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# A test of four plant species to reduce total nitrogen and total phosphorus from soil leachate in subsurface wetland microcosms

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## Abstract

Four wetland plant species (*Scirpus validus*, *Carex lacustris*, *Phalaris arundinacea*, and *Typha latifolia*) were grown in monoculture and as a four-species mixture to compare effectiveness of nutrient removal in controlled 18.93-l outdoor subsurface treatment wetland microcosms. A nutrient treatment that mimicked single-resident domestic effluent consisted of two levels of nitrogen (N) and phosphorus (P) [low (56 mg/l N and 31 mg/l P) and high (112 mg/l N and 62 mg/l P)] of nutrient solution applied three times weekly. The plants were established and maintained for one year before the nutrient treatment and monthly water sampling commenced; water sampling began July 31, 2001 and ended October 23, 2001. We tested four hypotheses: (1) vegetated microcosms are more effective at reducing concentrations of total N and total P from soil leachate than unvegetated, (2) there is a differential species effect on the potential to reduce N and P, (3) plant mixtures are more effective than monocultures at reducing N and P, and (4) the microcosms will be least effective at reducing N and P concentrations in October compared to August. We found support for hypotheses 1, 2, and 4, but our results are inconclusive for the third hypothesis. Total N and total P in the soil leachate were significantly higher from unvegetated microcosms compared to vegetated. *S. validus* was most effective and *P. arundinacea* was generally least effective at reducing N and P in monocultures, with treatment capabilities similar to unvegetated microcosms. The four-species mixture was generally highly effective at nutrient removal, however the results were not significantly different from the monocultures. At the end of the growing season (October) treatment efficiency was significantly less than earlier months, especially for the unvegetated treatment.
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## 1. Introduction

The characteristic properties of wetlands make them unique ecosystems; they contain anoxic soils, have varying hydrology, distinct nutrient cycling and are composed of plants tolerant of flooded conditions. Since wetlands are often found as a transitional zone between aquatic and terrestrial ecosystems, they can receive a wide array of dissolved substances through storm water runoff as well as through rivers, streams and water channels. Wetlands are able to transform and reduce these compounds, so they have been utilized for water treatment (Kadlec and Knight, 1996).

Widespread degradation of aquatic environments including lakes, streams and even the oceans can be attributed to the increase of agricultural practices, such as fertilization, tilling, pesticides and herbicides (Ratcliffe, 1984; Hooda et al., 2000). An increasing human population generates a greater agricultural demand and a concomitant increase in agricultural runoff. Increases in wastewater, whether agricultural, municipal or industrial create challenges for those seeking cost effective treatment methods to process this wastewater (Fraser et al., 2003; Steer et al., 2003). Wetlands have been constructed in order to engineer secondary water treatment facilities, and scientists have explored the

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fundamental biogeochemical processes that enable wetlands to treat this wastewater, and the utility of phytoremediation—the use of plants to treat wastewater (Brix, 1997; Carmen and Crossman, 2001).

As the numbers of constructed wetlands grow, an increasing amount of research throughout the world is being conducted in order to evaluate the efficiency of treatment wetlands (e.g. Steer et al., 2002 [Ohio, USA]; Nerella et al., 2000 [Texas, USA]; Badkoubi et al., 1998 [Iran]; Haberl et al., 1995 [Europe]; Greenway and Simpson, 1996 [Australia]). In Ohio, USA, 21 subsurface flow single-family treatment wetlands have been monitored throughout the northern half of the state for both organics and nutrient removal (Steer et al., 2002). The domestic treatment wetlands can reduce output of fecal coliform $88 \pm 27\%$, total suspended solids $56 \pm 53\%$, biochemical oxygen demand $70 \pm 48\%$, ammonia $56 \pm 31\%$ and phosphorus $80 \pm 20\%$ (Steer et al., 2002). Another study in Texas monitored eight household constructed subsurface flow wetlands (Nerella et al., 2000). After one year, the BOD removal efficiency was between 80% and 90%, however nutrient reduction was relatively low, below 40% (Nerella et al., 2000).

In all these published constructed wetland experiments there is a very wide range in treatment efficiency between wetlands and many of the experiments failed to meet all governmental standards, especially for total nitrogen and phosphorus (e.g. Rogers et al., 1991; Steer et al., 2002). In order to discover how to more efficiently construct treatment wetlands smaller scale mesocosm and microcosm experiments have been initiated to test the plant species composition (Gersberg et al., 1986; Tanner, 1996; Coleman et al., 2001).

The purpose of this research was to determine the relative effect specific plant species, as well as a four-species plant mixture, have on the reduction of total N (nitrogen) and total P (phosphorus) from the soil leachate in subsurface wetland microcosms. The four plants selected for our experiment were *Carex lacustris* Willd. (lake sedge), *Scirpus validus* Vahl. (softstem bulrush), *Phalaris arundinacea* L. (reed canarygrass) and *Typha latifolia* L. (broad-leaved cattail) (Gleason and Cronquist, 1991). We chose these species because they have been used in previously published wastewater treatment experiments (Gersberg et al., 1986; Coleman et al., 2001), and used in constructed wetlands (Cronk and Fennessy, 2001; Steer et al., 2002). These wetland plants are fast-growing, tall-stature, "clonal-dominants" (sensu Boutin and Keddy, 1993) that establish quickly, process a lot of energy, and are therefore considered suitable plants for treatment wetlands.

A microcosm experiment was designed to answer four questions: (1) Are vegetated microcosms better than non-vegetated microcosms at reducing N and P in the soil leachate? (2) Are there differential responses between the four wetland plants in their effectiveness at reducing N and P? (3) Does a four-species mixture relatively enhance the effectiveness of the removal of N and P compared to monoculture treatments? and (4) is there a difference between sampling dates with regard to N and P in the soil leachate? Our hypotheses, based on the research presented in the introduction, are (1) vegetated microcosms are more effective at reducing concentrations of total N and total P than unvegetated, (2) there is a differential species effect on the potential to reduce N and P, (3) plant mixtures are more effective than monocultures at reducing N and P, and (4) the microcosms will be least effective at reducing N and P concentrations in October compared to August.

## 2. Methods

### 2.1. Study site

The study was located at the Bath Nature Preserve in Bath, Ohio. Beginning in the summer of 2000, a 4-m tall, $20 \times 20$ m fence was constructed at the site location. The fencing was covered completely with bird netting and secured with a padlocked gated entry. An on-site weather station (Spectrum™ WatchDog Model 900ET) recorded temperature and rainfall throughout the study period.

### 2.2. Experimental design

The experiment was established in the fall of 2000. The experimental design was a six (plant type) by two (nutrient addition) factorial combination. The plant treatment had six levels: no plants, four monocultures, (*C. lacustris*, *S. validus*, *P. arundinacea* and *T. latifolia*), and a four-species mixture of these plants. The nutrient treatment had two levels (low and high). Each type of microcosm treatment had six replicates for a total microcosm count of 72. The microcosms were arranged in six rows. Two rows were placed back-to-back with an approximate 1-m section in between. The placement of microcosms was completely randomized.

### 2.3. Microcosms

The outdoor microcosms used to test the effectiveness of plants to treat wastewater were 18.93-l white buckets filled with soil to $\frac{3}{4}$ full, accounting for approximately 0.014 m$^3$ of soil. The microcosms were set up to mimic subsurface treatment wetlands; that is, wetlands where the water level is below the soil surface (Kadlec and Knight, 1996). The soil (Carlisle Muck) was taken from the Panzner Wetland Restoration Site in Copley, Ohio. This soil was used because it is a peaty wetland soil with high carbon and nutrient levels, which aids in the establishment of the plants. Three soil samples were taken at random, before the microcosms were filled, and

sent to Spectrum Analytic Inc. (Washington Court House, OH) to be analyzed for total nitrogen (TN), total phosphorus (TP), pH, and percent organic matter. Each microcosm had a hole in the bottom with a diameter of 1.9 cm that was plugged with a rubber stopper. A mesh net covering the hole was fastened on the inside of the microcosm in order to keep soil inside during the release of water for testing.

### 2.4. Plant establishment

In May of 2001, rhizomatous cuttings of each species from the field were collected. Each microcosm was planted with eight cuttings (approximately 10 cm in length) of each species in monocultures and two of each species in the four-species mixture microcosms. To control for the disturbance experienced by the vegetated microcosms during the planting of the rhizomes, the unvegetated microcosms were similarly disturbed but with no planting. The microcosms were monitored three times per week, and invasive seedlings detected were immediately removed.

The microcosms were watered three times weekly or as needed depending on the weather. The water level was kept constant at 5 cm below the level of the soil surface. Nutrient addition began in August 2001, at which time the plants were well established.

### 2.5. Nutrient addition

Rorison nutrient solution (Hendry and Grime, 1993) was used to simulate the high nutrient input levels typically found in single-family domestic treatment wetlands (Steer et al., 2002). The standard solution contained 56 mg/l of nitrogen (as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) and 31 mg/l of phosphorus (as $K_2HPO_4 \cdot 3H_2O$). For those microcosms receiving the lower level of nutrients 1 l of Rorison solution was added. For microcosms receiving the higher level of nutrients the concentration of the solution was doubled, but only 1 l was added (i.e. 112 mg/l of nitrogen and 62 mg/l of phosphorus). The nutrient addition was applied three times weekly.

### 2.6. Water sampling

A baseline measurement of water was taken on July 30, 2001, before the nutrient addition commenced, followed by monthly samples until October 23, 2001. Seventy-two 100 ml water samples were taken monthly, one from each of the microcosms. Soil leachate was drained from the bottom of the microcosms, through a pre-drilled hole. Water samples were immediately placed in a cooler, brought back to the lab and kept cool at 4 °C until analyzed, which was within 48 h of sampling. Total nitrogen (mg/l) and total phosphorus (mg/l) were run using the HACH Test'N Tube™ tests (HACH

Company, Loveland, CO) and a Spec 20 mass spectrometer.

### 2.7. Harvesting

During the month of November the above-ground plant biomass was harvested. The plants growing in mixture were separated to species. All plants were oven-dried for approximately 48 h at 80 °C and weighed.

### 2.8. Statistical analysis

All statistical tests were performed using Systat Version 8 by SPSS Inc. In all cases, significance was defined by $p < 0.05$. Six microcosms were removed from the analyses because vegetation failed to establish. A two-way ANOVA was used to determine significance of species and nutrient effect on biomass. Tukey's LSD was applied to test for significance between treatment means. Three-way ANOVAs were used to examine the effects of the three sampling periods (DATE), the two nutrient treatments (NUTR), and the species treatment (SPECIES) on total nitrogen and total phosphorus in the effluent water. Linear regressions were performed within each plant species to determine the possible effect of biomass on nitrogen and phosphorus reduction, but the results were non-significant and therefore we have not included these results.

## 3. Results

A number of baseline measurements were made before and during the experiment. Both the temperature and the total amount of rainfall were recorded during the study period by the weather station. The highest recorded temperatures occurred at the end of July and the beginning of August (37 °C). One night during the last week of August the temperature fell below freezing (−1 °C), but daytime temperatures were still high (27 °C). The lowest temperatures were recorded at the end of the study in October when nighttime temperatures were regularly below freezing. Rainfall was lowest during both July and September (6 cm/month). Rainfall in the remaining months during the study period ranged from 9.5 to 11.5 cm/month. Preliminary tests were run on the soil at the beginning of the experiment (Table 1). There was very little variation among the microcosms as noted by the small standard deviations.

The harvested plant dry biomass was analyzed comparatively between nutrient treatments (Fig. 1, Table 2). A two-way ANOVA (Table 2) showed that the biomass of the species was significantly different, however nutrients had no significant affect on biomass. *C. lacustris* had the greatest dry biomass at both nutrient levels, and post-hoc analysis determined that the difference

Table 1
Soil analysis of the Carlisle Muck used in the microcosms

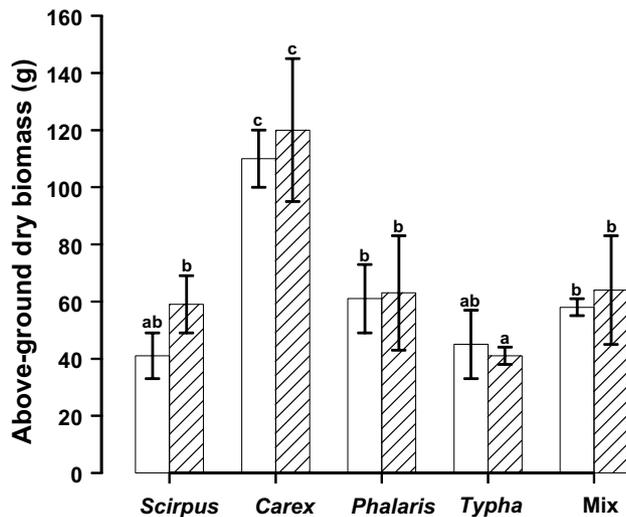|  | Mean | Standard deviation |
|---|---|---|
| Total nitrogen (mg/l) | 2.4 | 0.06 |
| Total phosphorus (mg/l) | 0.73 | 0.04 |
| pH | 5.83 | 0.06 |
| Organic matter (%) | 16.93 | 0.551 |



Fig. 1. Mean dry biomass (g) of total above-ground plants per microcosm between plant treatments and nutrient levels. Open bars are low nutrient and hatched bars are high nutrient treatments. Error bars represent standard deviation. Bars sharing the same letter are not significantly different based on Tukey's HSD.

Table 2
Two-way ANOVA to determine significance of species and nutrient effect on biomass

| Source | Sum-of-squares | df | F-ratio | P |
|---|---|---|---|---|
| NUTR | 593.198 | 1 | 2.578 | 0.116 |
| SPECIES | 33679.492 | 4 | 36.586 | <0.001 |
| NUTR * SPECIES | 691.802 | 4 | 0.753 | 0.563 |
| ERROR | 9896.132 | 43 |  |  |

In the table, NUTR refers to nutrient treatment, and SPECIES refers to the plant species treatment. "df" is degrees of freedom.
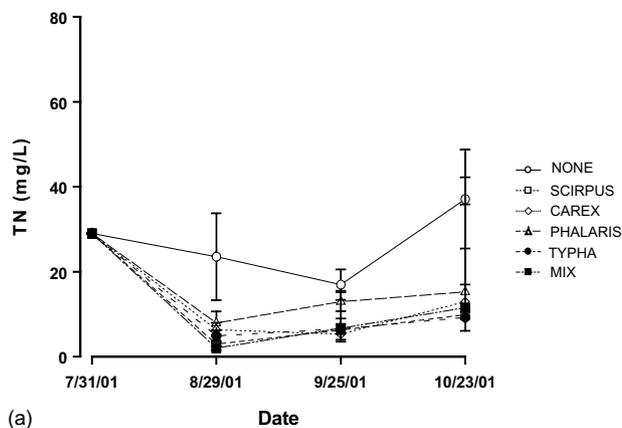
was statistically significant in comparison with all other species as well as the mixture.

Results from the three-way ANOVA for total nitrogen to detect effects of sampling date, nutrients and species factors are shown in Table 3. This analysis produced a significant effect of sampling date, nutrient and species factors. In addition, the interactions between date and nutrient, as well as date and species was significant. The significant date by nutrient combination result indicates that the high nutrient treatment result in higher nitrogen levels in the effluent. The significant date by species combination illustrates a trend of higher nitrogen levels measured later in the growing season when temperature has declined.
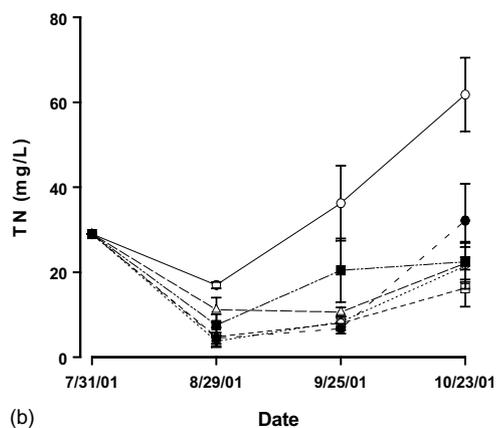
A comparison of total nitrogen by species treatment, and across sampling dates, is shown in Fig. 2a (low nutrients) and Fig. 2b (high nutrient). The 7/31/01 sampling date is a pre-treatment baseline measurement. Unvegetated microcosms show consistently and significantly higher nitrogen levels at all three post-treatment dates. At low nutrient (Fig. 2a), *P. arundinacea* has consistently higher nitrogen values than the other vegetated microcosms. The high nutrient treatment shows less consistent responses by date (Fig. 2b), but the mixture has the highest values of vegetated microcosms on 9/25/01, while *T. latifolia* has the highest values of vegetated microcosms on 10/23/01. The general trend is for increasing total nitrogen levels over time.

Table 4 shows the results from the three-way ANOVA for total phosphorus to detect effects of sampling date, nutrient and species factors. This analysis produced a significant effect of sampling date, nutrient and species factors. In addition, the interactions between date and species, as well as nutrient and species was significant. The nutrient by species response showed that *P. arundinacea* had significantly lower phosphorus levels at the high nutrient treatment.

A comparison of total phosphorus by species treatment, and across sampling dates, is shown in Fig. 3a (low nutrient) and Fig. 3b (high nutrient). The 7/31/01 sampling date is a pre-treatment baseline measurement. Unvegetated microcosms show the highest total phos-
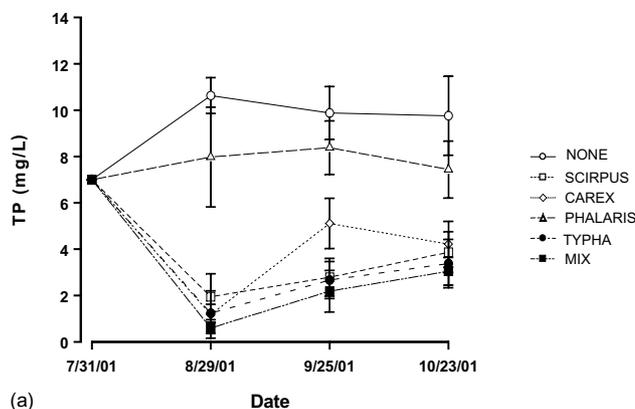
Table 3
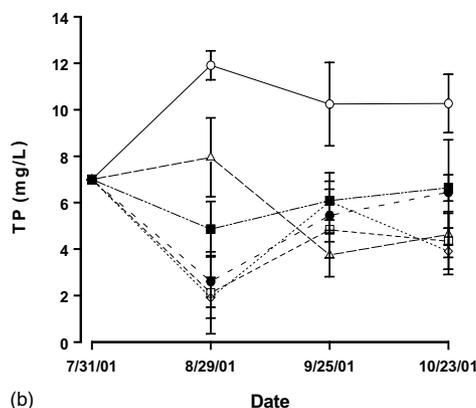Results of three-way ANOVA examining the effects of the three sampling periods (DATE), the two nutrient treatments (NUTR), and the species treatment (SPECIES) on total nitrogen in the outlet water

| Source | Sum-of-squares | df | F-ratio | P |
|---|---|---|---|---|
| DATE | 7453.415 | 2 | 32.169 | <0.001 |
| NUTR | 2065.655 | 1 | 17.831 | <0.001 |
| SPECIES | 13915.313 | 5 | 24.023 | <0.001 |
| DATE * NUTR | 1425.715 | 2 | 6.153 | 0.003 |
| DATE * SPECIES | 2230.425 | 10 | 1.925 | 0.045 |
| NUTR * SPECIES | 747.720 | 5 | 1.291 | 0.270 |
| DATE * NUTR * SPECIES | 1496.974 | 10 | 1.292 | 0.239 |
| ERROR | 18999.044 | 164 |  |  |

"df" is degrees of freedom.

Fig. 2. The mean total nitrogen (mg/l) in soil leachate for (a) low nutrient, and (b) high nutrient treatments over the study period. Error bars represent one standard error.



Fig. 3. The mean total phosphorus (mg/l) in soil leachate for (a) low nutrient, and (b) high nutrient treatments over the study period. Error bars represent one standard error.

Table 4
Results of three-way ANOVA examining the effects of the three sampling periods (DATE), the two nutrient treatments (NUTR), and the species treatment (SPECIES) on total phosphorus in the outlet water

| Source | Sum-of-squares | df | *F*-ratio | *P* |
|---|---|---|---|---|
| DATE | 48.970 | 2 | 3.385 | 0.036 |
| NUTR | 47.178 | 1 | 6.523 | 0.012 |
| SPECIES | 1365.292 | 5 | 37.751 | <0.001 |
| DATE * NUTR | 2.531 | 2 | 0.175 | 0.840 |
| DATE * SPECIES | 189.173 | 10 | 2.615 | 0.006 |
| NUTR * SPECIES | 191.539 | 5 | 5.296 | <0.001 |
| DATE * NUTR * SPECIES | 39.489 | 10 | 0.546 | 0.855 |
| ERROR | 1186.223 | 164 | | |

"df" is degrees of freedom.

phorus levels at low and high nutrient levels, and they do not change over time. Over time, vegetated microcosms show a general trend to increase. *P. arundinacea* has high phosphorus levels at low nutrient, but reduced levels at high nutrient on the last two sampling dates.

## 4. Discussion

Microcosms are extremely useful for controlled, mechanistic investigations (Fraser and Keddy, 1997), and have previously been used to test plants' ability to

treat wastewater (Gersberg et al., 1986; Coleman et al., 2001). Microcosms, however, have limitations. For example, the spatial scale does not generally reflect what occurs in nature, and abiotic conditions may be affected by the experimental conditions. In addition, research has shown that in constructed systems more than one year may be needed to reach "natural wetland conditions" (Sistani et al., 1996). Hence, our microcosms were being tested during their second growing season.

Our purpose was to determine if plants can effectively reduce the levels of total N and total P in the soil water leachate, and thereby be potentially useful in treating

single-family domestic wastewater in subsurface constructed wetlands. Biomass of the microcosms was not significantly different among the high and low nutrient treatments (Table 2, Fig. 1), which suggests that plant growth was not limited by nutrients. Bachand and Horne (1999) showed that an increase in biomass, both living and dead, enhanced rates of denitrification and improved removal efficiencies of nitrogen. We did not find a correlation between biomass and treatment efficiency, but perhaps given more time this would be detectable.

Our results support the first hypothesis that states plants can reduce total N and total P at a level significantly lower than unvegetated systems (Tables 3 and 4). In some cases, the reduction of N and P in the soil leachate was 10 times greater in vegetated microcosms than unvegetated microcosms (e.g. Fig. 2b, 10/23/01 for N and Fig. 3a, 8/29/01 for P). Our second hypothesis tested whether four wetland plant species were differentially effective at reducing N and P. We found that plant species do have a differential response to reducing N and P (Tables 3 and 4; Figs. 2 and 3). At low nutrients, both S. validus and the four-species mixture had significantly lower N and P in their soil leachate than the other plant treatments. The least effective species was P. arundinacea, which tended to have significantly higher N and P in the soil leachate than the other wetland plant treatments, and similar N and P concentrations as the unvegetated microcosms. However, the high nutrient treatment resulted in slightly different results for some of the species, which indicates that nutrient load might affect the treatment potential of plant species. For example, the Typha microcosms performed quite well at reducing N at low nutrient conditions (9.2 mg/l on 10/23/01, Fig. 2a), but performed poorly under high nutrient additions (31.5 mg/l on 10/23/01, Fig. 2b). Phalaris was consistently poor at reducing P at low nutrients across all dates (Fig. 3a), but under high nutrient conditions, Phalaris was one of the more effective plants at reducing P (Fig. 3b).

Gersberg et al. (1986) investigated constructed wetlands that were vegetated by S. validus, Phragmites communis, or T. latifolia or that were unvegetated in order to determine the efficiency among the treatments at reducing nitrogen. In all cases unplanted wetlands were less effective than any of the planted (Gersberg et al., 1986). However, amongst species, S. validus was the most effective, which supports our findings. T. latifolia was the least effective (Gersberg et al., 1986). Presumably due to the high ammonia loading, the T. latifolia plants began to yellow after three months and nearly all were dead after six months (Gersberg et al., 1986). Gersberg hypothesized that T. latifolia was least effective due to its shallow rooting zone and the inability

to create an effective environment for various microbial communities (Gersberg et al., 1986). Contrary to the finding of Gersberg et al. (1986), a study conducted by Coleman et al. (2001) found that T. latifolia was very efficient at removal of nutrients.

In our experiment, T. latifolia was the slowest to establish. Contrary to published reports (Clarke and Baldwin, 2002; Svengosouk and Mitsch, 2001), T. latifolia did not produce more biomass with high nutrient conditions. High nutrient levels appeared to stunt and even kill the T. latifolia plants; very few transplanted seedlings grew within the microcosms. Similar to results reported by Gersberg et al. (1986), the T. latifolia plants began to yellow after 2.5 months and were the first species to die off at the end of the season.

The third hypothesis was that a four-species plant mixture is more effective than a monoculture at reducing nitrogen and phosphorus. At low nutrients, the mixed microcosms consistently had among the lowest N and P concentrations in the soil leachate (Figs. 2a and 3a), but the only significant difference between a monoculture treatment was Phalaris for total P (Fig. 3a). At high nutrients, the mixed microcosms did not have the lowest N and P concentrations, and in fact had significantly higher P than Phalaris, Scirpus, and Carex on 10/23/01 (Fig. 3b). Therefore, our results do not support the hypothesis that mixtures have the potential to reduce N and P any more than monocultures. However, mixtures may provide other benefits over monocultures, such as enhanced tolerance to abiotic stress or enhanced treatment efficiency of other toxins or nutrients not measured in this study.

Coleman et al. (2001) tested unplanted versus planted monocultures and three-species mixture mesocosms (400 l troughs) with two levels of water depth. The plants used were Juncus effusus, T. latifolia, and Scirpus cyperinus. Their mixed treatment was quite effective at reducing nutrient level, possibly due to root partitioning of the soil. However, their results showed no significant difference between T. latifolia monocultures and the mixed system (Coleman et al., 2001).

The results of our experiment supported the fourth hypothesis; we found a general increase in N and P concentration within the leachate over time, with the highest levels in October. Temperature has been shown to play a role in the removal of nitrogen through enhanced denitrification processes (Bachand and Horne, 1999). Some of the decline in treatment at the end of the study might be attributed to lower temperatures and thus lower microbial activity as well as a reduction in macrophytic growth rates.

A review by Brix (1997) details the role macrophytes play within a constructed wetland system and how they are an integral part in nutrient cycling. Macrophytic

plants encourage the assimilation and breakdown of nutrients within a wetland system. They have the ability not only to bind high amounts of nutrients within their system, but also to create an environment conducive to decreasing nutrients. For example, (1) they provide surface area on their stems and leaves, which is necessary for microbial growth; (2) their roots provide a structure for microorganisms to adhere and perform the processes necessary for transformation of nutrients; (3) roots not only provide a place for microbes, but also serve to decrease erosion and increase the levels of oxygen, which provides for the oxidation of toxic substances like ammonia ($NH_3$) and nitrites ($NO_2$); (4) macrophytes are not only beneficial in the removal and transformation of nutrients, but are also aesthetically pleasing to homeowners where many of these systems are located (see Brix, 1997; Carmen and Crossman, 2001).

## 5. Conclusion

This study demonstrates the importance of macrophytes to reduce nutrient concentrations encountered by single-family domestic subsurface constructed wetlands, however the applicability of the results to actual constructed wetlands has yet to be determined. Vegetated microcosms had significantly lower total N and total P in their soil leachate than unvegetated microcosms. *S. validus* was shown to be effective overall at treating nutrient loaded water and should therefore be considered for the future design of single-resident treatment wetlands. *P. arundinacea* was generally least effective in monocultures, with treatment capabilities similar to unvegetated microcosms, but the performance of *Phalaris* at reducing phosphorus at high nutrient levels was relatively high. The four-species mixture microcosms were generally highly effective at nutrient removal and should also be considered for future design of single-resident treatment wetlands, however they did not show any significant difference between *Scirpus*, *Typha* and *Carex* in monoculture.

## Acknowledgements

## References

Bachand, P.A.M., Horne, A.J., 1999. Denitrification in constructed free-water surface wetlands: II. Effects of vegetation and temperature. Ecol. Eng. 14, 17–32.

Badkoubi, A., Ganjidoust, H., Ghaderi, A., Rajabi, A., 1998. Performance of a subsurface wetland in Iran. Water Sci. Technol. 38, 345–350.

Brix, H., 1997. Do macrophytes play a role in constructed treatment wetlands. Water Sci. Technol. 35, 11–17.

Boutin, C., Keddy, P.A., 1993. A functional classification of wetland plants. J. Veg. Sci. 4, 591–600.

Carmen, E.P., Crossman, T.L., 2001. Phytoremediation. In: Nyers, E.K. (Ed.), In situ Treatment Technology. Lewis Publishers, New York, pp. 391–435.

Clarke, E., Baldwin, A.H., 2002. Responses of wetland plants to ammonia and water level. Ecol. Eng. 18, 257–264.

Coleman, J., Hench, K., Garbutt, K., Sextone, A., Bissonnette, G., Skousen, J., 2001. Treatment of domestic wastewater by three wetland plant species in constructed wetlands. Water, Air Soil Poll. 128, 283–295.

Cronk, J.K., Fennessy, M.S., 2001. Wetland Plants: Biology and Ecology. Lewis Publishers, New York.

Fraser, L.H., Keddy, P., 1997. The role of experimental microcosms in ecological research. TREE 12, 478–481.

Fraser, L.H., Bradford, M.E., Steer, D.N., 2003. Human appropriation and treatment of fresh water: a global hydrology model incorporating treatment wetlands. Int. J. Environ. Sust. Dev. 2 (2), 174–183.

Gersberg, R.M., Elkins, B.V., Lyons, S.R., Goldman, C.R., 1986. Role of aquatic plants in wastewater treatment by artificial wetlands. Water Res. 20, 363–368.

Gleason, H.A., Cronquist, A., 1991. Manual of vascular plants of northeastern United States and Adjacent Canada, second ed. The New York Botanical Garden, Bronx, NY.

Greenway, M., Simpson, J.S., 1996. Artificial wetlands for wastewater treatment, water reuse and wildlife in Queensland, Australia. Water Sci. Technol. 33, 221–229.

Haberl, R., Perfler, R., Mayer, H., 1995. Constructed wetlands in Europe. Water Sci. Technol. 32, 305–315.

Hendry, G.A.F., Grime, J.P., 1993. Methods in Comparative Plant Ecology: A Lab Manual. Chapman & Hall, London.

Hooda, P.S., Edwards, A.C., Anderson, H.A., Miller, A., 2000. A review of water quality concerns in livestock farming areas. Sci. Total Environ. 250, 143–167.

Kadlec, R.H., Knight, R.L., 1996. Treatment Wetlands. Lewis Publishers, New York.

Nerella, S., Weaver, R., Lesikar, B.J., Persyn, R.A., 2000. Improvement of domestic wastewater quality by subsurface flow constructed wetlands. Bioresour. Technol. 75, 19–25.

Ratcliffe, D.A., 1984. The flora and vegetation of Britain: origins and changes—the facts and their interpretations: post medieval and recent changes in British vegetation the culmination of human influence. New Phytol. 98, 73–100.

Rogers, K.H., Breen, P.F., Chick, A.J., 1991. Nitrogen removal in experimental wetland treatment systems: evidence for the role of aquatic plants. Res. J. Water Poll. Control Fed. 63, 934–941.

Sistani, K.R., Mays, D.A., Taylor, R.W., 1996. Development of natural conditions in constructed wetlands: biological and chemical changes. Ecol. Eng. 12, 125–131.

SPSS, 1998. SYSTAT® 8.0. SPSS Inc., Chicago, IL.

Steer, D., Fraser, L.H., Boddy, J., Seibert, B., 2002. Efficiency of small constructed wetlands for subsurface treatment of single family domestic effluent. Ecol. Eng. 18, 429–440.

Steer, D., Aseltyne, T., Fraser, L.H., 2003. Life-cycle economic model of small treatment wetlands for domestic wastewater disposal. Ecol. Econ. 44, 359–369.

Svengosouk, L.S., Mitsch, W.J., 2001. Dynamics of mixtures of *Typha latifolia* and *Schoenoplectus tabernaemontani* in nutrient-enrichment wetland experiments. Am. Mid. Nat. 145, 309–324.

Tanner, C.C., 1996. Plants for constructed wetland treatment systems—a comparison of the growth and nutrient uptake of eight emergent species. Ecol. Eng. 7, 59–83.