). While erosion of particulate P from soils remains a dominant concern, the transport of dissolved or soluble P in surface runoff and subsurface flow is also a critical environmental issue (17).

Eutrophication caused by excessive inputs of nitrogen (N) and P is one of the most common impairment of surface waters in the United States (6). Phosphorus can be removed in bioretention areas by plant uptake, assimilation by microorganisms, sorption by soil substrate, or precipitation as insoluble compounds (3, 19). The effect of flooding or inundation on P uptake by plants is complicated and dependent upon soil or substrate type. In some alkaline soils that are relatively low in P, flooding may cause an increase in P availability, leading to a temporary increase in P uptake by plants. On the other hand, prolonged flooding can reduce P

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uptake by plants due to root dysfunction, damage, and death (2, 9). Upon flooding, a flood-tolerant species will generally acquire more minerals than flood-intolerant species (2, 14).

Rain gardens have become an accepted landscape practice in commercial and residential developments. Rain gardens are bioretention areas in the landscape designed to catch stormwater runoff and facilitate infiltration and treatment (7). In previous work, P concentrations in a pond that captured stormwater runoff typically ranged from 0.01 to 0.8 $mg \cdot liter^{-1}$ (ppm) P (5). When this stormwater was added to rain gardens, the concentration of P in the outflow from the rain garden was reduced. To elucidate uptake contributions from plants, research is needed to evaluate individual plant species for their ability to absorb P under flooded conditions. Therefore, the objective of this research is to evaluate the effect of P concentration in irrigation water on growth of a southeastern U.S. native grass *Muhlenbergia capillaris* (gulf muhly grass) in flooded and non-flooded conditions.

Materials and Methods

Run 1. On November 17, 2009, 140 [0.25 liter (2.25 in) liners] *Muhlenbergia capillaris* from Magnolia Gardens Nursery, Magnolia, TX, were planted into 3.8 liter (1 gal) containers filled with 2.5 liters (0.7 gal) substrate of 85:15 sand:peat by vol (8) amended with 1.2 $kg \cdot m^{-3}$ (2 $lbs \cdot yd^{-3}$) dolomitic limestone and 0.9 $kg \cdot m^{-3}$ (1.5 $lbs \cdot yd^{-3}$) micronutrient fertilizer (Micromax, Scott's Company, Marysville, OH). Plants were placed on a full sun nursery pad at the Paterson Horticulture Greenhouse Complex (PHGC) on the campus of Auburn University in Auburn, AL. Plants were top dressed on December 1, 2009, with 5.1 g (0.18 oz) 43N-0P-0K (Harrell's Professional Fertilizer Solutions, Sylacauga, AL) per container and 3.7 g (0.13 oz) 0N-0P-52.3K (Piedmont Fertilizer, Opelika, AL) per container. No P was added to the substrate. During the overwintering period, plants received daily overhead irrigation of 0.75 in (1.9 cm). At temperatures of –4C (25F) or lower, plants were covered with white polyethylene plastic with ventilation holes.

On March 30, 2010, (133 DAP) plants were moved into a greenhouse and placed on raised benches at the PHGC. Plants were grown in an unshaded 8 mm (0.3 in) twin-wall, polycarbonate covered greenhouse under natural photoperiods with a heating set point of 18.3C (65F) and ventilation beginning at 25.6C (78F). Flooding treatments were initiated on April 13, 2010. Plants were flooded using a pot-in-pot method. The container with the plant (with holes in the bottom to allow for drainage) was placed inside another container of the same size without drainage holes. To flood a container, tap water was added by hand to the container until the water level was even with the substrate level; additional water was added to the container daily as needed to keep the water level even with the substrate surface. Approximately 600 mL (0.2 gal) was added initially to each container to flood plants, and approximately 100 mL (0.03 gal) was added daily to each container to maintain the flood level. Plants were flooded for 0 (non-flooded) or 3 days.

Following flooding, plants were allowed to drain for 6 days by removing the outer container. No additional water was added to the flooded plants during the 6 days of draining. The flood/drain cycle was repeated for four additional cycles (total five cycles over six weeks). Non-flooded plants were irrigated by hand as needed. For each flood/drain cycle, plants were irrigated (non-flooded) or flooded with one of seven solutions, each with a different P concentration (source of P was 85% ortho-phosphoric acid, Fisher Scientific, Pittsburg, PA). Solutions contained 0, 0.1, 0.2, 0.3, 0.4, 0.5, or 0.8 $mg \cdot liter^{-1}$ (ppm) P (hereafter referred to as P irrigation rate). At the end of the 1st, 3rd, and 5th flood cycles, leachate was collected from five flooded and five non-flooded plants in each P irrigation rate. Leachate was collected from non-flooded plants using the Virginia Tech Pour-Through Method (20). Each pour-through of non-flooded plants was conducted by applying 500 mL (16.9 oz) of the same solution used for irrigation to the container. For flooded plants, leachate was collected from water that drained from the container when the exterior pot was removed. Leachate samples were placed in 125 mL (4.2 oz) amber bottles (Fisher Scientific, Pittsburg, PA) and stored in a cooler at 5C (41F) until analysis. Leachate water samples were analyzed using inductively coupled plasma spectrometry (ICP) (SpectroCiros CCD, Kleve, Germany). Run 1 was terminated on June 1, 2010 (195 DAP). Shoots were severed at the substrate level, and roots were rinsed to remove substrate. Shoots and roots were dried separately in an oven for 48 hours at 66C (150F), and shoot dry weight (SDW) and root dry weight (RDW) were determined.

Run 2. The above experiment (run 1) was repeated with the following similarities or modifications. On May 24, 2010, 96 [0.25 liter (2.25 in) liners] *M. capillaris* from Stepping Stone Nursery, Homestead, FL were potted into 3.8 liter (1 gal) containers as described above (substrate and amendments the same). All plants were top dressed with 5.1 g (0.18 oz) of 43N-0P-0K (Harrell's Professional Fertilizer Solutions, Sylacauga, AL) per container and 2.9 g (0.1 oz) 0N-0P-52.3K (Piedmont Fertilizer, Opelika, AL) per container. Plants were grown in a greenhouse as described above. Flooding treatments were initiated on June 7, 2010, (14 DAP) and were applied as described above. Solutions used for irrigation or flooding included 0, 0.2, 0.4, or 0.8 $mg \cdot liter^{-1}$ (ppm) P. Leachate was collected and analyzed as described above. The experiment was terminated and plants were harvested on July 20, 2010, (58 DAP) as described above. Shoot dry weight and RDW were determined as described above.

In both runs, plants were arranged in a completely randomized design. Treatments were in a P irrigation rate × flooding factorial combination for a total of 14 treatments in run 1 and 8 treatments in run 2. In run 1, there were nine replications per treatment; in run 2 there were six replications per treatment. Data were analyzed separately by run for significance of treatment main effects and interactions and regression analysis using Proc GLM with mean separation performed where appropriate using PDIFF ($P < 0.05$) (SAS Institute, Cary, NC). Leachate P concentrations were analyzed separately for each time of collection (1st, 3rd, or 5th flood cycle). Treatment main effects are presented when interactions were not significant.

Phosphorus isotherm. In order to characterize the P adsorption capacity of the substrate used, a P sorption isotherm was developed for the sand-peat substrate using solutions containing 0, 0.01, 0.05, 0.1, 0.2, 0.5, 1, 5, 10, 20, or 50 $mg \cdot liter^{-1}$ (ppm) P and based on techniques of Nair et al. (11) and Litaor et al. (10). The isotherm was determined using a substrate: P solution ratio of 2 g (0.07 oz):25 mL (0.85 oz). The suspension was equilibrated for 24 h in polyethylene tubes on a reciprocal shaker, then centrifuged at 2500 × *g*

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Table 1. Effect of flooding duration and concentration of phosphorus (P) in flooding or irrigation water (P-rate) on shoot dry weight (SDW) and root dry weight (RDW) of *Muhlenbergia capillaris* grown in a greenhouse April to June (run 1) and June to July (run 2).

Run 1		
Flooding duration (days)	**SDW (g)**	**RDW (g)**
0	6.2a[z]	5.1a
3	4.2b	4.4b
P-rate (mg·liter^{-1})		
0.0	4.8	5.3
0.1	5.0	3.7
0.2	4.7	4.1
0.3	4.4	4.8
0.4	6.0	4.9
0.5	5.1	5.5
0.8	6.2	4.8
Significance[y]	Linear (P = 0.0230)	Cubic (P = 0.0002)
Equation	y = 1.77x + 4.58	y = –42.7x^3 + 50.9x^2 – 13.8x + 5.1
Run 2		
Flooding duration (days)	**SDW (g)**	**RDW (g)**
0	6.4a	5.1a
3	4.9b	2.0b

[z]Lowercase letters denote mean separation between flooding duration using PDIFF P < 0.05 (SAS Institute, Cary, NC).

[y]Regression analysis using Proc GLM (SAS Institute, Cary, NC).

for 30 min. Samples were then filtered through 0.45 µm filter paper and analyzed for P using ICP.

Results and Discussion

In both runs, SDW and RDW were higher in non-flooded plants (0 day flood duration) than flooded plants (3 d flood duration) (Table 1), however even under flooded conditions, the plants continued to produce new root and shoot growth. *Muhlenbergia capillaris* is a facultative upland plant in the southeast and usually occurs in non-wetlands but occasionally is found in wetlands (12). Results of this research indicate that *M. capillaris* appears to be able to tolerate the challenges of fluctuating hydrology as would be expected in a rain garden including short periods of inundation followed by drying. Flooded plants in this study did not receive any irrigation during their draining period to allow the substrate to dry. Examining the extent of drought tolerance of this taxon may provide additional information regarding its suitability for use in rain gardens.

In run 1, SDW increased linearly with an increasing P irrigation rate, while RDW changed cubically with increasing P irrigation rates (Table 1). Although statistically significant, this cubic trend may not be biologically significant but instead indicates the lack of consistent effect of P irrigation rate on root growth. There was no effect of P irrigation rate on SDW or RDW in run 2 (data not shown). Results suggest that the low rates of P irrigation rate used in this study do not affect plant growth as dramatically as does hydrology. The P supplied by irrigation or flood water or the substrate appeared sufficient to support plant growth and survival since regardless of treatment, no plants appeared nutrient deficient (visual observation), and no plants died. In treatments where no P was added, P was still detected in leachate suggesting the presence of P in the substrate initially that was subsequently released over time (Table 2).

Following the 1st flood cycle in run 1 (April 2010), P concentration in leachate increased linearly with an increasing P irrigation rate in non-flooded plants; there was no effect of P irrigation rate on P concentration in leachate in flooded plants (Table 2). The relatively consistent P concentration in leachate under flooded conditions is likely due to the fact that duration of flooding allowed P sorption to come to an equilibrium between the solution and the substrate. Additionally, due to plant uptake the maximum sorption (see below) of the substrate was not achieved. Following the 3rd flood cycle in run 1 (May 2010), P concentration in leachate was higher in flooded plants [0.07 mg·liter^{-1} (ppm)] than non-flooded plants [0.03 mg·liter^{-1} (ppm)]. Following the 5th flood cycle of run 1 (June 2010), P concentration in the leachate of flooded and non-flooded plants increased linearly with increasing P irrigation rate (Table 2). This increase in P concentration leachate of the flooded plants that occurred over time suggests that by the end of the experiment most if not all substrate sorption sites had been filled. In cases when P irrigation rate had an effect on leachate P concentration, the concentration of P in leachate was lower than that applied via irrigation or flooding, suggesting removal either by the plant or the substrate. The concentrations in P irrigation rates were based on previous studies that used stormwater

Table 2. Effect of flooding duration and concentration of phosphorus (P) in flooding or irrigation water (P-Rate) on concentration of P in leachate of *Muhlenbergia capillaris* grown in a greenhouse, April to June 2010 (run 1, 1st and 5th flood/drain cycles).

	Leachate P (mg·L^{-1})	
	Flooding duration (days)	
P-Rate (mg·L^{-1})	**0**	**3**
April 2010		
0	0.03	0.04
0.1	0.00	0.08
0.2	0.04	0.07
0.3	0.07	0.06
0.4	0.12	0.08
0.5	0.12	0.07
0.8	0.18	0.06
Significance[z]	Linear (P<0.0001)	NS
Equation	y= 0.23x+0.007	NS
June 2010		
0	0.02	0.04
0.1	0.02	0.02
0.2	0.09	0.05
0.3	0.06	0.15
0.4	0.14	0.15
0.5	0.22	0.13
0.8	0.34	0.12
Significance	Linear (P < 0.0001)	Linear (P = 0.0129)
Equation	y = 0.42x – 0.009	y = –0.12x + 0.06

[z]Regression analysis using Proc GLM (SAS Institute, Cary, NC).

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from a local impoundment to irrigate rain gardens at the Davis Arboretum, Auburn University, AL (5), and results of testing local watersheds surrounding Auburn, AL (1). Our results indicate that rain gardens or other bioretention areas utilizing a substrate of 85:15 sand:peat and *M. capillaris* may contribute to P removal from stormwater runoff.

Following the 1st flood cycle in run 2, P concentration in leachate was not affected by P irrigation rate or flooding (data not shown). Following the 3rd and 5th flood cycles P concentration in leachate was higher, in flooded [0.43 and 0.53 mg·liter^{-1} (ppm)] than in non-flooded [0.00 and 0.05 mg·liter^{-1} (ppm)] plants. This is consistent with others (13) who reported that repeated flooding can cause a release of P from soils. Also, as in run 1, P concentration in leachate increased over time as substrate sorption sites filled. In the current research, higher P in leachate from flooded plants than non-flooded plants may also have been a result of the flooded plants receiving additional water containing P each day to maintain flooding. The reason for the lack of significant effect of P irrigation rate on P concentration in leachate in run 2 is not clear. Instead, as in run 1, plant growth seemed to be more affected by flooding than P irrigation rate. Although plant growth in this experiment was not sufficient to remove all P from leachate, it was still reduced compared to the concentration of P in irrigation or flood water.

Phosphorus adsorption isotherms indicated that when P irrigation rate was < 0.5 mg·liter^{-1} (ppm) P, 100% P adsorption occurred (data not shown). For P irrigation rates > 0.5 mg·liter^{-1} (ppm) [0.5, 1, 5, 10, 20, and 50 mg·liter^{-1} (ppm)], adsorption declined with increasing P irrigation rate indicating that the substrate adsorption sites were saturated with adsorbed P. This is reflected in the leachate P concentration in the 1st cycle of run 1 non-flooded conditions (Table 2). Also, due to the continued supply of P to the substrate from both flooding and irrigation, the substrate was not likely to contribute as much to P removal as plant growth and uptake.

In recent decades, much landscape research has focused on xeric systems and plant drought tolerance. Recently, however, pressures are increasing to maintain a clean water supply and manage water resources by remediating stormwater runoff quality. Identification of suitable species for nutrient uptake and flood tolerance are important as a means to manage stormwater runoff catchment systems such as rain gardens, reservoirs, constructed wetlands, and bioretention areas. Identifying appropriate plant materials and substrate or soil mixtures to remediate watershed nonpoint source pollutant concerns such as P will continue to increase in importance as water quality issues and stormwater regulations drive implementation of stormwater practices. The results of this research suggest *M. capillaris* thrives in hydrologic conditions of short periods of flooding followed by periods of soil drying similar to that of a typical rain garden or bioretention system. The ability of *M. capillaris* to maintain root and shoot growth across all treatments in this research indicates that it would be suitable for inclusion in rain garden and bioretention areas. Furthermore, at low P irrigation concentrations P is either taken up by *M. capillaris* or adsorbed to the substrate. Finally, the effect of flooding on concentration of P in leachate suggests that flooding increased plants' length of exposure to P and thus allowed time for plants to adsorb P from solution and decrease leaching.

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