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The interacting effects of temperature and plant community type on nutrient removal in wetland microcosms

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Abstract

Treatment wetlands can remove nutrients from inflow sources through biogeochemical processes. Plant composition and temperature play important roles in the nutrient removal efficiency of these wetlands, but the interactions between these variables are not well understood. We investigated the seasonal efficiency of wetland macrophytes to reduce soil leachate concentrations of total nitrogen and total phosphorus in experimental microcosms. Each microcosm contained one of six vegetation treatments: unplanted, planted with one of four species (*Carex lacustris*, *Scirpus validus*, *Phalaris arundinacea* and *Typha latifolid*) in monoculture or planted with an equal abundance of all four species. Microcosms were also subjected to two temperature treatments: insulated microcosms and microcosms exposed to environmental conditions. A constant nutrient solution containing 56 mg/l N and 31 mg/l P was added to all microcosms three times a week. Water samples were analyzed monthly for total dissolved nitrogen and total dissolved phosphorous. Microcosms exhibited a typical pattern of seasonal nutrient removal with higher removal rates in the growing season and lower rates in the winter months. In general, planted microcosms outperformed unplanted microcosms. Among the plant treatments, *Carex lacustris* was the least efficient. The four remaining plant treatments removed an equivalent amount of nutrients. Insulated microcosms were more efficient in the winter and early spring months. Although a seasonal pattern of nutrient removal was observed, this variation can be minimized through planting and insulation of wetlands.

Keywords: Treatment wetlands; Phytoremediation; Eutrophication; Nitrogen; Phosphorous; Seasonality; Microcosms

1. Introduction

Human activities have had a large effect on global biogeochemical cycles. Through agricultural practices, urbanization, industrialization and other alterations, humans have increased the input of nutrients into biogeochemical cycles, especially nitrogen (Vitousek et al., 1997) and phosphorous (Reckhow and Simpson, 1980). Nutrient enrichment, or eutrophication, of aquatic ecosystems can cause an increase in algae and aquatic plants, loss of component species and loss of ecosystem function (Smith et al., 1999). Eutrophication is the largest water quality problem throughout the world (Carpenter et al., 1998). For example, 61% of 2048 water bodies located in the United States failed to meet EPA standards with regard to total nitrogen and total phosphorous (Smith et al., 1997a,b). Wetlands have been investigated as a possible solution to these global eutrophication and water quality problems (Mitsch et al., 2001; Fraser et al., 2003).

Total nitrogen and total phosphorous removal in treatment wetlands can range from 3–98% to 31–99% respectively (Spieles and Mitsch, 2000; Steer et al.,

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2002). On average, the removal of nitrogen and phosphorous from these systems is about 50% (Verhoeven and Meuleman, 1999). Plants and seasonal temperature change are two factors that greatly affect the nutrient removal efficiency of these systems. Plants, such as species from the genera *Typha*, *Scirpus*, and *Phragmites* play an important role in the biogeochemical cycle of treatment wetlands (Brix, 1997; Wood et al., 1999; Schutes, 2001; Fraser et al., 2004).

Wetlands are affected by solar radiation and ambient temperatures, which cycle on an annual and daily basis. These abiotic factors mediate the temperature of the wetland environment causing cyclical patterns in evapotranspiration, photosynthesis and microbial activity (Kadlec, 1999). Laboratory studies demonstrate the optimum temperature for nutrient removal to be 30 °C (Wood et al., 1999). ''Biological zero'' or 5 °C is where biological processes drastically slow down or cease (Mitsch and Gosselink, 1993). More specifically, nitrification rates in wetlands become inhibited at water temperatures of about 10 °C and rates drop rapidly at 6 °C (Werker et al., 2002). Phosphorous removal is affected less because it is dominated by sediment adsorption as opposed to biological processes. Many treatment wetlands in temperate climates often operate at a much lower level of nutrient removal efficiency in the colder months (Spieles and Mitsch, 2000). Wittgren and Maehlum (1997) state that nitrogen cycling is inhibited in colder months due to the decrease of oxygen availability. Furthermore, extreme temperatures inhibit the growth rate of nitrogen reducing bacteria (Spieles and Mitsch, 2000). Conversely, constructed wetlands have effectively run at atmospheric temperatures of −4 °C in northern China and −20 °C in Norway (Yin and Shen, 1995; Werker et al., 2002). These wetlands are often insulated by some natural or artificial means. Many cold-climate wetlands are also specifically designed to operate in cold conditions.

Little research has been done on the interacting effects of the role of plants in treatment wetlands and seasonal temperature changes on nutrient removal efficiency. The objective of this experiment was to investigate the seasonal changes in efficiency with respect to plant communities using microcosms. This study also explored the use of insulation as a method for increasing nutrient removal efficiency by mediating wetland temperature. The major questions to be answered were: (1) How does nutrient removal change throughout the seasons with respect to different plant communities? (2) Do plants exhibit species-specific rates of nutrient removal? (3) Does insulating microcosms improve their effectiveness in extreme temperatures by mediating soil temperature? We expected that all microcosms would be more efficient in the growing season as compared to the winter months. Based on previous research, nutrient removal was expected to be greatest in microcosms containing polycultures during all seasons. Throughout the experiment, planted microcosms should be more efficient than unplanted microcosms. It was also predicted that insulated microcosms should provide a more suitable environment for nutrient removal.

2. Methods

2.1. Study site

This experiment was carried out in an enclosed area at the Bath Nature Preserve in Bath, Ohio (41°06′39″ N, 81°51′42″ W). The research area consisted of fencing that was 2.4 m tall and enclosed a 15.2 × 15.2 m area of grassland within the preserve. The fencing was covered with bird netting and secured with a locked gate entry. The area was mowed and outfitted with three 189.3-l water tanks, which provided water throughout this experiment. A weather station (Watchdog) was placed in the research area and recorded the ambient temperature at 15-min intervals throughout the study period.

2.2. Microcosms

The microcosms used in this experiment consisted of 18.93-l buckets (28.58 cm diameter and 35.88 cm high) that were a quarter filled with soil. The microcosms were filled with approximately 0.02 m^3 of highly organic soil (Carlisle muck), which was taken from the Panzner Wetland Restoration Site in Copley, Ohio. Each microcosm had a 2 cm hole in the bottom plugged with a removable rubber stopper to allow drainage. A mesh lining was glued to the bottom of the bucket to prevent soil loss during drainage. Four 2 cm holes were also drilled into the sides of the buckets at a point about 5 cm above the soil surface. These holes were evenly distributed around the circumference of the bucket. The purpose of these holes was to ensure that the water levels in the buckets did not exceed 5 cm above the soil surface.

2.3. Experimental design

The experimental design for this study involved a six by two factorial with six replicates. Hence, the experiment consisted of 72 microcosms. Each microcosm contained one of six vegetation treatments. These treatments consisted of second year wetland plants in monoculture or an equal mixture of these species. There was also a set of unplanted microcosms, which acted as a control. The four species of wetland plants used were *Carex lacustris*, *Scirpus validus*, *Phalaris arundinacea*, and *Typha latifolia*. Rhizomatous cuttings from wild plants were planted in the microcosms in May 2001. Each microcosm was planted with six cuttings of each

species in monoculture and two of each species in the combination treatments. Each cutting was approximately 12 cm in length. The majority of the microcosms had well established plant communities by August of 2001. However, some plants did not survive the winter of 2001–2002. Hence, additional rhizomatous cutting were added to the microcosms in April 2002 using the same procedure. By June 2002, all of the microcosms housed a well-established plant community. Throughout the experiment, microcosms were hand weeded regularly to remove any invading plants. The surrounding research area was also kept mowed to ensure that the surrounding, natural plant growth was not interfering with the plant communities within the microcosms.

In addition to the vegetation treatment, half of the microcosms were also subjected to an insulation treatment. At the research site, holes of the approximate size of the microcosms were drilled into the ground using a gas-powered auger. These holes were then fitted with a sleeve of Reflectix©insulation to provide further insulation, as well as to fortify the structure of the hole. Half of the microcosms were placed in these holes so the soil levels of the bucket were slightly below the actual soil level. The remaining microcosms were left at regular soil level in order to be exposed to the environment.

Microcosms were arranged into six pair wise exposed and insulated blocks for a total of 12 blocks. The order of the pair wise arrangement was randomly assigned. Within each block, the six vegetation treatments were randomly distributed. Six Stowaway Tidbit Weatherproof and Waterproof Temperature Loggers (Forestry Suppliers Inc.) were placed in randomly designated insulated and exposed microcosms. These data loggers were buried about 15 cm below the soil surface. The locations of the data loggers were changed weekly to compensate for the low quantity of loggers being utilized. However, this became difficult in the winter months when the top layer of soil was often frozen. Hence, the loggers were moved to new locations as the weather permitted during these colder months. These loggers took and stored readings of soil temperature every 30 min.

2.4. Nutrient additions

The experiment officially began in June 2002 and ran for one year. A modified Rorison solution of 56 mg/l $Ca(NO_3)_2$ and 31 mg/l K_2HPO_4 was added three times a week to all microcosms (Hendry and Grime, 1993). Microcosms were also watered at a frequency that kept the water level at approximately the soil surface. In order to avoid ice formation in the microcosms, a higher concentration Rorison solution was added once a week in the colder months. Nutrient additions were added at approximately midday throughout the duration of the experiment. Nutrient additions were standardized so that on a monthly basis each microcosm received 672 mg/l $Ca(NO_3)_2$ and 372 mg/l K_2HPO_4. These nutrient loads are similar to those of actual treatment wetlands.

2.5. Sampling regime and laboratory analysis

A 125 ml sample of filtered effluent (using a 50 μm sieve) was taken from each of the microcosms during the last few days of each month, with the exception of February. Due to extremely low temperatures, the samples for February were taken during the first week of March. At the time of sampling, each microcosm was also drained of any remaining water in an attempt to mimic natural systems. All water samples were collected in Nalgene© bottles and stored at approximately 4 °C within a laboratory refrigerator until further analysis. The data logger information was also obtained during each sampling event using Boxcar 3.7 software (Forestry Suppliers).

To ensure accurate results, all water samples were analyzed for total dissolved nitrogen (TDN) and total dissolved phosphorous (TDP) within five days of sampling. This analysis involved a modified Kjeldahl technique utilizing HACK Test N′ Tube kits. Percent absorbance was obtained from the samples using a HACH DR/4000 Spectrometer. The absorbance was transformed into concentration values for TDN and TDP using a standardized curve.

2.6. Calculations and statistical analysis

With the exception of June through August, biweekly means and standard error were calculated for each set of data loggers. Only monthly averages were available for June through August. Temporary logger malfunction caused gaps in the data set. Consequently, no further statistical analysis could be done on this data.

All statistical analyses were done using Systat Version 8 (SPSS, 1998). Data were analyzed for homoscedasticity and normality of residuals. Monthly mean values and standard errors for TDN and TDP were calculated for each cross treatment. A general linear model was applied to determine potential block effects for TDN and TDP. TDN and TDP were analyzed with a repeated measures two-way analysis of variance (ANOVA). These ANOVA were used to test the effects of the plant treatment and insulation treatment on effluent nutrient concentrations, as well as the interacting effects of these treatments. The monthly sampling was set as the repeated measures.

A set of one-way ANOVA tests was run to detect the significance of the insulation treatment on each plant treatment. A final set of ANOVA tests was run to detect the monthly significance of the insulation treatment. For all aforementioned statistical tests, the significance level was set at $p < 0.05$.

3. Results

Mean data logger and ambient temperature readings are represented in Fig. 1. All temperature readings were highest in the month of July. These maximum temperature readings were as follows: insulated = 23.0 °C ± 0.1, exposed = 24.4 °C ± 0.1 and ambient = 23.0 °C ± 0.1. Temperature readings were the lowest from 1/19/03 to 1/25/03. These minimum temperature readings were as follows: insulated = −0.7 °C ± 0.1, exposed = −8.9 °C ± 0.1 and ambient = −11.1 °C ± 0.1. There was little difference between data logger readings within the temperature treatments as demonstrated by the low standard errors.

Monthly mean TDN readings for each plant treatment within the two insulation treatments are represented in Fig. 2. The unplanted treatment demonstrated a maximum TDN output within the exposed treatment in January (100.6 mg/l ± 4.2) and a minimum value in July (24.7 mg/l ± 3.3) within the insulated microcosm group. *Scirpus validus* demonstrated a maximum and minimum TDN output within the exposed treatment in January (87.1 mg/l ± 3.4) and August (4.7 mg/l ± 0.5). *Carex lacustris* demonstrated a maximum and minimum TDN output within the exposed treatment in January (95.7 mg/l ± 1.8) and September (15.3 mg/l ± 3.6). *Phalaris arundinacea* demonstrated a maximum and minimum TDN output within the exposed treatment in February (88.7 mg/l ± 3.0) and July (8.9 mg/l ± 2.3). *Typha latifolia* demonstrated a maximum and minimum TDN output within the exposed treatment in January (92.6 mg/l ± 1.4) and August (10.2 mg/l ± 2.7). The combination of plants demonstrated a maximum TDN value within the exposed treatment in January (75.7 mg/l ± 4.1) and a minimum value in July (9.8 mg/l ± 3.2) within the insulated microcosm group. The results of a two-way repeated measures ANOVA (Table 1) show that both the plant and insulation treatments had a significant effect on TDN output ($p < 0.001$). The interacting effect of the plant and insulation treatments was also significant ($p < 0.05$). The effects of month, month and plant treatment interactions and month and insulation treatment interactions were all significant ($p < 0.001$).

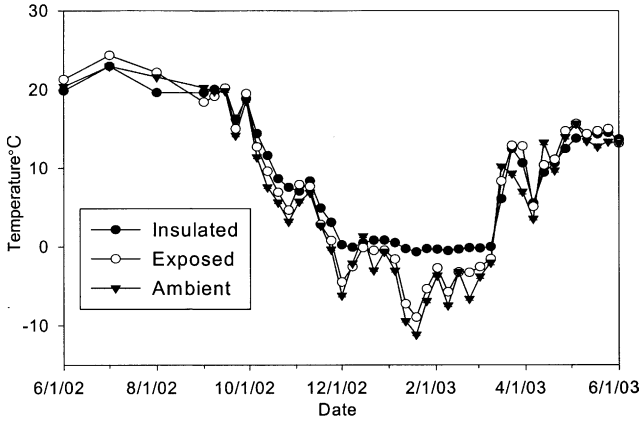

Fig. 1. Mean temperature readings from the microcosm data loggers and the weather station.

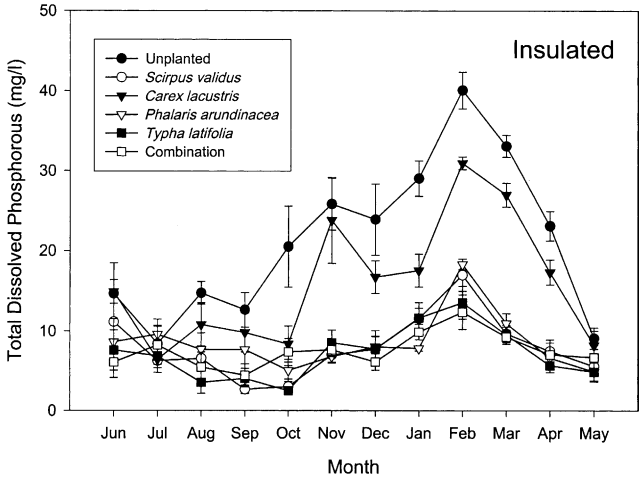

Fig. 2. Monthly mean total dissolved nitrogen values for each plant treatment within the insulation treatments. Error bars represent ±1 SE.

Monthly mean TDP readings for each plant treatment within the two insulation treatments are represented in Fig. 3. The unplanted treatment demonstrated a maximum TDP output within the exposed treatment in February (42.9 mg/l ± 2.1) and a minimum value in July (8.4 mg/l ± 2.2) within the insulated microcosm group. *Scirpus validus* demonstrated a maximum and minimum TDP output within the exposed treatment in February (23.1 mg/l ± 1.9) and August (7.8 mg/l ± 0.3). *Carex lacustris* demonstrated a maximum and minimum TDP output within the exposed treatment in February (37.2 mg/l ± 2.2) and September (4.5 mg/l ± 0.8). *Phalaris arundinacea* demonstrated a maximum and minimum TDP output within the exposed treatment in February (24.6 mg/l ± 0.6) and July (4.9 mg/l ± 1.9).

Table 1
Two-way repeated measures ANOVA for total dissolved nitrogen

Source	SS	DF	*F*-value	*P*-value
Between subjects				
Plant	141,167.241	5	115.988	0.001
Insulation	11,405.891	1	46.858	0.001
Plant × insulation	3327.458	5	2.734	0.027
Error	14,604.974	60		
Within subjects				
Month	560,457.878	11	420.326	0.001
Month × plant	45,526.841	55	6.829	0.001
Month × insulation	13,745.929	11	10.309	0.001
Month × plant × insulation	7245.579	55	1.087	0.316
Error	80,003.3630	660		

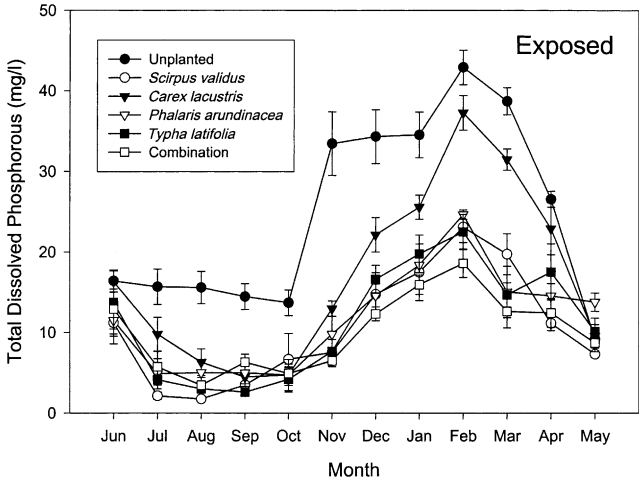

Fig. 3. Monthly mean total dissolved phosphorous values for each plant treatment within the insulation treatments. Error bars represent ±1 SE.

Typha latifolia demonstrated a maximum and minimum TDP output within the exposed treatment in February (22.5 mg/l ± 2.2) and September (2.6 mg/l ± 0.4). The combination of plants demonstrated a maximum and minimum TDP value within the exposed treatment in February (18.6 mg/l ± 1.8) and August (3.5 mg/l ± 1.0). The results of a two-way repeated measures ANOVA (Table 2) show that both the plant and insulation treatments had a significant effect on TDP output ($p < 0.001$). The effects of month, month and plant treatment interactions and month and insulation treatment interactions were all significant ($p < 0.001$). The combined interaction of month, plant and insulation was also significant ($p < 0.05$).

Throughout the study period, the TDN output of insulated microcosms was significantly less than that of exposed microcosms for *Phalaris arundinacea* ($p < 0.05$), *Typha latifolia* ($p < 0.05$) and the plant combination ($p < 0.05$) (Table 3). Similarly, the TDP output of insulated microcosms was less than that of exposed microcosms for *Scirpus validus* ($p < 0.05$), *Phalaris arundinacea* ($p < 0.05$), *Typha latifolia* ($p < 0.001$) and the plant combination ($p < 0.05$) (Table 4). For all plant treatments, the insulated microcosms exhibited significantly less TDN output than exposed microcosms for the following months: December ($p < 0.05$), January ($p < 0.001$), April ($p < 0.05$) and May ($p < 0.001$) (Table 5). For all plant treatments, the insulated microcosms exhibited significantly less TDP output than exposed microcosms for the following months: June ($p < 0.05$), December ($p < 0.05$), January ($p < 0.001$), February ($p < 0.05$), March ($p < 0.05$), April ($p < 0.05$) and May ($p < 0.001$) (Table 6).

Table 2
Two-way repeated measures ANOVA for total dissolved phosphorous

Source	SS	DF	*F*-value	*P*-value
Between subjects				
Plant	24,218.410	5	96.941	0.001
Insulation	1569.975	1	31.421	0.001
Plant × insulation	356.052	5	1.425	0.228
Error	2997.907	60		
Within subjects				
Month	27,480.241	11	119.057	0.001
Month × plant	8017.503	55	6.947	0.001
Month × insulation	2958.042	11	12.816	0.001
Month × plant × insulation	1692.846	55	1.467	0.018
Error	13,848.940	660		

Table 3
One-way ANOVA analysis of total nitrogen data to determine effects of the insulation treatment on each plant treatment

Plant	Source	SS	DF	*F*-value	*P*-value
Unplanted	Insulation	252.082	1	0.258	0.613
	Error	138,914.022	142		
Scirpus validus	Insulation	1351.628	1	1.597	0.208
	Error	120,217.370	142		
Carex lacustris	Insulation	148.450	1	0.145	0.704
	Error	145,345.465	142		
Phalaris arundinacea	Insulation	5242.074	1	6.495	0.012
	Error	114,611.712	142		
Typha latifolia	Insulation	3678.887	1	4.394	0.038
	Error	118,891.002	142		
Combination	Insulation	4002.239	1	6.798	0.010
	Error	83,601.697	142		

Table 4
One-way ANOVA analysis of total phosphorous data to determine effects of the insulation treatment on each plant treatment

Plant	Source	SS	DF	*F*-value	*P*-value
Unplanted	Insulation	426.433	1	3.027	0.084
	Error	20,004.755	142		
Scirpus validus	Insulation	212.253	1	4.480	0.036
	Error	6728.052	142		
Carex lacustris	Insulation	2.268	1	0.022	0.883
	Error	14,896.370	142		
Phalaris arundinacea	Insulation	392.278	1	10.116	0.002
	Error	5506.212			
Typha latifolia	Insulation	637.550	1	14.233	0.001
	Error	6360.899	142		
Combination	Insulation	224.328	1	8.926	0.003
	Error	3568.545			

4. Discussion

The objective of this study was to examine the effects of season on the nutrient removal efficiency of various types of plant communities using microcosms. The experiment also set out to investigate if there are species-specific rates of nutrient removal and to explore the use of insulation for the increase of nutrient

Table 5
One-way ANOVA analysis of total nitrogen data to determine the monthly effects of the insulation treatment

Month	Source	SS	DF	*F*-value	*P*-value
June	Insulation	199.341	1	1.634	0.205
	Error	8541.470	70		
July	Insulation	10.467	1	0.054	0.817
	Error	13,508.004	70		
August	Insulation	28.743	1	0.070	0.792
	Error	28,609.021	70		
September	Insulation	136.891	1	0.440	0.509
	Error	21,791.870	70		
October	Insulation	43.264	1	0.216	0.644
	Error	14,030.095	70		
November	Insulation	1979.463	1	2.644	0.108
	Error	52,405.144	70		
December	Insulation	2622.232	1	8.203	0.006
	Error	22,376.118	70		
January	Insulation	3863.952	1	16.502	0.001
	Error	16,390.498	70		
February	Insulation	43.984	1	0.359	0.551
	Error	8571.791	70		
March	Insulation	437.335	1	0.996	0.322
	Error	30,731.821	70		
April	Insulation	7902.633	1	10.690	0.002
	Error	51,748.989	70		
May	Insulation	7883.515	1	23.817	0.001
	Error	23,170.636	70		

Table 6
One-way ANOVA analysis of total phosphorous data to determine the monthly effects of the insulation treatment

Month	Source	SS	DF	*F*-value	*P*-value
June	Insulation	180.114	1	4.184	0.045
	Error	3013.372	70		
July	Insulation	96.216	1	2.592	0.112
	Error	2598.307	70		
August	Insulation	92.725	1	2.971	0.089
	Error	2184.923	70		
September	Insulation	11.849	1	0.449	0.505
	Error	1847.646	70		
October	Insulation	30.695	1	0.622	0.433
	Error	3452.818	70		
November	Insulation	1.448	1	0.013	0.909
	Error	7647.915	70		
December	Insulation	986.827	1	12.828	0.001
	Error	5384.972	70		
January	Insulation	976.739	1	14.635	0.001
	Error	4671.755	70		
February	Insulation	674.571	1	6.191	0.015
	Error	7627.172	70		
March	Insulation	538.976	1	4.805	0.032
	Error	7851.257	70		
April	Insulation	720.430	1	12.875	0.001
	Error	3916.993	70		
May	Insulation	187.669	1	15.849	0.001
	Error	828.885	70		

removal. It was hypothesized that all microcosms would experience a decrease in nutrient removal during the winter months as compared to the growing season. Plant polycultures were predicted to remove the greatest amount of nutrients. Also, insulated microcosms were predicted to be more effective in removing nutrients than exposed microcosms.

During the year long study, all microcosms removed large amounts of both nitrogen and phosphorous compared to large-scale natural and treatment wetlands. These high rates of nutrient removal may be due to the small treatment area of the microcosms (Tanner et al., 1995). The microcosms exhibited a definite pattern in seasonal nutrient removal. In general, nutrient removal was highest during the growing season (June–October) and lowest in the cold months (November–March). Nutrient removal then increased again in April and May and was associated with an increase in temperature and plant growth. This pattern of nutrient removal is similar to that of many natural and treatment wetlands (Mitsch and Gosselink, 1993; Spieles and Mitsch, 2000; Tanner, 2001b). There was less variance in seasonal phosphorous removal when compared to nitrogen. This difference in variance is demonstrated in many treatment wetlands and may be due to year-round sedimentary binding of phosphorous (Kadlec and Knight, 1996; Wittgren and Maehlum, 1997).

Planted microcosms almost always outperformed unplanted microcosms in removing nutrients. This has been observed in various microcosm and full-scale experiments (Hunter et al., 2001; Juwarkar et al., 1995; Zhu and Sikora, 1995; Fraser et al., 2004). This pattern was also demonstrated in a natural wetland comparing nutrient retention in unvegetated patches and patches containing *Juncus effusus* (Mann and Wetzel, 2000). In general, planted microcosms were also less vulnerable to monthly fluctuations in nutrient removal efficiency. The elevated nutrient uptake in planted systems may be directly due to plant processes and more stable year-round temperatures in planted systems (Hill and Payton, 2000). The direct uptake of nutrients by macrophytes has been shown to be significant in various experiments. For example, a microcosm experiment demonstrated that uptake from *Scirpus validus* accounted for over 90% of nitrogen removal and over 74% of phosphorous removal (Hunter et al., 2001). Similar experiments concluded that aquatic plants were responsible for approximately 90% of nitrogen removal (Rogers et al., 1991).

Unplanted microcosms also consistently removed large concentrations of nutrients. Some unplanted microcosms were colonized by duckweed (*Lemna* spp.), which have been shown to remove nutrients from wastewater in Czechoslovakian treatment wetlands (Vymazal, 2002). The unplanted microcosms that remained free of duckweed also reduced nutrient levels to a great extent. This suggests that microbial processes are the major pathways of nutrient removal in all the microcosms and plants provided supplemental nutrient removal. There is also a great deal of research support-

ing microbial processes as the impetus behind nutrient removal in treatment wetlands. The monitoring of four wetlands used to treat dairy wastewater demonstrated that plant nutrient uptake was variable with nitrogen and phosphorous removal ranging from 3–19% to 3–60% respectively (Tanner et al., 1995). In addition, experiments on full-scale wetlands valued direct uptake by plants as only 7% for nitrogen and 8% for phosphorous (Tanner, 2001a). It is assumed that plant-mediated microbial processes were the major pathways of nutrient removal in these systems. In this experiment, macrophytes may be removing some nutrients through direct uptake and providing an environment for more intense microbial activity. Microbial communities are often more numerous and diverse in planted treatment wetlands as compared to wetlands with little or no vegetation (Ottova et al., 1997; Werker et al., 2002). This could be due to the diverse microhabitats within the soil structure created by plant root growth. Plants may also be supplying carbon to the microbes, which allows these microbes to be more successful in surviving and removing nutrients (Lin et al., 2002).

Contrary to some studies, there was no clearly defined pattern of species-specific nutrient removal found in this experiment (Bachand and Home, 2000; Gersberg et al., 1986; Lin et al., 2002). This specificity is often due to nutrient needs, plant physiology and plant morphology. *Scirpus validus*, *Phalaris arundinacea*, *Typha latifolia* and the plant combination communities all exhibited near equal nutrient removal efficiency for most of the year. In other treatment wetland research, *Typha* spp. removed greater amounts of nutrients as compared to *Scirpus* spp. (Coleman et al., 2001). Similar research showed *Scirpus* spp. to outperform *Typha* spp. in nutrient removal efficiency (Bachand and Home, 2000; Gersberg et al., 1986). *Phalaris* spp. was shown to be of greater or equal nutrient removal efficiency when compared to *Typha* spp. and *Scirpus* spp. depending on the type of nitrogen species (Zhu and Sikora, 1995). These results exhibit the complexity of wetlands systems, as well as research involving these systems.

It has been hypothesized that plant polycultures outperform plant monocultures in removing nutrients (Coleman et al., 2001; Kadlec and Knight, 1996; Karpiscak et al., 1996). In this experiment, the polycultures and their associated communities tended to outperform the other plant treatments in certain months. The growing season of wetland plants is species-specific with some species having longer growing seasons than other species. This phenomenon supports the theory of using polycultures to maximize nutrient removal (Scholes et al., 1999).

The *Carex lacustris* community was the least efficient of the plant treatments. This may be due to *Carex lacustris* having lower amounts of below ground biomass or a different root morphology than the other plants utilized in this experiment. Consequently, these characteristics could have lead to the lower abundance or diversity of soil microbes when compared to the other plant communities. These results are different than those of a similar experiment involving the same wetland plant species. The previous experiment found that *Carex lacustris* community removed nutrients at an intermediate level, while the *Phalaris arundinacea* community was the least efficient of the plant treatments (Fraser et al., 2004). The differences in results may be due to the relative maturation of the microcosm communities. Fraser et al. (2004) experimented on microcosms that had established for one year, whereas our experiment was conducted on 2–3 year-old planted microcosms. Studies demonstrate that both treatment and natural wetlands often are more efficient at removing nutrients after the wetland community is allowed to develop (Biesboer, 1984; Maehlum and Stalnacke, 1999; Werker et al., 2002). An important community characteristic that is directly related to nutrient removal is the development of the root zone (Wood et al., 1999). The root zone of the microcosms containing *Phalaris arundinacea*, a plant with high amounts of belowground biomass, may have developed greatly over the past year. In relation to this concept, the roots of *Phalaris arundinacea* have been known to release high concentrations of organic carbon that facilitates microbial nutrient removal pathways (Zhu and Sikora, 1995).

Temperature and the use of insulation was the other variable being investigated in this experiment. The insulation treatment in this experiment had a varied effect on nutrient removal. Overall, insulation had no effect on nutrient removal during the growing season. However, insulated microcosms were often significantly more effective in the winter months, as well as the early spring months. During the warmer months both sets of microcosms must have experienced the same rates of heat absorption. Insulated microcosms were probably able to better retain this heat in the winter. With respect to plant treatment, insulation only affected planted microcosms. As demonstrated by the data logger information, soil temperature of insulated microcosms was only greater than that of exposed microcosms in the winter months. Hence, this difference may have lead to higher amounts of nutrient removal during the winter. The insulation may also have protected the belowground biomass of the macrophytes allowing for more vigorous growth in the early spring. This could be the reason for differences in nutrient removal between the insulation treatments during early spring.

Nutrient removal in treatment wetlands has been shown to be temperature dependent. Hence, nutrient removal is often a primary factor when designing cold-climate treatment wetlands (Werker et al., 2002). Low technology methods of combating cold-climate effects include construction of subsurface flow wetlands and the allowance of high retention times (Maehlum and

Stalnacke, 1999; Werker et al., 2002). In addition, cold climate wetlands may also operate successfully due to insulation by a natural vegetation layer, snow cover or a thin ice layer (Smith et al., 1997a,b; Werker et al., 2002; Wittgren and Maehlum, 1997). Incoming wastewater may also provide a heat source for many treatment wetlands (Kadlec, 2001). More complex methods of insulation have also been shown to allow for year-round operation of cold-climate wetlands. These methods include the enclosure of treatment wetland systems, as well as the addition of industrial byproducts to the wetland substrate (Merlin et al., 2002; Peterson and Teal, 1996).

5. Conclusion

This experiment demonstrated that there is a distinct seasonal pattern of nutrient removal within treatment wetland microcosms. Microcosms removed the greatest amount of nutrients during the growing season and lesser amounts in the colder months. However, seasonal differences in nutrient removal can be minimized through the use of plant growth and insulation. Planted microcosms outperformed unplanted microcosms stressing the importance of macrophytes in treatment wetland operation. Plants play a supplemental role in nutrient cycling to microbial processes, which seem to be the major pathway of nutrient removal in these microcosms. Within planted microcosms, the *Carex lacustris* community performed the poorest. Hence, monocultures of *Carex lacustris* and other sedges may not be the optimal plants to utilize in treatment wetlands. More reliable results may have been attained with temperature and insulation effects if this experiment was performed in a green house or climate controlled structure. This study also focused on only a few variables that may affect treatment wetlands. Studies of other variables and interactions are needed to better understand these systems. These types of microcosm studies should be an impetus for further large-scale experiments that deal with wetland design and function. The coupling of these two research strategies could lead to better management of these systems. Consequently, these wetlands could be operated more successfully in temperate and cold climate regions.

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