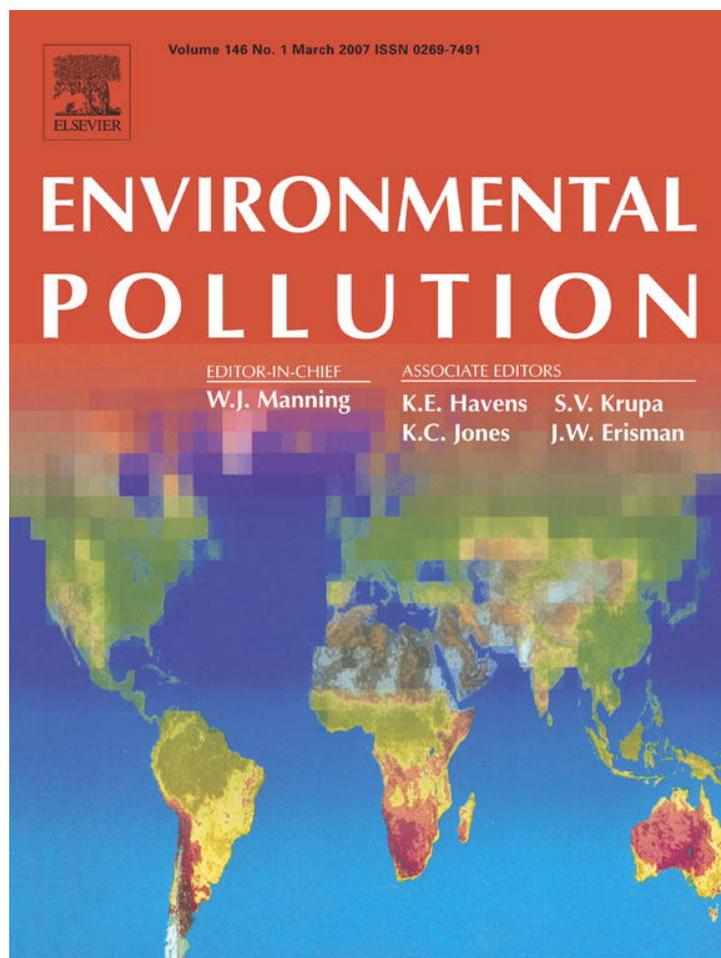


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Plant senescence: A mechanism for nutrient release in temperate agricultural wetlands

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Release of sequestered nutrients during plant senescence.

Abstract

The beneficial uptake of nutrients by wetland plants is countered to some extent by nutrient release back into the aquatic environment due to vegetative die-back. This current study examined whether *Leersia oryzoides*, a common wetland plant, exhibits luxury uptake of nutrients from simulated farm runoff. The study also tested whether with subsequent decomposition, these nutrients are released back into the water column. When exposed to elevated (>2 mg/L N and P) runoff, *L. oryzoides* assimilated significantly higher concentrations of nitrogen ($p < 0.001$) and phosphorus ($p < 0.001$) in above-ground biomass as compared to non-enriched treatments (<0.05 mg/L N and P). Subsequently, senescence of enriched above-ground biomass yielded significantly higher concentrations of phosphorus (2.19 ± 0.84 mg P/L). Using *L. oryzoides* as our model, this study demonstrates nitrogen and phosphorus sequestration during the growing season and release of phosphorus in the winter. © 2006 Elsevier Ltd. All rights reserved.

Keywords: Nutrients; Wetlands; Assimilation; Drainage ditch; *Leersia oryzoides*

1. Introduction

Temperate wetlands have numerous mechanisms by which nutrients are assimilated and transformed. One such mechanism of nutrient removal is plant nutrient uptake. Wetland plants generally remove only very small quantities (<5%) of nutrients from influent waters (Hammer, 1992). This amount is insignificant compared to loading rates measured flowing into many treatment wetlands (Brix, 1994). Aquatic plant uptake of nutrients is of quantitative importance in low-loaded wetland systems, such as drainage ditches associated with agricultural runoff (Pevery, 1985; Richardson, 1985). Drainage ditches are primary intercept wetlands in the mitigation of nutrient-containing runoff in agricultural landscapes (Cooper et al., 2002). Increasingly, agricultural drainage ditches are

being examined in terms of their nutrient retention and processing capabilities. One important component of these capabilities is the plant community. These plants present a unique situation with regards to nutrient cycling because during the growing season they may incorporate nutrients from the surrounding environment into new tissues. However, upon senescence, those nutrients may be released back into the environment. This dynamic aspect of nutrient flux in wetlands, of which there is little understanding, has received little attention by managers and researchers.

A distinct seasonal response in vegetation phenology is noted in temperate wetland systems, where there is growth in the summer and senescence in the winter (Dolan et al., 1981). Emergent macrophytes leach nutrients rapidly upon death and decomposition into the water column (Boyd and Hess, 1970; Gaudet, 1977; Johnston, 1991). Examples include *Typha latifolia* L. and *Phragmites australis* (Cav.) Trin. ex Steud., where leaves lost 90–93% of their potassium, sodium, nitrogen and phosphorus after 20 days of submerged tissue

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senescence (Boyd and Hess, 1970; Nichols and Keeney, 1972; Gaudet, 1977).

With the possibility of using vegetation for pollution control, there have been several efforts to determine relationships between nutrient status of emergent macrophytes and their environment (Klopatek, 1978). Certain plants exhibit high nutrient use efficiency and accumulate high concentrations of nutrients within above- and below-ground structures. The occurrence of increased nutrient concentrations in the plant tissue is termed luxury uptake, a mechanism by which plants assimilate higher than necessary concentrations of nutrients for normal metabolic functions (Cronk and Fennessy, 2001). Greenway (1997) compared nutrient levels in plant tissues between high nutrient loaded wetlands and control wetlands and documented luxury uptake whereby total phosphorus and total nitrogen levels were 2 mg P/g and 7 mg N/g higher in high loaded wetlands relative to control wetlands. It has been hypothesized that this increased uptake might be a means by which the plant prepares for unfavorable times or environmental stress such as freezing and temporal variability in water depth (Farahbakshazad and Morrison, 1997). It has been noted in most studies that plant contribution to nutrient removal is often only temporary because of the nutrient loss at senescence (Cronk and Fennessy, 2001). Few studies (Gaudet, 1977; Kuehn and Suberkropp, 1998; Kuehn et al., 1999) have examined the associated release of nutrients of wetland species following senescence. We suggest using agricultural drainage ditches as model wetlands to examine the occurrence of nutrient release from certain wetland plants during decomposition.

The objectives of this study were to (1) evaluate whether *Leersia oryzoides*, given the opportunity, will sequester high concentrations of nutrients from an applied concentrated nutrient load of nitrogen (N) and phosphorus (P), and (2) evaluate the leaching of N and P back into the water column with subsequent above-ground tissue senescence. This information is important for characterizing the nutrient dynamics of wetlands during dormant periods, which are often overlooked.

2. Methods and materials

These experiments were conducted at the University of Mississippi Field Station (UMFS) greenhouse. The purpose of these experiments was to address questions developed as a result of previous work at the UMFS (Davis and Holland, 1998) that focused on the dynamics and relationships between nutrients and wetland vegetation. *Leersia oryzoides* (L.) Sw. dominated the majority (>50% aerial cover) of the treatment and control ditches. UMFS ditches were selected, as they formed the basis of a previous simulated nutrient release experiment in 2003 by the United States Department of Agriculture (USDA)—Agricultural Research Service.

For the first objective, above-ground portions of *L. oryzoides* were harvested from ditches during the summer at the height of plant nutrient uptake and the growing season (June 2004—3 months post USDA experiment). Experimental ditches and control ditches were exposed to similar flow and water volumes over the 2-year USDA experiment. The mean mass loading per experimental ditch, every 2 weeks, was 3.8 g N/P/m³, per runoff experiment, with a total injection volume of 220 L over 4 h. Flow rates varied from 36 to 38.9 L/min. Water was pumped into each ditch from groundwater from the UMFS with below detectable limits of nitrate, ammonia and phosphorus. Eight bulk shoot samples of *L. oryzoides* were randomly selected from four

vegetated treated ditches and two untreated control ditches. Each bulk sample was air-dried, ground and analyzed for carbon, total nitrogen and total phosphorus. Total nitrogen and carbon were determined using an elemental analyzer (detection limit ≥ 0.9 mg/L), while total phosphorus was determined using an inductively coupled plasma optical emission spectrophotometer (detection limit ≥ 0.8 mg/L) (Isaac and Johnson, 1985). These procedures and protocols were also used to analyze above-ground vegetation used in the winter senescence experiment. Initial nutrient concentrations were subtracted between summer and winter to determine seasonal differences in nutrient contents of tissues as well as to provide an initial base nutrient load for senescence over the winter.

The senescence experiment, testing assimilated nutrient release, ran over a 12-week period from December 2004 to February 2005 and consisted of shoot samples collected from the following three treatments: (1) (enriched) nutrient enriched ditch, (2) (non-enriched) control ditch, and (3) control with no vegetation in decomposition bags. Each replicate (eight in each of the enriched and non-enriched; four in the control) consisted of an individual 104-L plastic container. Containers were placed in a greenhouse, and treatments were randomly assigned. Each container was filled with approximately 95 L groundwater from the UMFS. Baseline nitrogen and phosphorus concentration of the UMFS groundwater was 2.6 mg N/L and 0.002 mg P/L. A dry weight of 800 g *L. oryzoides* above-ground tissue was placed in decomposition bags in each container of standing water for 12 weeks. The decomposition bags had a 5-mm mesh diameter and were weighted to submerge the vegetation simulating the submergence of vegetation in the field over winter. The processes determining the rate of decomposition of submerged litter include microbial decay, leaching and microbial uptake (Davis and van Der Valk, 1978). Mesh bag techniques are appropriate for assessing the impact of these processes on nutrient release to surface water. Water samples were taken on a weekly basis and analyzed for nitrate, ammonia and total phosphorus. Nitrate was determined using a Dionex Ion Chromatograph (detection limit ≥ 0.5 mg/L) fitted with a conductivity detector. Total phosphorus and ammonia were determined analytically using the ammonia persulfate and phenate digestion methods, respectively (Clesceri et al., 1998). The control was used primarily to show that water nutrient concentrations did not fluctuate throughout the course of the experiment.

Differences between nutrient enriched and non-enriched vegetation in the first objective were analyzed with two-sample equal variance Student *t*-tests. Differences between weekly nutrient concentrations between nutrient-enriched, non-enriched and control containers were analyzed by ANOVA and a post hoc Tukey HSD. Both statistical analyses used alpha values of $p = 0.05$.

3. Results

A comparison of nutrient contents of *L. oryzoides* in enriched and non-enriched ditches at the height of the growing season revealed statistically significant differences in above-ground tissue nutrient concentrations (Fig. 1). Total nitrogen content was significantly ($p < 0.001$) higher in enriched (13.20 ± 2.08 mg/g) than non-enriched vegetation (8.4 ± 0.5 mg/g) (Fig. 1). Similarly, total orthophosphate (TOP) was significantly ($p < 0.001$) higher in enriched (3.85 ± 0.25 mg/g) than non-enriched vegetation (1.91 ± 0.32 mg/g). These results also revealed significant effects on C:N and C:P ratios within plant tissue. The C:N ratio of enriched *L. oryzoides* was 38.6, in comparison to 208 for the non-enriched treatment.

Winter nutrient concentrations of enriched and non-enriched plant tissues were significantly lower than those collected in the summer. Prior to decomposition, nutrient enriched vegetation had nitrate, ammonia and TOP concentrations of 7.8 mg N/g ($n = 8$), 2.12 mg NH₄-N/g ($n = 8$) and 5.02 mg P/g ($n = 8$), respectively. Similarly, nutrient

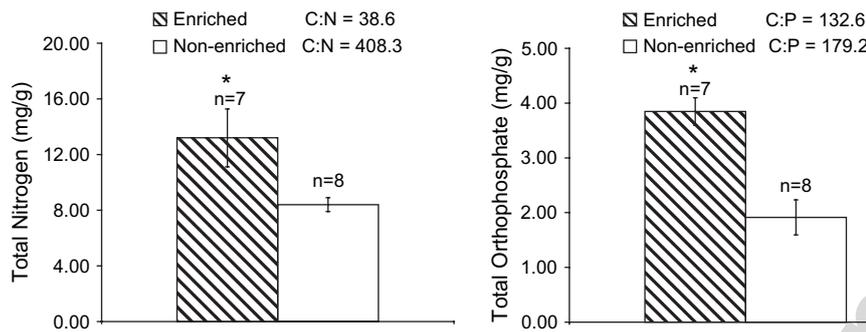


Fig. 1. A comparison of mean total nitrogen and phosphorus concentrations (\pm SE) between nutrient enriched and non-enriched *Leersia oryzoides* in summer 2004. * $p < 0.001$.

concentrations for the non-enriched treatment were 5.91 mg N/g ($n = 8$), 2.02 mg $\text{NH}_4\text{-N/g}$ ($n = 8$) and 1.68 mg P/g ($n = 8$). Between summer and winter, C:N and C:P ratios remained consistent or increased slightly, ranging from 38.6–48.2 and 208–215 (C:N), and 125.4–132.6 and 166.3–179.8 (C:P) for enriched and non-enriched treatments, respectively.

Higher tissue phosphorus concentrations in summer (prior to decomposition) resulted in significantly higher phosphorus concentrations being released when plants senesced in winter (Fig. 2). TOP concentrations increased rapidly in the first eight days and then remained relatively constant for the duration of the experiment (Fig. 2). The difference is evident throughout the course of the experiment, and at termination, where the water column of the enriched vegetation had a higher ($p < 0.05$) concentration than non-enriched vegetation. The control indicated that background water nutrient concentrations did not change throughout the course of the experiment. Thus, changes in nutrient concentrations within each treatment's water column were a result of the release of nutrients through tissue senescence. However, there was a slight reduction over time in TOP concentration without the presence of

an outlet (Fig. 2). Release of P from decomposing plant material also depends on the C:P ratio, which was 125.4 and 166.3 for the enriched and non-enriched vegetation, respectively.

Winter nitrate levels were highest in the controls and remained around 2.63 mg $\text{NO}_3\text{-N/L}$ throughout the experiment (Fig. 3). Nitrate levels within enriched and non-enriched containers were significantly reduced from control levels to below detection limits (0.5 mg $\text{NO}_3\text{-N/L}$) after 1 week. This suggests that nitrate was apparently transformed as fast as it was produced in the stages of decomposition. Even though plant tissue concentrations in enriched and non-enriched containers were initially different, the resultant leachate nutrient concentration from tissue senescence was the same. There were no statistically significant differences in ammonia concentrations between enriched, non-enriched and control treatments (Fig. 4).

4. Discussion

Luxury uptake theory states that, given the opportunity, wetland vegetation will sequester higher loads of nutrients

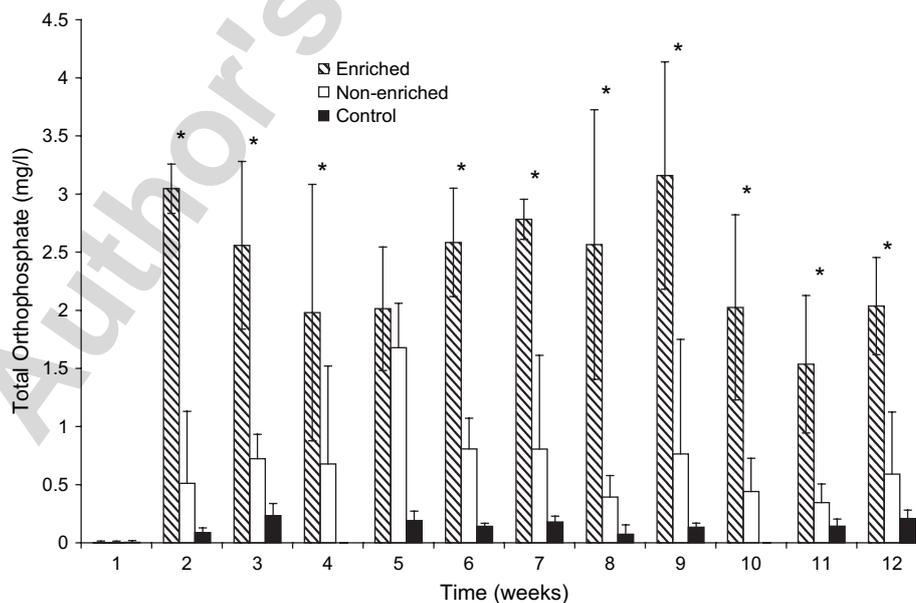


Fig. 2. Mean total orthophosphate concentrations (\pm SE) in the water column as a result of *Leersia oryzoides* tissue senescence, December 2004–February 2005. * $p < 0.05$, ANOVA.

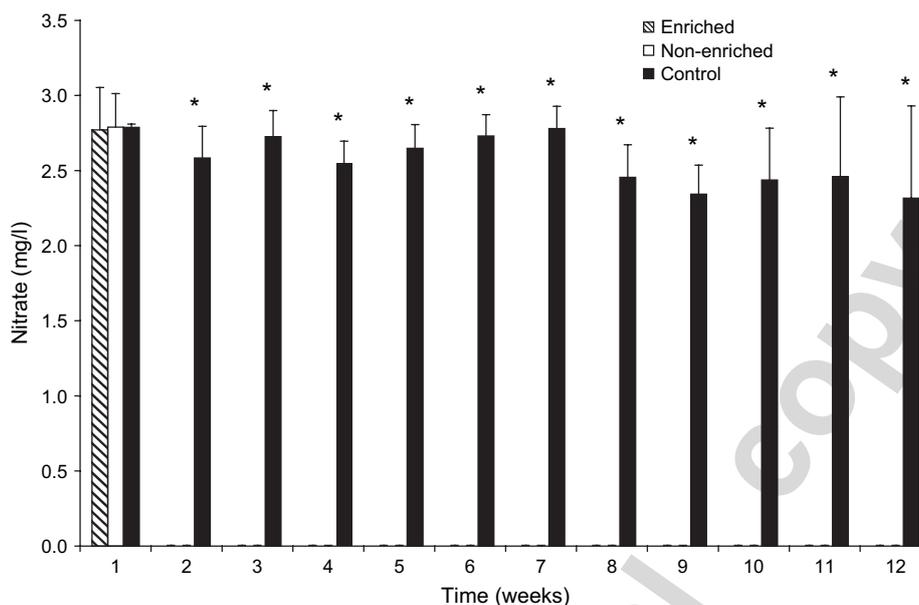


Fig. 3. Mean nitrate concentrations (\pm SE) in the water column as a result of *Leersia oryzoides* tissue senescence, December 2004–February 2005. * $p < 0.05$, ANOVA.

even though they are not needed for growth or other metabolic functions (Cronk and Fennessy, 2001). During this period of uptake, inorganic nutrients are converted to organic matter. *Leersia oryzoides* is a perennial emergent wetland species, which when exposed to elevated nutrient runoff concentrations, assimilates higher concentrations of N and P in its above-ground plant tissue. Thus, *L. oryzoides* has a high nutrient use efficiency (NUE). A plant with high NUE decreases the potential for nutrient loss, increases plant nutrient concentrations and maximizes nutrient adsorption and mobilization (Baligar et al., 2001). *Phragmites australis* is another well-

studied aquatic macrophyte, that when given the opportunity will exhibit luxury uptake (Farahbakshazad and Morrison, 1997; Kern and Idler, 1999; Meuleman et al., 2002). Farahbakshazad and Morrison (1997) suggested that in highly loaded systems, luxury plant uptake with rhizome storage dominates nitrogen removal. Luxury uptake is not limited to aquatic vegetation. Japanese Larch (*Larix kampeferi* Sarg.), an upland deciduous species, exhibits induced luxury nutrient consumption (Qu et al., 2003). Qu et al. (2003) demonstrated that Japanese Larch at high nutrient concentrations yielded a higher nutrient uptake of nitrogen, produced more above-ground biomass and

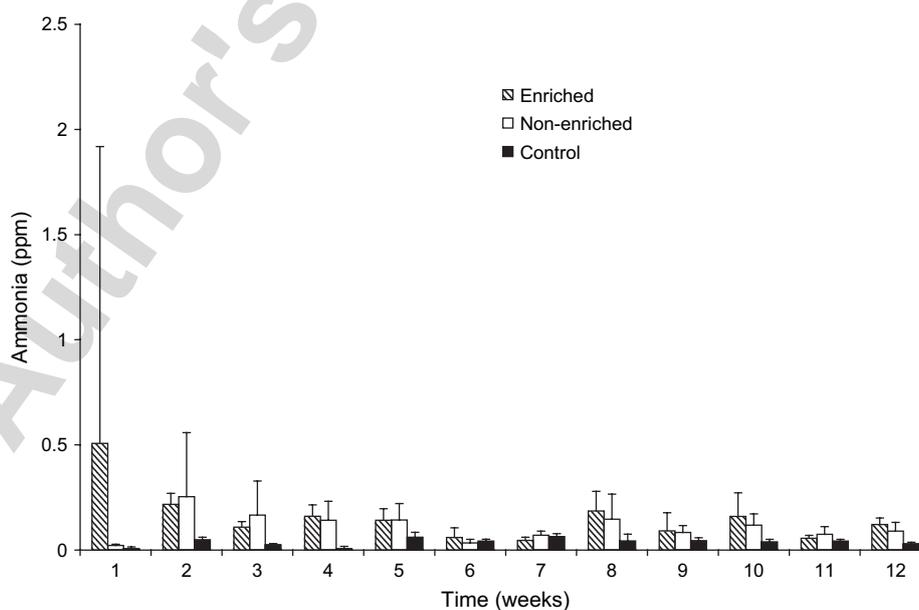


Fig. 4. Mean ammonia concentrations (\pm SE) in the water column as a result of *Leersia oryzoides* tissue senescence and microbial metabolism, December 2004–February 2005.

had higher foliar nitrogen concentrations, suggesting that higher nutrient loads induced luxury nutrient consumption within the plant. However, in environments where seasonal shifts in temperature cause senescence of the above-ground tissue of many wetland species, that luxury capacity will release sequestered nutrients back into receiving waters. Whether or not uptake and storage is negated is difficult to conclude since this study did not examine the below-ground component and how its nutrient content changed through time. However, *L. oryzoides* is capable of luxury uptake, as evidenced by above-ground tissue nutrient content and through senescence its nutrient enriched above-ground tissue releases more P than tissue that has not undergone luxury uptake. Release of accumulated nutrients has received far less attention in the literature than the ability of nutrient uptake (Boyd and Hess, 1970; Jordan et al., 1999; Vymazal et al., 1999), even though decomposition of aquatic macrophytes is an important avenue for the return of assimilated nutrients back into the aquatic environment (Hill, 1979). Upon breakdown, organic nutrients are more readily utilized by consumer and decomposer organisms. Under senescence, nutrient-enriched above-ground vegetation releases higher concentrations of P than non-enriched plants. The rapid increase in water column P in week 1 follows the rapid leaching of soluble material suggested to occur in the first 0–8 days (Hill, 1979). After this time period, much slower degradation occurs. This pattern of nutrient release upon decomposition is widely reported in the literature (Nichols and Keeney, 1972; Kuehn and Suberkropp, 1998; Cronk and Fennessy, 2001). Furthermore, plant material that was enriched had lower C:N and C:P ratios, and resulted in greater amounts of organic and inorganic P accumulating in the water column (Nichols and Keeney, 1972).

Many investigations of phosphorus dynamics (Doughty, 1930; Bache, 1964; Patrick and Khalid, 1974; Cooke, 1992) have concentrated on phosphorous release from sediments under aerobic and anaerobic conditions. Phosphorus released during senescence of aquatic macrophytes could constitute another potential source of internal P to outflow and downstream environments. Release of nitrate and nitrite may be negated by denitrification, nitrate ammonification as well as immobilization into microbial, algal and fungal biomass and protein (Davis and van Der Valk, 1978; Laanbroek, 1990). Accumulation of N in the form of protein by the growing population of microorganisms has been suggested to be responsible for measured net declines in N species from the water column. Nichols and Keeney (1972) found that macrophytes contained enough P to sustain the microbial population thus allowing the net accumulation of N-protein in the population and a net decrease of N from the water and sediment. Another possible explanation for a lack of inorganic N in the system may be an artifact of the C:N ratio. The C:N ratio was above 25 for both the treated (38) and untreated containers (201). C:N tissue ratios greater than 25 indicate that N levels in the decomposing plant litter were not high enough to support release of inorganic N from the associated microbial population (Davis and van Der Valk, 1978). Furthermore, once that ratio began to increase, microorganisms may have compensated for this deficit by

extracting N from the surrounding water, further lowering the aqueous N concentrations in the water column (Davis and van Der Valk, 1978). High C:N ratios could also have been an artifact of the translocation of nutrients such as N and P to below-ground storage structures between the growing season and senescence.

Plants exposed to agricultural runoff can contribute to the assimilation of nutrients. With senescence, this assimilation function may be buffered to some extent with subsequent translocation of nutrients to below-ground storage structures, reducing above-ground tissue concentrations. Phosphorus data suggest that P will be leached into the surrounding system during senescent periods such as with winter dormancy. Nitrogen species such as nitrate and ammonia were consistently low throughout the duration of the experiment suggesting that denitrification, transforming nitrogen species to dinitrogen, may have been an important process.

5. Conclusions

The fundamental premise of this research lies in the temporal heterogeneity of wetland mitigation components such as plant nutrient uptake. This study shed light on the sequestration capacity of a particular temperate wetland plant and its associated nutrient release in seasonal environments. Wetland plants have the capacity to sequester high loads of nutrients. However, upon the conclusion of the growing season when senescence occurs, a portion of those sequestered nutrients may be released and can potentially have negative impacts on downstream aquatic receiving systems.

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References

- Bache, B.W., 1964. Aluminum and iron phosphate studies relating to soils. Reactions between phosphate and hydrous oxides. *Journal of Soil Science* 15, 110–116.
- Baligar, V.C., Fageria, N.K., He, Z.L., 2001. Nutrient use efficiency in plants. *Communications in Soil Science and Plant Analysis* 32, 921–950.
- Boyd, C.E., Hess, L.W., 1970. Factors influencing shoot production and mineral nutrient levels in *Typha latifolia*. *Ecology* 51, 296–300.
- Brix, H., 1994. Functions of macrophytes in constructed wetlands. *Water Science and Technology* 29, 71–78.
- Clesceri, L.S., Greenberg, A.E., Eaton, A.D., 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th ed. American Public Health Association, Washington DC.
- Cooke, J.G., 1992. Phosphorus removal processes in a wetland after a decade of receiving a sewage effluent. *Journal of Environmental Quality* 21, 733–739.

- Cooper, C.M., Moore, M.T., Bennett, E.R., Smith, J.S., Farris, J.L., 2002. Alternative environmental benefits of agricultural drainage ditches. *Verhandlungen der Internationale Vereinigung für Limnologie* 28, 1678–1682.
- Cronk, J.K., Fennessy, M.S., 2001. *Wetland Plants: Biology and Ecology*. Lewis Publishers, New York.
- Davis, C.B., van Der Valk, A.G., 1978. The decomposition of standing and fallen litter of *Typha glauca* and *Scirpus fluviatilis*. *Canadian Journal of Botany* 56, 662–675.
- Davis, S.A., Holland, M.M., 1998. Vascular plant species native to Northern Mississippi for use in constructed wetlands for wastewater treatment, In: Mississippi Water Resources Conference, Raymond, MS, pp. 10–19.
- Dolan, T.J., Bayley, S.E., Zoltek, J.J., Hermann, A.J., 1981. Phosphorus dynamics of a Florida freshwater marsh receiving treated wastewater. *Journal of Applied Ecology* 18, 205–219.
- Doughty, J.L., 1930. The fixation of phosphate by a peat soil. *Soil Science* 29, 23–35.
- Farahbakshazad, N., Morrison, G.M., 1997. Ammonia removal processes for urine in an upflow macrophyte system. *Environmental Science and Technology* 31, 3314–3317.
- Gaudet, J.J., 1977. Uptake, accumulation and loss of nutrients by papyrus in tropical swamps. *Ecology* 58, 415–422.
- Greenway, M., 1997. Nutrient content of wetland plants in constructed wetland receiving municipal effluent in tropical Australia. *Water Science and Technology* 35, 135–142.
- Hammer, D.A., 1992. Designing constructed wetlands systems to treat agricultural nonpoint source pollution. *Ecological Engineering* 3, 1–34.
- Hill, B.H., 1979. Uptake and release of nutrients by aquatic macrophytes. *Aquatic Botany* 7, 87–93.
- Isaac, R.A., Johnson, W.A., 1985. Elemental analysis of plant tissue by plasma emission spectroscopy: collaborative study. *Journal of the Association of Official Analytical Chemists* 68, 499–510.
- Johnston, C.A., 1991. Sediment and nutrient retention by freshwater wetlands: effects on surface water quality. *Critical Reviews in Environmental Control* 21, 491–565.
- Jordan, T.E., Whigham, D.F., Hofmockel, K., Gerber, N., 1999. Restored wetlands in crop fields control nutrient runoff. In: Vymazal, J. (Ed.), *Nutrient Cycling and Retention in Natural and Constructed Wetlands*. Backhuys Publishers, Leiden, pp. 49–60 (Chapter 4).
- Kern, J., Idler, C., 1999. Treatment of domestic and agricultural wastewater by reed bed systems. *Ecological Engineering* 12, 13–25.
- Klopatek, J.M., 1978. Nutrient dynamics of freshwater riverine marshes and the role of emergent macrophytes. In: Good, R.E., Whigham, D.F., Simpson, R.L. (Eds.), *Freshwater Wetlands: Ecological Processes and Management Potential*. Academic Press, New York, pp. 195–215.
- Kuehn, K.A., Gessner, M.O., Wetzel, R.G., Suberkropp, K., 1999. Decomposition and CO₂ evolution from standing litter of the emergent macrophyte *Erianthus giganteus*. *Microbial Ecology* 38, 50–57.
- Kuehn, K.A., Suberkropp, K., 1998. Decomposition of standing litter of freshwater emergent macrophyte *Juncus effusus*. *Freshwater Biology* 40, 717–727.
- Laanbroek, H.J., 1990. Bacterial cycling of minerals that affect plant growth in waterlogged soils: a review. *Aquatic Botany* 38, 109–125.
- Meuleman, A.F.M., Beekman, J.P., Verhoeven, J.T.A., 2002. Nutrient retention and nutrient-use efficiency in *Phragmites australis* stands after wastewater application. *Wetlands* 22, 712–721.
- Nichols, D.S., Keeney, D.R., 1972. Nitrogen and phosphorus release from decaying water milfoil. *Hydrobiologia* 42, 509–525.
- Patrick, W.H.J., Khalid, R.A., 1974. Phosphate release and sorption by soils and sediments: effect of aerobic and anaerobic conditions. *Science* 186, 53–55.
- Peeverly, J.H., 1985. Element accumulation and release by macrophytes in a wetland stream. *Journal of Environmental Quality* 14, 137–143.
- Qu, L., Qureshi, A.M., Koike, T., 2003. Root growth characteristics, biomass and nutrient dynamics of seedlings of two larch species raised under different fertilization regimes. *Plant and Soil* 255, 293–302.
- Richardson, C.J., 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Science* 228, 1424–1427.
- Vymazal, J., Dusek, J., Kvet, J., 1999. Nutrient uptake and storage by plants in constructed wetlands with horizontal sub-surface flow: a comparative study. In: Vymazal, J. (Ed.), *Nutrient Cycling and Retention in Natural and Constructed Wetlands*. Backhuys Publishers, Leiden, pp. 85–100 (Chapter 6).