Transactions of the Illinois State Academy of Science
(1997), Volume 90, 3 and 4, pp. 103-112

received 12/13/96
accepted 4/10/97

Carbon, Plant, and Temperature Control of Nitrate Removal from Wetland Mesocosms

Mark B. David[1]*, Lowell E. Gentry[1], Karen M. Smith[1], and David A. Kovacic[2]

[1]University of Illinois
Department of Natural Resources and Environmental Sciences
W-503 Turner Hall
1102 South Goodwin Av.
Urbana, IL 61801

[2]University of Illinois
Department of Landscape Architecture
101 Temple Buell Hall
611 East Lorado Taft Drive
Champaign, IL 61820

*Corresponding author (m-david@uiuc.edu)

ABSTRACT

Constructed wetlands have been developed to remove agricultural non-point source pollution from tile drainage waters in the Midwest, but their effectiveness and function are not known. This study investigated the interaction of C availability and temperature on NO_3^- removal from water columns in a constructed wetland. Experimental mesocosms (20.32 cm diameter PVC pipes) were buried upright to a depth of 15 cm into wetland sediments to enclose a 7.5 L water column (23 cm depth). Six mesocosms were placed in areas with bare soil and six were placed in areas supporting reed canary grass (*Phalaris arundinacea*). Treatments were either NO_3^- additions (10 mg NO_3^--N L^{-1} increase in concentration in water column) or NO_3^- plus glucose additions (10 mg NO_3^--N L^{-1} and 50 mg C L^{-1} increases in water column) to the mesocosms during April and June. In April, (11-12°C water temperature) over a 7 day time span, NO_3^- concentrations in the overlying water decreased approximately 50% in non-grass treatments, with or without glucose additions. All or nearly all of the NO_3^- was removed from the grass mesocosms in April, and glucose additions did not increase the removal rate. In June (27°C water temperature) NO_3^- concentrations decreased to zero for all treatments in 48 hours or less. Presence of grass did not affect the rate of NO_3^- decrease; however, glucose additions increased the rate to < 24 hours. When calculated on a mass basis in the NO_3^- only mesocosms, removal of NO_3^- was 0.25 and 0.42 g NO_3^--N m^{-2} d^{-1} in the April non-grass and grass treatments, respectively, and 1.6 and 1.4 g NO_3^--N m^{-2} d^{-1} in the June corresponding treatments. Calculated Q_{10} values of NO_3^- removal per day for non-grass and grass treatments were 3.3 and 2.2, respectively. Depending on amounts and seasonal timing of inputs of NO_3^- to

the wetlands, mesocosm results suggest that large amounts of NO_3^- can be removed from the overlying water by a combination of sediment and plant mechanisms.

INTRODUCTION

Constructed wetlands have been used to remove a variety of pollutants from various sources, including sewage effluent, mine drainage, and urban runoff. This is due to the ability of wetlands to retain a wide range of nutrients and toxic metals (Gambrell, 1994; Kadlec and Knight, 1996). Recently, studies have been evaluating the feasibility and capability of constructed wetlands to remove nutrients and pesticides from agricultural non-point source pollution (Kovacic et al., 1996). This is particularly important in Illinois, where large amounts of agricultural chemicals enter surface waters that are often used as drinking water sources. Agricultural non-point source pollution can cause NO_3^- concentrations in surface waters of Illinois to exceed health advisory standards (Osborne and Kovacic, 1993).

Riparian buffer strips have been shown to reduce inputs of agricultural chemicals to surface waters in some agricultural areas, although the removal mechanisms for various chemicals have not been clearly identified (Hill, 1996). However, in Illinois nearly 40% of agricultural land is artificially drained with perforated tile lines (Fausey et al., 1995) that shunt runoff to surface waters, bypassing any riparian zones. Constructed wetlands can be positioned to intercept tile drainage, allowing chemicals such as NO_3^- to be removed before drainage waters enter surface waters (Kovacic et al., 1996). Nitrate can be removed from wetland water by two main processes: microbial denitrification and plant uptake. Organic carbon (C) is needed as an electron donor, and can be a major limiting factor controlling denitrification (McCarty and Bremner, 1993), along with availability of NO_3^- (Seitzinger, 1994). Plants can add C to wetland ecosystems through litter (above- and below-ground) and rhizopshere sources, as well as incorporate NO_3^- into biomass. A constant source of mineralizable C is needed in the wetland sediments to keep denitrification processes active. Because denitrification is a microbial process, it is temperature dependent and denitrification may be limited during winter and early spring conditions (Hill, 1996).

In east-central Illinois, five wetlands have been constructed to receive tile drainage from agricultural fields (Kovacic et al., 1996). In the subsurface drainage tiles that transport agricultural runoff to these wetlands, we found high NO_3^- concentrations (David et al., 1997), but low concentrations of dissolved organic C (DOC). We hypothesized that C may be limiting denitrification in these wetlands, and that a C source, from either plants or added glucose, could enhance denitrification. In addition to C availability, we hypothesized that seasonal variations in temperature and plant growth would also affect the rate of decrease of NO_3^- from these wetlands. Therefore, our objective was to investigate the interaction of C availability and temperature on NO_3^- removal from the water column in a constructed wetland.

METHODS

Our study site was located along the Embarras River about 20 km south of Champaign-Urbana, Illinois. Five wetlands were constructed in 1994 to intercept tile drainage lines from corn-soybean agricultural fields (Kovacic et al., 1996). They were constructed by excavating soil from land that was in pasture to form a berm near the river, which allowed tile water to be detained in wetlands ranging in size from 0.5 to 2 ha. Within each wetland area, strips of soil were removed for berm construction, which left variable areas of undisturbed soil. The experiments presented here were on relatively undisturbed soil between excavated strips.

Following construction, soil samples were collected by depth (0-10, 10-30, 30-50, and 50-100 cm) at approximately eight locations in each wetland, four in disturbed areas and four in relatively undisturbed areas. Results reported here are for the undisturbed soils where the mesocosm studies were conducted. Soil samples were air-dried, sieved (2 mm), and subsamples ground to 40 mesh (~0.4 mm). Oven-dry mass was determined at 105°C, and used to correct all measurements on air-dry soil. Sieved soil was used for determination of pH in water (1:1 soil:solution ratio; Blume et al., 1990) and extractable phosphorus (P) (Olsen and Sommers, 1982). Ground soil was analyzed for total organic matter by loss-on-ignition (combustion at 450°C for 18 h), organic C using a LECO analyzer (Nelson and Sommers, 1982), and total N by Kjeldahl digestion (Bremner and Mulvaney, 1982) followed by measurement of NH_4 using an automated phenate method (APHA, 1989).

Experimental mesocosms were used to determine the rate and mechanisms of NO_3^- removal in one of the constructed wetlands. Twelve-20.32 cm inside diameter PVC pipes were used as mesocosms and buried upright to a depth of 15 cm into the sediment to enclose a 7.5 L water column (23 cm depth). Six of the mesocosms were placed in areas with reed canary grass (*Phalaris arundinacea*) and six in areas with bare soil. Treatments (three replicates each) were then randomly applied to each group of mesocosms that consisted of either NO_3^- additions (10 mg NO_3^--N L^{-1} increase in concentration in water column) or NO_3^- plus glucose additions (10 mg NO_3^--N L^{-1} and 50 mg C L^{-1} increases in water column). Initial concentrations of NO_3^- and C varied slightly following the additions due to ambient concentrations present. Nitrate was added (as $Ca(NO_3)_2 \bullet 4H_2O$) to bring the concentration to at least 10 mg NO_3^--N L^{-1} because tile water entering the wetlands is generally between 5-15 mg NO_3^--N L^{-1} (Kovacic et al., 1996).

Following the additions of NO_3^- and C, mesocosm water was sampled 4 or 5 times in 7 days, depending on the rate of decrease of NO_3^- from the water column, including an initial sample after the additions were made. Samples were obtained by gently mixing the overlying water in each mesocosm (without sediment disturbance) before sampling and then collecting a small volume of water (100 ml). All solutions were immediately returned to the laboratory, and pH was determined by glass electrode. Samples were then filtered (Whatman GF/C glass fiber) and analyzed for NO_3^- and sulfate (SO_4^{2-}) by ion chromatography, dissolved organic carbon (DOC) using a Dohrmann DC-80 C analyzer, NH_4^+ by automated phenate, and ortho-phosphate by colorimetric techniques (APHA, 1989).

Temperature of both the water and soil in the mesocosms was recorded during each sample collection using a thermocouple probe and digital meter. After the completion of each experiment, all above-ground reed canary grass in the mesocosms was harvested and dried to constant mass at 65°C to determine grass biomass. Reed canary grass was the only plant species present in the mesocosms. In this study, removal of NO_3^- refers to a decrease in concentration of NO_3^- in the water column.

All data were analyzed using SAS for Windows v. 6.11 and the GLM procedure with a repeated measures analysis of variance (ANOVA) technique. For most of the solutes of interest, the interaction term (treatment*day) was significant ($p < 0.01$), so that we analyzed individual days to test specific treatment effects. To examine rates of change, we used regression to fit a line to the concentration of individual solutes versus time in each replicate during each experiment. The slopes were then compared using ANOVA. Duncan's multiple range test was used for mean separation when treatment effects were significant to test a specific response.

RESULTS

Wetland soils were rich in organic matter (Table 1), probably a result of alluvial parent material and previous land use as a pasture. Both organic C and N concentrations down to 100 cm were much greater than nearby agricultural fields (David et al., 1994), and indicated that large pools of C and N were present in the soil before wetland construction. The C:N ratio was 13:1 down to a depth of 50 cm suggesting that the organic matter present was well decomposed and varied little with depth. Soil pH was near neutral at all depths, and extractable P was enriched in the upper soil, decreasing from 38 mg P kg^{-1} in the 0-10 cm depth to 7 mg P kg^{-1} in the 50-100 cm depth. The soil chemistry measured before diversion of tile drainage water onto these soils generally indicate that available C levels, at least initially, might be high enough to support the microbial activity needed for denitrification.

Soil and water temperatures were higher in the June experiment (24.9 to 27.3°C) compared to April (9.9 to 12.0°C), with water temperatures always greater than the soil (Table 2). Grass biomass was also greater in all mesocosms during June compared to April.

For the April mesocosm period (Figure 1), NO_3^- concentrations in the overlying water decreased in all treatments ($p < 0.01$). Over the seven day period, approximately one-half of the NO_3^- was removed from non-grass treatments, regardless of whether C was added. On day 7, all or nearly all of the NO_3^- was removed from grass mesocosms. The addition of glucose did not increase the rate of NO_3^- removal in either soil or grass mesocosms ($p > 0.05$). Therefore, although glucose decreased rapidly in the mesocosms, it did not appear to control NO_3^- removal rates. For treatments without glucose, ambient concentrations of dissolved organic C were high (10-12 mg C L^{-1}) and constant. For all treatments, SO_4^{2-}-S concentrations remained high (18-21 mg S L^{-1}) and unchanged throughout the experiment ($p < 0.01$). Solution pH (about 8.0) had little change during the experimental period, and concentrations of ortho-P and NH_4^+-N were low and showed no response to treatment ($p < 0.01$).

For the June period (Figure 2), NO_3^--N concentrations decreased to zero for all treatments in 48 hours or less. Presence of grass did not make a difference in rate of NO_3^- decrease ($p > 0.05$), however, the addition of glucose increased the rate of NO_3^- removal to within 24 hours ($p < 0.01$). For glucose treatments, most of the additional C was removed within 48 hours. As in April, the ambient concentrations of DOC were high. Sulfate concentrations were lower in the June (1-7 mg SO_4^{2-}-S L^{-1}) compared to April and decreased for all treatments except the non-grass treatment without glucose ($p < 0.05$). As in the April experiments, solution pH was about 8.0 and was greater in the soil mesocosms compared to the grass on day 5 ($p < 0.01$). Ortho-P and NH_4^+-N had no response to treatment ($p > 0.05$).

DISCUSSION

During both experimental periods, NO_3^- concentrations decreased rapidly in all treatments. This may have been due to both plant uptake and denitrification. We presume the NO_3^- removal from mesocosms without grass was due to denitrification and not diffusion of NO_3^- into the soil, because Cl concentrations (data not shown) remained stable over time. When grass was present, we could not distinguish between denitrification and plant uptake. Grass, however, did increase NO_3^- removal in April ($p < 0.05$), but not in June ($p > 0.05$). Although grass biomass was greater in June (Table 2), the demand for NO_3^- by the grass may have been less at this time, and/or the microbes may have outcompeted the grass for NO_3^-. Johengen and LaRock (1993) found that plant mesocosms had the highest removal efficiencies for NO_3^-, but similar to our results, found that sediment-only mesocosms also removed nearly as much NO_3^-. This supported their conclusion that much of the removal processes occurred at the substrate surface (Johengen and LaRock, 1993).

The addition of an easily degradable C source increased NO_3^- removal for June treatments, but did not affect NO_3^- removal in April. Although ambient DOC concentrations were high, C was apparently still limiting in June because added glucose stimulated NO_3^- removal. When temperatures were cool and microbial activity was presumed lower (as in the April mesocosms), the added glucose had no effect. The ambient DOC present may not have been in an easily degradable form, thus limiting microbial activity during warm conditions in June.

Nitrate was removed more quickly in June when both water and soil temperatures were more than twice as high as April temperatures. In addition, we suspect the redox potential was lower in June, as evidenced by the decrease in concentration of SO_4^{2-}-S in all treatments, except non-grass mesocosms without glucose.

When calculated on a mass basis in the NO_3^- only mesocosms, removal of NO_3^- was 0.25 and 0.42 g NO_3^--N m^{-2} d^{-1} in the April non-grass and grass treatments, respectively, and 1.6 and 1.4 g NO_3^--N m^{-2} d^{-1} in the June corresponding treatments. Calculated Q_{10} values of NO_3^--N removal per day for the non-grass and grass treatments were 3.3 and 2.2, respectively. These Q_{10} values indicate that enzymatic reactions are taking place, and show the importance of temperature in controlling NO_3^- removal rates. Temperature seemed to be a more important controlling factor for NO_3^- removal than C levels in these mesocosm experiments. Also, because we added NO_3^- to the mesocosms and the

experiments were short-term, NO_3^- was probably not limiting to denitrification. Other studies have shown clear effects of NO_3^- concentration on NO_3^- removal rates in wetland or riparian ecosystems, but have not shown such a strong response to temperature (Phipps and Crumpton, 1994; Seitzinger, 1994; Nelson et al., 1995).

Recent estimates of tile NO_3^- inputs on a daily basis (David et al., 1997) to the wetland where the mesocosm experiments were conducted range from about 10-20 kg NO_3^--N d^{-1} in the winter to early spring and 20-40 kg NO_3^--N d^{-1} in late spring to early summer from precipitation events. This amount of tile NO_3^- is distributed over an approximately 1.5 ha wetland area. With April rates of NO_3^- removal averaging 0.34 g NO_3^--N m^{-2} d^{-1} in the mesocosms, this would give an estimated NO_3^- removal rate (or potential) of 5.1 kg NO_3^--N d^{-1} for the entire wetland. If the water was retained in the wetland for several days to weeks and entered in pulses of 10-20 kg NO_3^--N d^{-1} that were spread out over time, the wetlands should be able to remove most of the NO_3^- during this period. June rates calculated in this way were 22 kg NO_3^--N d^{-1}, again indicating that NO_3^--N removal rates in the wetlands could remove substantial NO_3^- if the water detention time was long enough. It is not known what winter (January - March) NO_3^- removal rates would be like, since temperatures would be closer to 0°C. However, assuming that the Q_{10} values calculated for the higher temperature range were valid at these low temperatures, rates would be expected to be 2.75 times lower than our April measurements at about 12°C, or about 0.12 g NO_3^--N m^2 d^{-1} at 2°C. This would lead to an estimated NO_3^- removal of 1.8 kg NO_3^--N d^{-1} for the wetland at 2°C.

CONCLUSIONS

Nitrate removal occurred in all treatments, and denitrification was presumed to be the dominant mechanism. Higher temperatures of the water and soil in mesocosms enhanced NO_3^- removal in June versus April. Added glucose stimulated NO_3^- removal, except when microbial activity was presumed low due to cooler temperatures. When glucose was added in June, NO_3^- removal and C disappearance were closely related. The redox potential may have been lower in June than in April based on the decrease in SO_4^{2-}-S concentrations. Calculated NO_3^- removal rates for the wetland based on mesocosms gave estimates of 5.1 and 22 kg NO_3^--N d^{-1} removal of NO_3^- for the entire wetland for the conditions present in the April and June experiments. Depending on amounts and seasonal timing of inputs of NO_3^- to the wetlands, mesocosm results suggest that large amounts of NO_3^- can be removed from the overlying water.

REFERENCES

APHA. 1989. Standard Methods For The Examination of Water and Wastewater. 17th Edition. American Public Health Association, Washington, DC.

Blume L. J., Schumacher B. A., Schaffer P. W., Cappo K. A., Papp M. L., van Remortel R. D., Coffey D. S., Johnson M. G. and Chaloud D. J. 1990. Handbook of Methods for Acid Deposition Studies Laboratory Analyses for Soil Chemistry. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Las Vegas, Nevada. EPA/600/4-90/023.

Bremner J. M. and Mulvaney C. S. 1982. Nitrogen-total. p. 595-624. *In* Methods of Soil Analysis. Part 2. Chemical and Microbial Properties (A. L. Page, R. H. Miller and D. R. Keeney, Eds). Agronomy Monograph 9. American Society of Agronomy,

David, M.B., L.E. Gentry, D.A. Kovacic and K.M. Smith. 1997. Nitrogen balance in and export from an agricultural watershed. Journal of Environmental Quality (in press).

David, M.B., D.A. Kovacic, L.E. Gentry and K.D. Konyha. 1994. Nitrogen dynamics of agricultural watersheds in central Illinois. p. 110-115. *In* M. Davis (ed.) Research on Agricultural Chemicals in Illinois Groundwater: State and Future Directions IV. Proc. Fourth Annual Conference. Southern Illinois University, Carbondale, IL.

Fausey, N.R., L.C. Brown, H.W. Belcher, R.S. Kanwar. 1995. Drainage and water quality in Great Lakes cornbelt states. J. Irrigation Drainage Eng. 121:283-288.

Gambrell, R.P. 1994. Trace and toxic metals in wetlands-a review. J. Environ. Qual. 23:883-891.

Hill, A.R. 1996. Nitrate removal in stream riparian zones. J. Environ. Qual. 25:743-755.

Johengen, T.H. and P.A. LaRock. 1993. Quantifying nutrient removal processes within a constructed wetland designed to treat urban stormwater runoff. Ecol. Eng. 2:347-366.

Kadlec, R.H. and R.L. Knight. 1996. Treatment Wetlands. Lewis Publishers, Boca Raton, FL. 893 p.

Kovacic, D.A., M.B. David, and L.E. Gentry. 1996. Grassed detention buffer strips for reducing agricultural nonpoint-source pollution from tile drainage systems. p. 88-97. *In* M. Davis (ed.) Research on Agricultural Chemicals in Illinois Groundwater: State and Future Directions VI. Proc. Sixth Annual Conference. Southern Illinois University, Carbondale, IL.

McCarty, G.W. and J.M. Bremner. 1993. Factors affecting the availability of organic carbon for denitrification of nitrate in subsoils. Biol. Fert. Soils 15:132-136.

Nelson D. W. and Sommers L. E. 1982. Total carbon, organic carbon, and organic matter. p. 539-579. *In* Methods of Soil Analysis. Part 2. Chemical and Microbial Properties (A. L. Page, R. H. Miller and D. R. Keeney, eds). Agronomy Monograph 9. American Society of Agronomy, Madison, WI.

Nelson, W.M., A.J. Gold and P.M. Groffman. 1995. Spatial and temporal variation in groundwater nitrate removal in a riparian forest. J. Environ. Qual. 24:691-699.

Osborne, L.L. and D.A. Kovacic. 1993. Riparian vegetated buffer strips in water-quality restoration and stream management. Freshwater Biol. 29:243-258.

Olsen, S.R. and L.E. Sommers. 1982. Phosphorus. p. 403-430. *In* Methods of Soil Analysis. Part 2. Chemical and Microbial Properties (A. L. Page, R. H. Miller and D. R. Keeney, eds). Agronomy Monograph 9. American Society of Agronomy, Madison, WI.

Phipps, R.G. and W.G. Crumpton. 1994. Factors affecting nitrogen loss in experimental wetlands with different hydrologic loads. Ecol. Eng. 3:399-408.

Seitzinger, S.P. 1994. Linkages between organic matter mineralization and denitrification in eight riparian wetlands. Biogeochemistry 25:19-39.

Figure 1. Mean solution concentrations, with standard errors, of pH, NO_3^--N, ortho-P, dissolved organic carbon (DOC), NH_4^+-N, and SO_4^{2-}-S by treatment in the April mesocosm experiment.

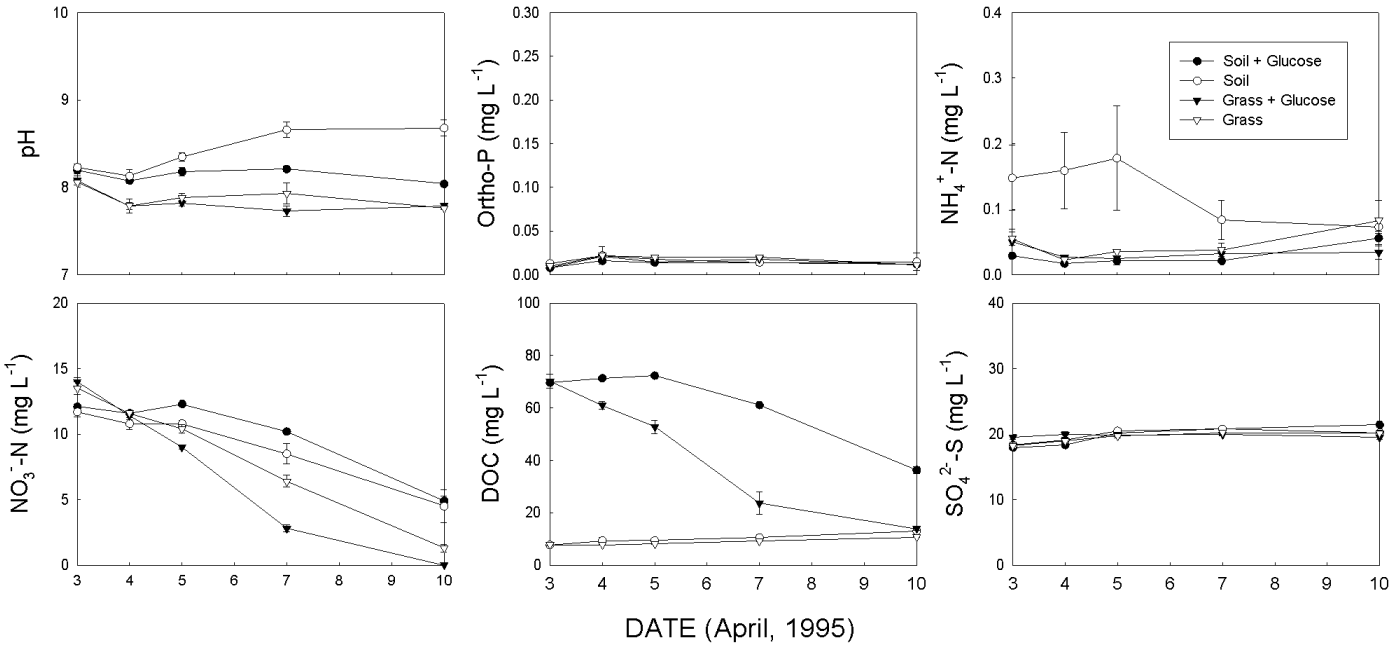

Figure 2. Mean solution concentrations, with standard errors, of pH, NO_3^--N, ortho-P, dissolved organic carbon (DOC), NH_4^+-N, and SO_4^{2-}-S by treatment in the June mesocosm experiment.

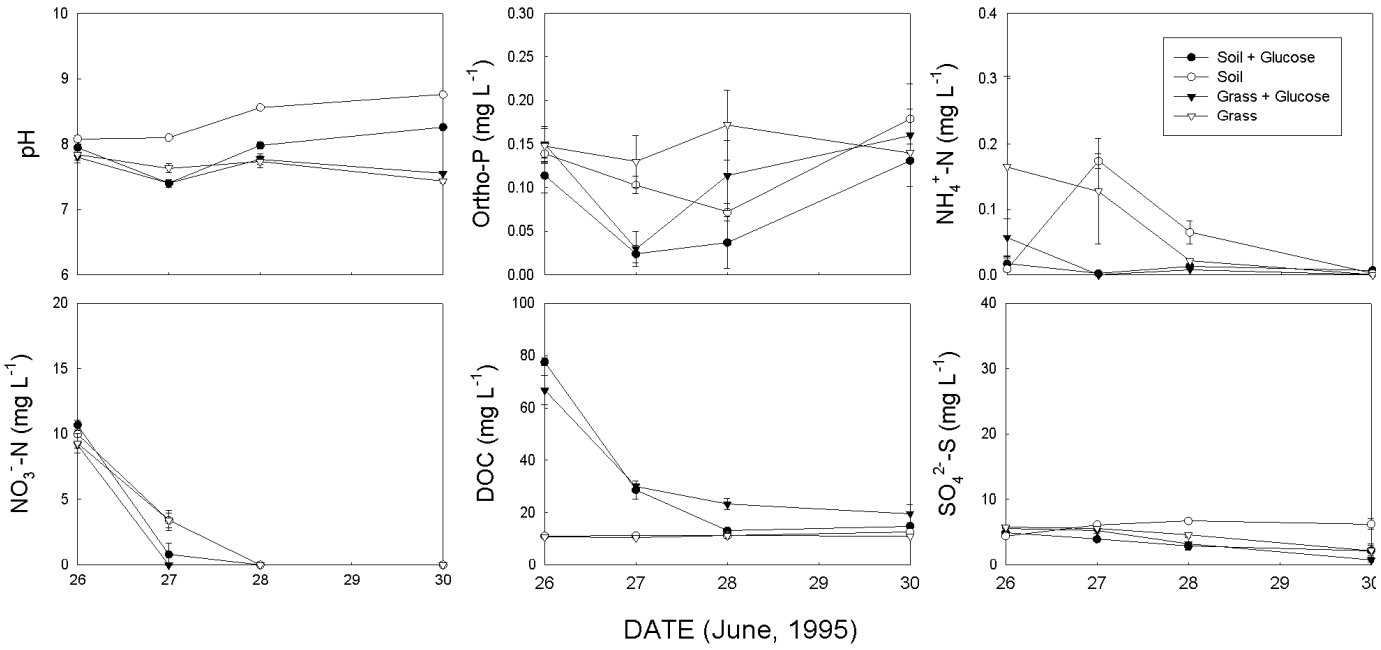

Table 1. Selected characteristics of wetland soils used for mesocosm experiments by depth. Mean with standard error in parenthesis (n=4).

Depth (cm)	pH	Loss-on-ignition (%)	Organic C (%)	Total N (mg kg^{-1})	C:N (mol/mol)	Extractable P (mg kg^{-1})
0-10	6.94 (0.05)	8.9 (0.1)	3.6 (0.1)	2944 (296)	13.0 (0.5)	38 (2)
10-30	6.76 (0.06)	7.9 (0.3)	2.9 (0.1)	2590 (83)	13.0 (0.4)	17 (5)
30-50	6.85 (0.04)	7.0 (0.5)	2.7 (0.2)	2402 (95)	13.3 (0.4)	14 (3)
50-100	7.01 (0.10)	4.9 (0.5)	1.6 (0.2)	1236 (194)	15.3 (0.7)	7 (2)

Table 2. Total grass biomass and temperatures for spring and summer mesocosm experiments. Mean of three replicates with standard errors in parentheses for each vegetative treatment for total grass biomass. Mean of six replicates with standard errors in parentheses for grass vs. non-grass mesocosms for soil and water temperatures.

Parameter	Spring 1995	Summer 1995
Grass Biomass	g m^{-2}	
grass	805 (54)	1403 (11)
grass + C	734 (77)	1178 (138)
Temperature	°C	
Grass		
Soil	9.9 (0.6)	24.9 (0.4)
Water	11.2 (1.3)	26.9 (0.4)
Non-grass		
Soil	10.5 (0.7)	25.9 (0.5)
Water	12.0 (1.2)	27.3 (0.5)