Phosphorus Removal and Accumulation by Sweet Basil (*Ocimum basilicum*) Grown in Floating Treatment Wetlands

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Significance to Industry: Floating treatment wetlands (FTWs) are a useful tool for removing excess nutrients and pollutants from production runoff water at ornamental nurseries. This plant-based treatment system is relatively low maintenance, and when performing optimally, reduces prevalence of nuisance aquatic weeds without the use of chemicals. When FTWs are successful, our surface water resources are protected from increased nutrient pollution, nutrients are removed from the internal cycle of the pond, biomass is harvested for use as a compost material, or plant material is grown for profit while cleaning water. The selection of proper plant species is essential for optimal water filtration. Sweet basil (*Ocimum basilicum*) was screened for its potential to cleanse water and to concentrate nutrients. Potentially FTWs could grow a marketable crop as an alternative marketing system, while recycling nutrients otherwise lost to runoff.

Nature of Work: Runoff from ornamental nurseries typically contains high amounts of phosphorous and nitrogen, which can cause eutrophication in the surrounding bodies of water (1,2). Eutrophication can lead to algal blooms and subsequent declines of water body dissolved oxygen, creating an anoxic environment in which aquatic animals cannot survive (3). Floating treatment wetlands can be deployed in waters too deep for aquatic plants to survive and can adapt to changing water levels (3, 4). Microbial populations colonizing the root systems of plants within FTWs and uptake of nutrients into tissues are two methods by which FTWs facilitate removal of phosphorous, nitrogen, and other fine suspended solids from water (1, 2, 5). Both aquatic and terrestrial plants can be used in FTWs (1). Some plant species can accumulate higher concentrations of nutrients within tissues, thus when selecting plants for inclusion within FTWs, a plants' ultimate capacity to both fix nutrients and facilitate their removal from water are important factors. We evaluated the capacity of sweet basil (*Ocimum basilicum*) established in experimental FTWs, exposed to moderate and high nutrient loading rates, to fix nutrients within tissues and to stimulate remediation of nutrients from aqueous solution.

Eight experimental units (EUs) were established with a mixture of *O. basilicum* 'Genovese' and *O. basilicum* 'Nuphar.' Each EU consisted of a 100 gal Rubbermaid® stock tank, filled with 90 gallons of water on which a 2' x 2' solid core foam buoyant mat with 10 precut holes was floated (50% surface cover). Ten basil liners (5 of each cultivar), roots washed and wrapped with ½" thick coir pressed fiber to stabilize the plant, were placed individually into a 5 cm diameter aerator cup and seated within the foam mat. The leaves of each basil plant were held above the water surface, while the root system was completely submerged.

The moderate (5.22 ± 1.12 mg/L N and 1.15 ± 0.09 mg/L P) and high (14.2 ± 2.36 mg/L N and 2.70 ± 0.17 mg/L P) nutrient loading rates were imposed using a 15-5-15 water soluble fertilizer (The Scotts Company LLC, Marysville, OH) supplied to respective EUs at a 7-day hydraulic retention. Water quality parameters were monitored on a weekly basis and included total nitrogen (TN), total organic carbon (TOC), minerals, pH, dissolved oxygen, and temperature (°C). Total N and TOC were determined via TOC/TN analysis using a Shimadzu TOC-V $_{CPH}$ total organic carbon analyzer with TNM-1 total nitrogen measuring unit (Shimadzu Scientific Instruments, Kyoto, Japan) and mineral concentrations (P, K, Ca, Mg, Zn, Cu, Mn, Fe, S, Na, B, and Al) were quantified via inductively coupled plasma emission spectrophotometer (ICP-ES, 61E Thermo Jarrell Ash, Franklin, MA). Plant height and root length (cm) were measured every seven days. After six weeks, three representative plants were harvested from each EU, weighed, dried, reweighed, and separate root and shoot tissues submitted to the Clemson University Agricultural Service Lab for tissue analysis. We discuss results from both water and plant tissue analysis for total P only, as P is typically the nutrient limiting eutrophication in fresh water systems.

Changes in concentration from influent to effluent as impacted by the presence of FTWs established with *O. basilicum* were quantified, and the influence of fertilizer treatment on the growth of (root length and shoot height) and P accumulation by *O. basilicum* in FTWs were evaluated. Treatment impacts on the above factors were quantified using analysis of variance ($\alpha > 0.05$) and means were separated using Tukey's LSD. Data were analyzed using JMP v10.0 (SAS Institute, Cary, NC).

Results and Discussion: Floating treatment wetlands established with sweet basil facilitated removal of P from simulated production runoff (Fig. 1). Phosphorus export from EUs with the moderate fertilizer treatment was reduced by 64% from influent levels; while P export from High fertilizer treatment was reduced by 36%. Though these reductions were significant ($P < 0.0001$), P concentrations in effluent of both moderate and high treatments were still excessive and could contribute to increased rates of eutrophication, as eutrophication can be stimulated with soluble reactive P concentrations as low as 50 ppb.

Basil plants exposed to both the high and moderate treatments grew over time (Figure 2). Maximum shoot height (harvestable size) was attained between weeks 5 and 6 (Figure 2A). The basil plants exposed to high fertilizer treatments grew more than those plants exposed to moderate fertilizer treatments (Table 1A). Root growth of basil was variable over time for both treatments, with the roots of basil plants exposed to the high treatment growing slightly longer than those of the moderate (Table 1B). We recorded a decline in root length between 27 July and 7 August (Fig. 2B). This senescence of root tissues may have been caused by either temperature stress as water temperatures increased by 3C over that 10 day period (from 27 to 30 C) or by decreased oxygen saturation (2.1 mg/L to 1.7 mg/L) around the root systems associated with higher water temperature during that same time period (6).

The mass of P removed per unit area of FTWs (g/m^2) when basil plants were harvested differed between the high and moderate treatments (*Fertilizer,* Table 2). The mass of P fixed in the shoot system of the basil plants was much greater than the P fixed in the plant root systems (*Organ,* Table 2, Fig. 3). More P was fixed in the shoots of basil plants exposed to the high fertilizer treatment than plants exposed to the moderate fertilizer treatment, while a similar mass of P was fixed by the root systems, without regard to fertilizer treatment.

When considering plant harvest as a means to facilitate complete removal of nutrients from aquatic systems, it is important to differentiate how plants partition nutrients. Because basil plants fixed the majority of absorbed P in their shoot systems, plant harvest within these FTWs could involve successive shoot harvest and continued regrowth from the root system. Despite the greater mass of P fixed in the shoot system of the basil plants exposed to the high fertilizer treatment, only 36% of the loaded P was removed, the effluent P concentration could still induce deleterious environmental effects associated with eutrophication. Two options could enhance the rate of P removal and include additional retention time within the pond system before water release and/or additional surface area coverage with planted FTWs.

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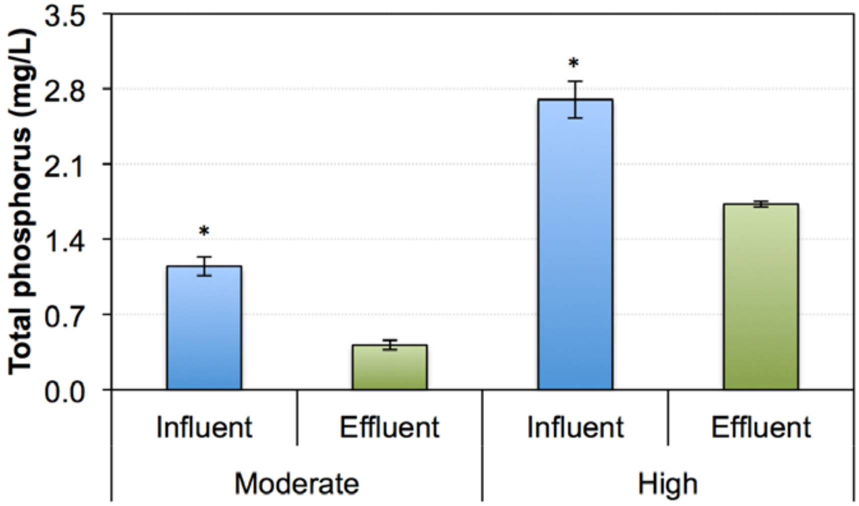

Figure 1. Change in total P concentration of influent and effluent as influenced by floating treatment wetlands established with Sweet basil (*Ocimum basilicum*). High and moderate fertilizer loads were refreshed weekly and the experiment was conducted over a 6 week period. Bars represent mean values for influent and effluent P concentration over time ± the standard error of the mean. The * designates a significant difference between influent and effluent values ($P < 0.0001$) for both moderate and high fertilizer treatments.

Table 1: Shoot height (A) and root length (B) of Sweet basil (*Ocimum basilicum*) established in floating treatment wetlands for 6-weeks as impacted by fertilizer (high, moderate). Means with the same letters are not significantly different, Student's t test ($\alpha \leq 0.05$).

		2-Way ANOVA	DF	*P > F*
A	Shoot	Week	1	< 0.0001
		Fertilizer	1	< 0.0001
		Week x Fertilizer	1	0.0478
		Level	*LS Mean*	
		High	36.8 A	
		Moderate	29.0 B	
B	Root	Week	1	0.0067
		Fertilizer	1	0.0050
		Week x Fertilizer	1	0.7661
		Level	*LS Mean*	
		High	18.0 A	
		Moderate	15.4 B	

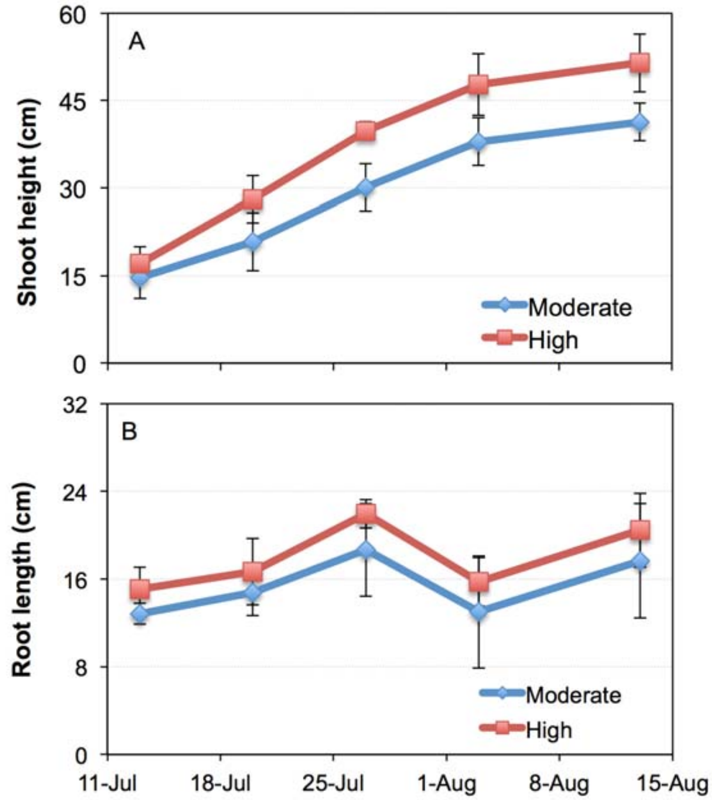

Figure 2. Shoot (A) and root (B) growth (mean ± standard error) of Sweet Basil (*Ocimum basilicum*) established in floating treatment wetland over a 6-week period after exposure to 2 fertilizer loading rates (moderate and high).

Table 2: Changes in phosphorus accumulation (g/m^2/6 weeks) and allocation within Sweet basil (*Ocimum basilicum*), grown in floating treatment wetlands over a 6-week period, as influenced by fertilizer (moderate and high), and organ (shoot and root).

2-Way ANOVA	DF	*P > F*
Fertilizer	1	< 0.0001
Organ	1	< 0.0001
Fertilizer x organ	1	< 0.0001

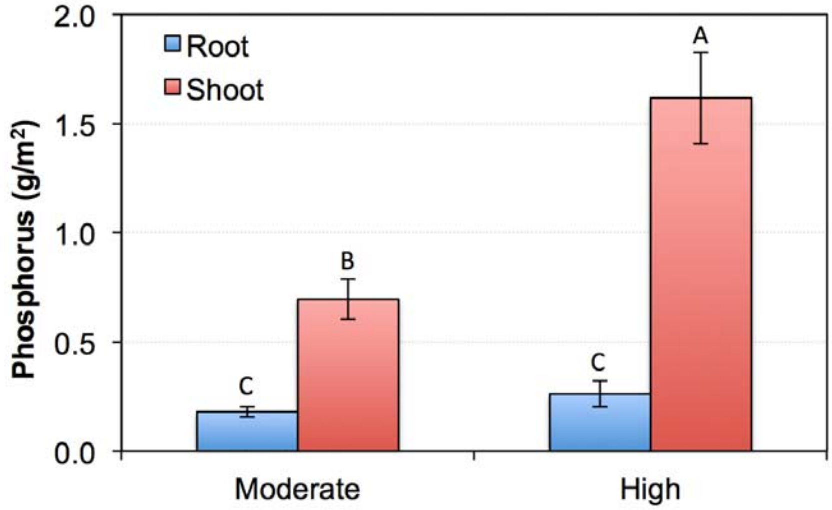

Figure 3. Total phosphorous content (mean ± standard error) of shoots and roots of Sweet basil (*Ocimum basilicum*) grown in floating treatment wetlands and exposed to either moderate or high nutrient loads over an 6-week experimental period. Means with different letters are significantly different, Student's *t* test ($\alpha \leq 0.05$).