PROJECT COMPLETION REPORT

The Importance of Denitrification to the Efficiency of Wastewater Treatment in Forested Wetlands

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Abstract

Wastewater, even after secondary treatment, typically contains high concentrations of nutrients that can cause eutrophication of receiving waters and deterioration of water quality. Therefore, there has been much interest in the use of natural wetlands as a simple and energy-efficient means of removing nutrients from wastewater and improving water quality. The utilization of a wetland for tertiary treatment of wastewater is based on the ability of the wetland to act as a nutrient sink (ie., outputs are less than inputs). One of the most important processes in wetland ecosystems that influences their capacity as a nitrogen sink is the gaseous exchange of nitrogen with the atmosphere, known as denitrification. Since denitrification represents a loss of nitrogen to the atmosphere, this mechanism tends to be most favorable for the removal of nitrogen. The objectives of this research project were to 1) determine the temporal and spatial ambient rates of denitrification and compare these rates to those of sediments amended with increased concentrations of nitrate comparable to concentrations of total nitrogen in the sewage effluent to be discharged; and 2) determine the proportion of total denitrification that can be attributed to direct utilization of nitrate loaded into the wetland, as compared to nitrate produced via nitrification within the wetland. Although nitrate is readily denitrified, short-term incubation rates are relatively low which is attributed to the presently low nitrate concentrations and subsequent reduced denitrifying microbial population in the wetland sediments. Nitrate concentrations varied seasonally associated with increased flooding during spring. Rates of nitrification coupled with denitrification were investigated with nitrogen-15 isotopes. Nitrification is limited in the wetland sediments; therefore, controls the rate of total nitrogen loss from the system. Maximum rates of nitrification depend largely on oxygen availability which may be enhanced by the rooting system of certain aquatic macrophytes which provide oxygen microsites within reduced sediments.

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Project Objectives

Wastewater, even after secondary treatment, contains high concentrations of nutrients that can cause eutrophication of receiving waters and deterioration of water quality. Therefore, there has been much interest in the use of natural wetlands as a simple and energy-efficient means of removing nutrients from wastewater. This study is concerned with quantifying the rates in which the primary nutrient nitrogen is lost from a forested wetland scheduled to receive secondary effluent in September 1990. High concentrations of nitrogen are expected to be a major constituent of the discharged effluent from the sewage treatment facility in Thibodaux, Louisiana. Denitrification, the reduction of nitrate to nitrogen gas, is a key process by which nitrogen is lost to the atmosphere, however, this process is somewhat limited by the low concentrations of nitrate that are typically found in secondary sewage. Organic-nitrogen and ammonium-nitrogen are predominate in typical secondarily treated wastewater. In order for denitrification to occur these reduced nitrogen species must first be oxidized to nitrate via nitrification. Maximizing rates of nitrification, and coupling this process to denitrification, should result in maximum loss of nitrogen from forested wetlands.

Denitrification is a major pathway for nitrogen loss from sediments to the atmosphere, primarily through a series of biochemical processes: mineralization of organic nitrogen and nitrification of NH_4^+-N, followed by denitrification (Reddy and Patrick 1984). Nitrate concentrations in wetland systems are generally low, primarily due to rapid denitrification and slow rates of nitrification (Reddy et al. 1989). Upon the discharge of sewage effluent, new sources of nitrogen are introduced and the influx of nitrogen is significantly increased in the forms of organic and ammonium-N. Thus the pathway for the loss of this introduced nitrogen to the atmosphere is nitrification followed by denitrification. Since denitrification occurs readily in the presence of nitrate, and nitrate is negligible in typical sewage effluent, the rate limiting process in forested wetlands tends to be nitrification with respect to denitrification. Therefore, the purpose of this study was to (1) determine the temporal and spacial scale of denitrification rates and (2) determine the conditions necessary to enhance the coupling of nitrification and denitrification so as to maximize the removal of nitrogen from the system. These goals are to be achieved by completing the following specific objectives:

1. Determine temporal and spacial ambient rates of denitrification and compare these rates to sediments amended with increased concentrations of nitrate comparable to concentrations of total nitrogen in the sewage effluent to be discharged.

2. Determine the proportion of total denitrification that can be attributed to direct utilization of nitrate loaded into the wetland, as compared to nitrate produced via nitrification within the wetland.

Objective 1 will allow for making predictions on the rates of denitrification that can be expected upon the discharge of nitrogen-loaded effluent. By treating sediments with high concentrations of nitrate, we can determine whether or not these sediments at least have the capacity to handle the projected loading rates. This is a most basic question relative to the success of this project; therefore, with increased concentrations of nitrate induced an increase in the rate of denitrification is expected.

Objective 2 is a more functional question dealing with the loss of nitrogen via two alternate pathways: the utilization of nitrate directly loaded into the wetland and the utilization of nitrate produced in situ via nitrification. The first pathway is refered to as direct denitrification, and the latter, coupled denitrification. Since there is typically negligible nitrate concentrations in sewage effluent, the coupled pathway is expected to be most important to the loss of total nitrogen from the system. The approach in this study was to simulate in the laboratory the conditions which optimize for the coupling of nitrification and denitrification under near ambient conditions.

Related Research

In most biological environments, and in all wastewater treatment processes, phosphorus exists as phosphate and does not change chemical valence. Nitrogen on the other hand cycles between several chemical valences. Therefore, the biological control of nitrogen in effluents is much more complex and difficult than various other components of the effluent. The environmental concerns with wastewater nitrogen are multi-faceted and have been documented as including health, oxygen demand and eutrophication (Barth 1978, Kemp and Day 1984). The successful utilization of natural wetlands for the tertiary treatment of wastewater will depend to a large extent on the control of nitrogen.

In a regional review comprised by Nixon and Lee (1986) mass balance studies performed on eleven sites consisting of various types of wetlands receiving wastewater in the Southeastern United States (Region 3) reveal in all cases that wetlands act as sinks for total nitrogen and phosphorus with respect to adjacent waters. The net uptake of nitrogen was variable ranging from 25% to 74% of inputs with a loading range from 1.7 to 51 g N m^{-2} yr^{-1}. However, it is noted that in only two of the eleven studies denitrification measurements were taken. Furthermore, in spite of the fact that there have been more attempts to measure denitrification in the wetlands of this region than any other area of the country, there remains far less than an adequate assessment of the importance of this process in the tertiary treatment of wastewater.

The major mechanism for removing nitrogen from wastewater applied to wetlands seems to be denitrification (Kemp and Day 1984, Nichols 1983, Reddy et al. 1989). In natural wetland systems the net exchange of nitrogen is controlled by the relative rates of nitrogen fixation and denitrification. One of the rate limiting steps controlling denitrification is the supply of nitrate (Twilley and Kemp 1986). Nitrate can be supplied either directly into the system, or by producing nitrate in situ via nitrification. In wetlands treated with wastewater, the input of nitrogen is increased significantly, requiring increased rates of denitrification to maintain a balance between inputs and outputs. The major dilemma however is in the species of nitrogen that is most dominant in the discharged effluent (e.g. organic-N and ammonium-N). Dierberg and Brezonik (1984) found in a study of cypress domes in Florida that even if conditions were near optimum for denitrification, the small levels of nitrate and nitrite pumped to the sewage domes limit the significance of denitrification in the total nitrogen budget. Furthermore, they suggest that a more favorable design and efficient operation of the treatment would be generating higher levels of nitrate in the wastewater (nitrification), which would greatly enhance the potential use of wetlands as nutrient sinks.

In a review by Nichols (1983) wetlands exposed to high loading rates (54 g m^{-2} yr^{-1} and as high as 428 g m^{-2} yr^{-1}) removed only a small percentage of nitrogen. Yet, in the laboratory under constant anaerobic conditions, soils from these same wetlands denitrified more than 90% of added NO_3-N at nitrogen concentrations as high as typically found in wastewater effluent. Studies such as this indicate that wetland sediments have the capacity to handle high loading rates of nitrogen given the proper environmental conditions. These environmental conditions include a combination of redox potentials, temperature, pH, organic matter content, hydrology, vegetation, and presence of benthic fauna. Together these factors will determine the temporal and spacial scales of denitrification coupled with nitrification in response to nutrient enrichment (Knowles 1982, Nichols 1983, Reddy et al. 1989). Therefore, the successful utilization of natural wetland systems for the tertiary treatment of wastewater will largely depend on understanding the ecological conditions that maximize rates of nitrification, and enhance the coupling of nitrification and denitrification.

Methods and Procedures

Site Description: The study area consists of a cypress/tupelo bottomland hardwood forested area in Terrebonne Parish about 10 km southwest of Thibodaux, Louisiana (Fig. 1). A ridge approximately 500 m wide runs from northeast to southeast separating two shallow water areas; one of which is the control plot and the other the experimental (flooded) plot where

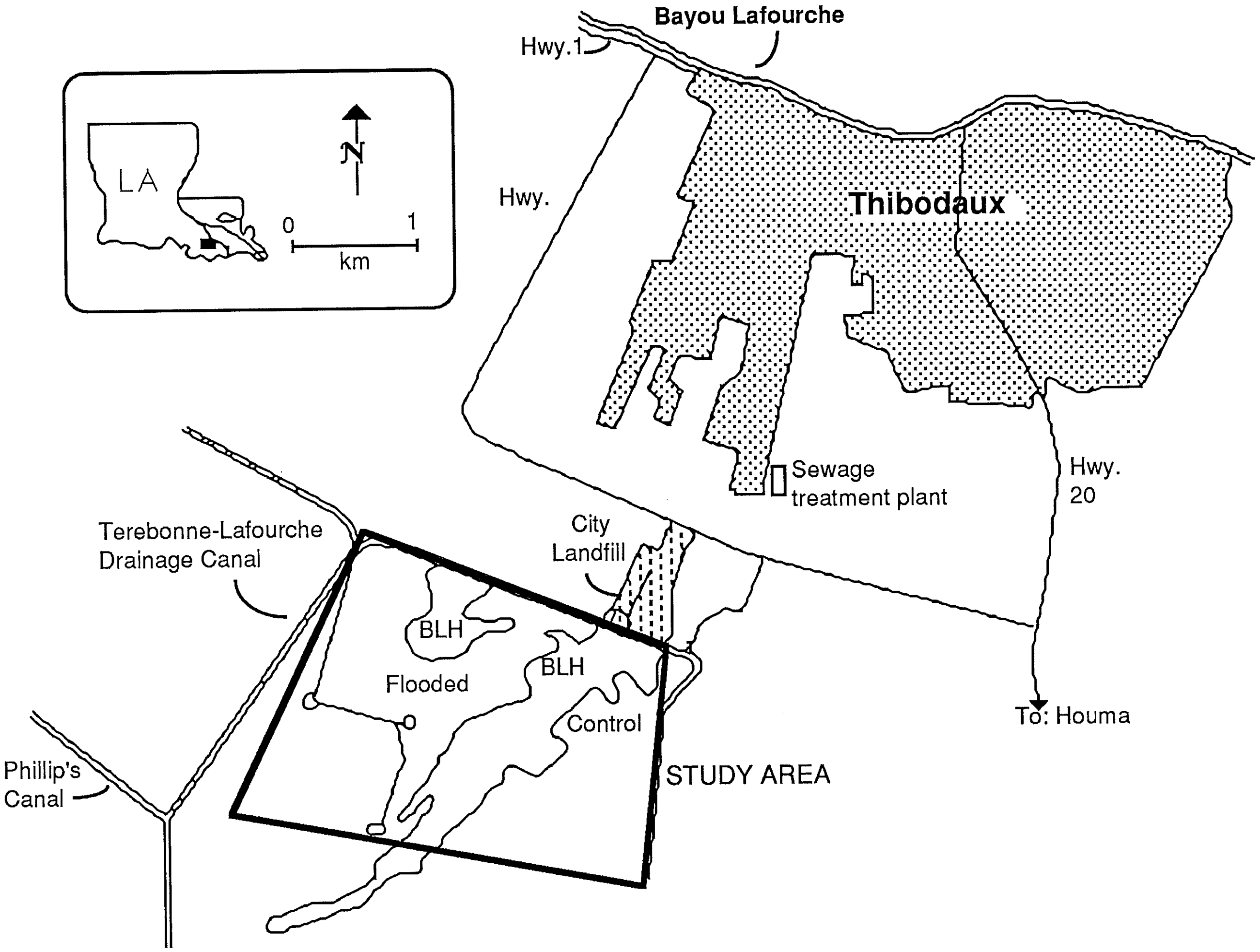

Figure 1. Map of the Thibodaux area showing the location of the city landfill and proposed wastewater application area (outlined in dark). The bottomland hardwood (BLH) ridges are populated with oaks, sweetgum, ash, elm, and maples. The flooded and control areas are populated with mainly ash, willow, maple, and cypress trees.

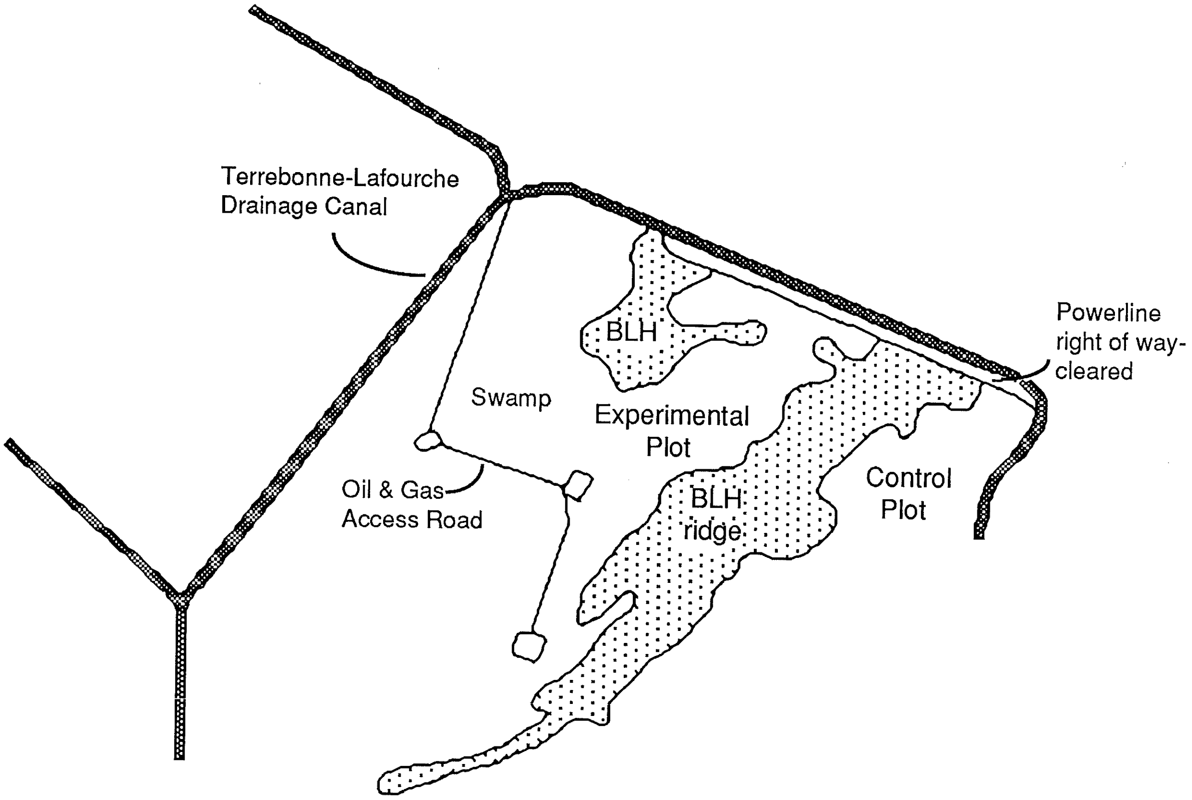

Figure 2. Map of the study area showing the location of the Control and Experimental plots. The experimental (flooded) plot is where the wastewater application will take place.

wastewater effluent will be discharged. A specific area encompassing approximately 15 square meters was chosen for sampling in each plot and was not deviated throughout the entire sampling period so as to avoid sediment diversity. The entire wetland encompasses 1205 ha. The shallow water areas appear to be flooded for most of the year while the ridge area is flooded only during periods of extremely high water (Fig. 2). The flooded plot consists of a 230 ha section that is confined on the west and south by a spoil bank created by the construction of an oil and gas access road, on the north by the Terrebonne Lafourche Drainage Canal, and the east by the bottomland hardwood ridge. Outflow is limited in which the water will flow southward overland through the swamp forest and exit at a point where the ridge and oil and gas access road nearly join. The projected loading rate of effluent at the site is 4 million gallons per day over 230 ha or about 225 cm/yr. The average concentration of total nitrogen in secondary effluent is 20 mg/L for an equivalent loading rate of approximately 0.12 g N m^{-2} d^{-1} or 44 g N m^{-2} yr^{-1}.

Denitrification Studies (Acetylene Block): Intact sediment cores from stations in the flooded and control areas of the study site were incubated under near ambient conditions in the laboratory to simulate the response of each soil type to wastewater enrichment. These sediment cores were used to measure rates of denitrification under ambient concentrations compared to those with increased concentrations of nitrate. The acetylene block technique was used to measure rates of denitrification. Acetylene inhibits the further reduction of nitrous oxide (N_2O) to nitrogen gas which allows for an indirect measurement of nitrogen gas production (Sorensen 1978). A total of 24 cores were taken per experiment: 6 for each treatment (ambient and 3 amendments) of which two were used for each of three subsampling times. The undisturbed sediment samples were taken with modified 60 cc syringes lined with pinholes at 1 cm intervals for 7 cm beginning at the 10 cc mark to allow for 10 ml of overlying water. The pinholes were sealed with silicon vacuum gel and a strip of duct tape was placed over them to prevent leakage. Approximately 30 cc of sediments were extracted and a 10 cc headspace containing the overlying water was maintained. The top of the syringes were sealed with serum bottle stoppers. Water obtained from the sampling site was filtered through a vacuum pump filter system (GF/F 0.1 um) and the overlying water in the amended cores was replaced by solutions enriched with 0.05 mM, 1.0 mM, and 3.0 mM KNO_3. Following water replacement the cores were injected with 1 ml of acetylene saturated water (1.6 ml C_2H_2/1 ml H_2O) through the pinholes in which complete inhibition was assumed as discribed in Sorenson (1978). At time intervals of 5, 60, and 180 minutes 1 ml subsamples were taken from the overlying water and placed in 5 ml vacutainers. These samples were then treated with mercury chloride saturated water (0.1 ml) to prevent further microbial activity. After the overlying water was sampled, the sediments in each core were placed in a 50 ml centrifuge tube and centrifuged at 2000 rpm for 20 minutes. One millileter pore water samples were then placed in 5 ml vacutainers and shaken vigorously on a vortexer for 1 minute to equilibrate the gas concentrations. The tubes were then assayed for nitrous oxide with a Hewlett-Packard 5890 gas chromatograph using the electron capture detector (ECD). Denitrification rates were determined from the difference of the overlying water and porewater nitrous oxide concentrations at t_o and those measured after 3 hours.

Coupled Nitrification/Denitrification (N15 Analyses): Denitrification in sediments was determined by measuring rates of $^{15}N_2$ production from $^{15}NH_4$ amended cores. This method allows for the determination of the relative importance of NO_3 produced from nitrification of NH_4 to total denitrification rates. Cores with water amended with 3 mM $^{15}NH_4$ (95-99% atom % ^{15}N) represent rates of denitrification coupled to nitrification. Intact sediment cores were acquired from the field with 6 inch acrylic cylinders (180cm^2). A headspace of approximately 900 cm^3 in each core was periodically sampled for $^{15}N_2$ during the incubations with 5 ml vacutainers. The samples were then analyzed for atom % ^{15}N excess using a JASCO ^{15}N emission spectrometer.

The importance of oxygen availability to nitrification was tested by comparing cores periodically aerated with cores that were left unaerated. The cores were incubated at the sediment temperature determined in the field upon collection. The cores were incubated for nine days and sampled at 1, 3, 5, 7, and 9 days following initiation of the study. The experiment was terminated on day 9 for sediment analysis. On each day, the overlying water was sampled for pH, dissolved oxygen and nutrients, sealed with plexiglass top, and following 6 h incubation the water was sampled again for these constituents. Redox potentials and pH were measured at the beginning of each sampling day and dissolved oxygen was measured using an Orbisphere oxygen meter.

At the termination of each experiment, 50 g subsamples from each 2 cm deep section of a core to a depth of 6 cm were extracted with 50 ml of 2N KCL. The samples were shaken for 30 minutes, centrifuged at 2000 rpm, and three subsamples were combined and filtered through Whatman # 4 filters for a final volume of 150 ml. The supernatant was prepared for emission spectrometric assay of $^{15}NH_4$ content by modification of the steam distillation technique (Bremner 1965; Keeney and Nelson 1982), and the condensate was collected directly onto 0.035 g of an ion exchange molecular sieve (Union Carbide W-85) according to Lipschultz et al. (1985). The ion sieve was collected on 25 mm A/E filter, dried at 60 C, and stored in aluminum foil until assayed for ^{15}N content. A specific volume of the remaining sample was dried at 60 C for 48 h, weighed and assayed for total ^{15}N concentration according to Fiedler and Proksch (1975).

The ^{15}N content of all materials were analyzed by emission spectrometry using the Dumas technique for sample preparation as described in Fiedler and Proksch (1975). Material was placed in 6 mm OD pyrex tubes containing a 1:1 mixture of CaO and cuprox (CuO). The tubes were attached to a manifold, evacuated to 5 millitorr, and then argon is added to pressure of 1 torr (following two purges of argon to clean the system). The tubes were sealed, heated to 550 C for no less than 10 h, and allowed to slowly cool to room temperature. ^{15}N content was analyzed with a JASCO ^{15}N emission spectrometer.

Sediment and Porewater Analyses: Sediments were collected monthly at each site with a 7 cm^2 (7.5 cm diameter) acrylic core and analyzed for pH, oxidation/reduction potentials (redox), and porewater nitrogen (NH_4 and NO_3/NO_2). pH and redox measurements were taken with an Orion (model SA250) portable meter. pH was taken in the overlying water and redox measurements were taken at 1 cm intervals to a depth of 10 cm using platinum wire and standard calomel electrode standardized with a pH 4 and quinhydrine solution. At 1 cm intervals subsamples were taken to a depth of 8 cm, centrifuged at 2000 rpm for 20 min in 50 ml centrifuge tubes, and the supernatant (porewaters) was filtered (GF/F glass fiber). Porewater nitrogen (NH_4 and

NO_3/NO_2) was analyzed with an ALPKEM RFA/2 autoanalyzer.

Principle Findings

Environmental Factors:

Soil temperature data (Fig. 3) indicate moderate change over the duration of the sampling period. The sediments tend to prevent temperature extremes by providing an insulating effect exhibited by minimum temperature of 18 ^{o}C in the winter months and a maximum of 27 ^{o}C in the summer. In the overlying water temperatures were slightly higher than the porewater by 2 to 3 ^{o}C and were subject to more variability associated with changes in air temperatures.

The pH measurements (Fig. 4) in both the control and flooded plots remain near neutral. In the overlying water the pH tends to be slightly acidic, whereas the porewater pH at 2cm tends to be slightly alkaline. The pH measurements demonstrate a buffering due to the little significant change throughout the sampling period.

Redox (oxidation/reduction) potentials (Fig. 5) differed in the overlying water as compared to the porewater measurements. The overlying water measurements in both the control and flooded plots remain high and do not drop below 350 mV, with the exception of 6 August. This reading is believed to be an artifact due to the low overlying water and difficulty in sampling. Porewater measurements at 2 cm were 100 - 200 mV lower than the overlying water in the late winter months in both sites, and continue to decrease during the summer months. In the control plot, porewater measurements in July and August decrease to nearly - 200 mV, whereas in the flooded plot redox potentials remain relatively steady at about 200 mV.

Dissolved oxygen measurements (Fig. 6) in overlying water taken at various locations in the control and flooded plots exhibit a peak in the month of March at approximately 8 ppm with a subsequent decline to readings of about 2 ppm June to August. These data are comparable to high redox potentials observed in the overlying water in both plots (Fig. 5); however, redox potentials

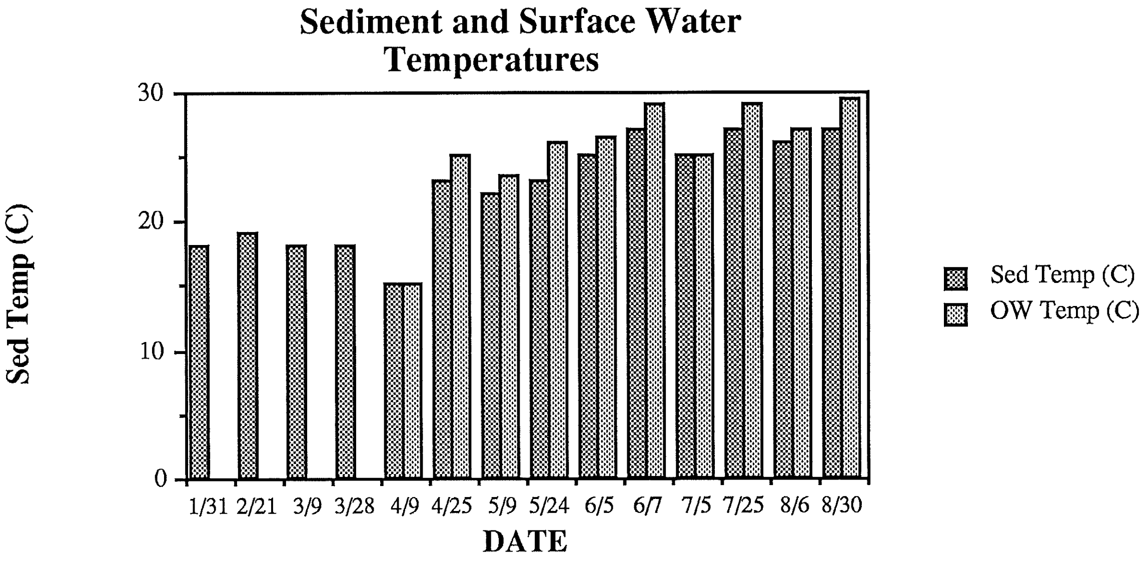

Figure 3. Overlying water and porewater temperatures for Control and Experimental plots (Jan - Aug, 1990). Note: Overlying water measurements were only taken from April through August.

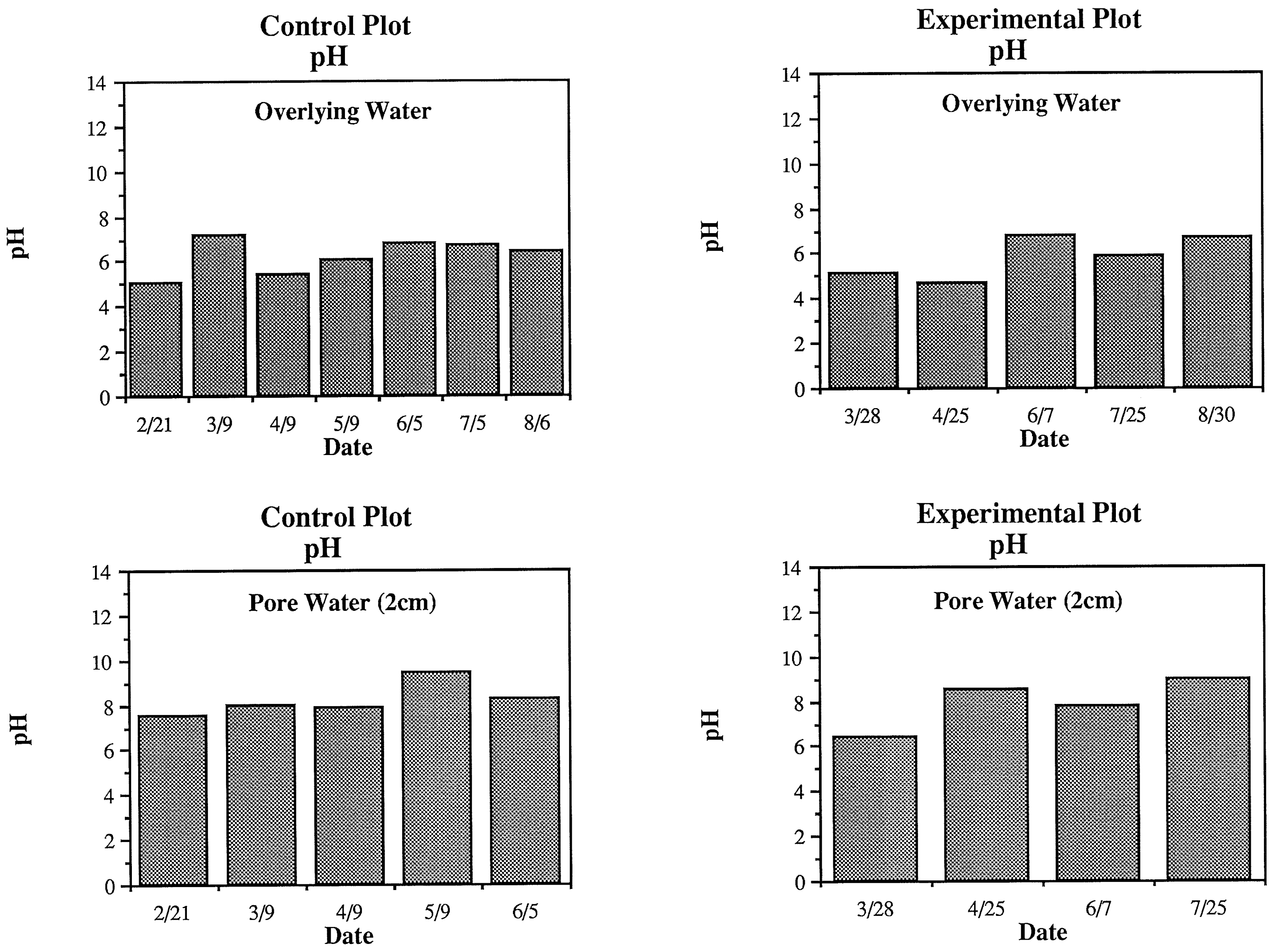

Figure 4. Monthly pH measurements of overlying water and porewater (2cm) in control and experimental plots.

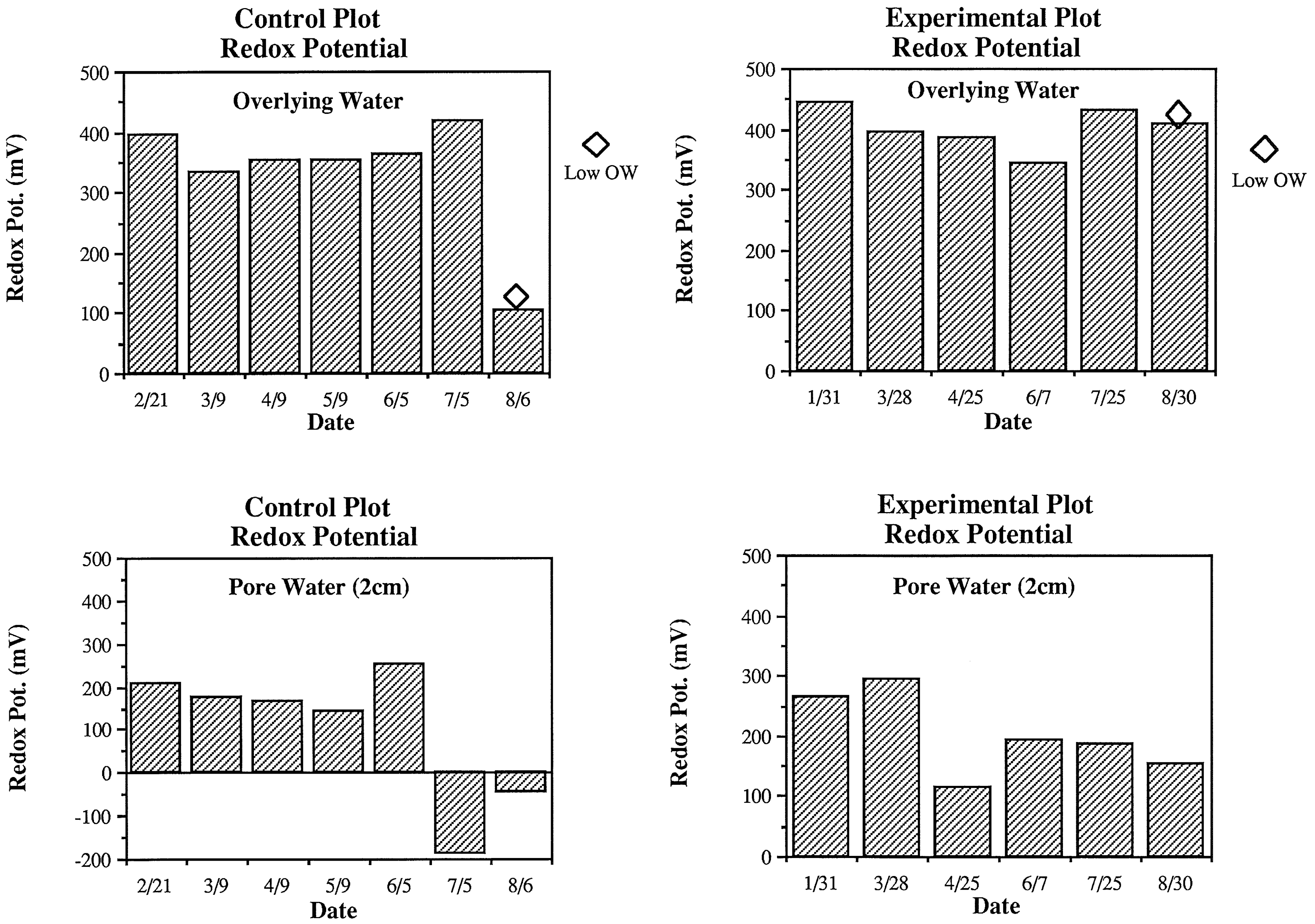

Figure 5. Monthly redox potentials (mV) of overlying water and porewater (2cm) in control and experimental plots.

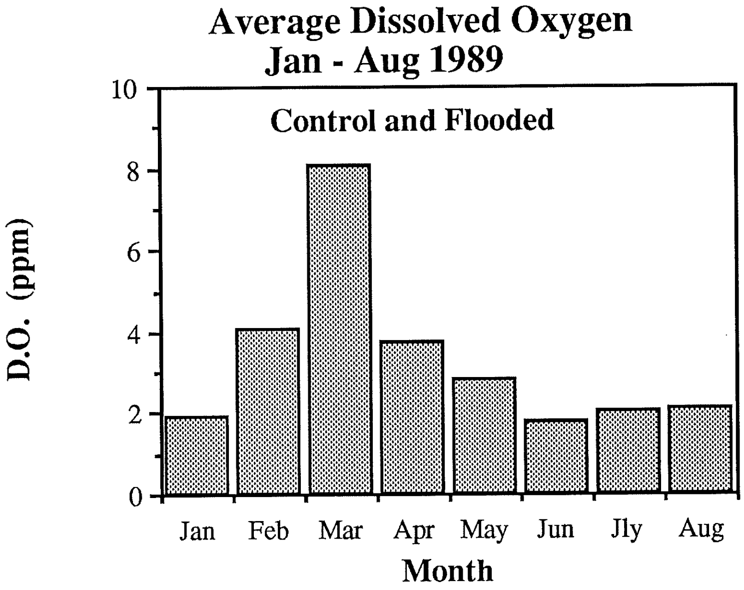

Figure 6. Average dissolved oxygen concentrations taken throughout various locations in both the control and experimental (flooded) plots. (data from Conner and Day, 1989).

remain near 400 mV throughout the summer months. While these observances suggest that low dissolved oxygen concentrations even at 2 ppm are sufficient enough to maintain relatively high redox potentials in the overlying water, a lack of oxygen diffusion in the sediments results in lower redox conditions as observed in the porewaters.

Concentrations of ammonium in overlying water are lower than in the 2 cm porewater measurements (Fig. 7). Again, an exception is the August measurement in the control plot attributed to sampling error. Therefore, in both plots, surface water ammonium is negligible relative to porewater measurements. In both plots the 2 cm porewater measurements tend to peak at above 150 ug-atm/L in the winter months and decline in March and April to less than 100 ug-atm/L. This may be due to seasonal vegetation blooms and increased rates of nitrification. However, in June and July porewater ammonium tends to increase once again followed by variability in the summer.

Ammonium concentrations in the overlying water were also measured at several locations throughout the study area and averaged for each month (Fig. 9). Concentrations increased in February, however there was a subsequent decrease in March and April, again followed by an increase in May. While these concentrations are significantly lower, they do follow the same trend seen in the ammonium concentrations observed in 2 cm porewater.

Nitrite/nitrate concentrations (Fig. 8) tend to be very low in both the overlying water and porewater for each of the plots throughout the duration of the sampling period. Nevertheless, there are some changes in concentrations which correlate with changes seen in ammonium measurements. For example, in the control plot the overlying water concentration increased in May (Fig. 8) when ammonium concentrations also increased (Fig. 9). In the control plot porewater concentrations, there is a significant increase in NO_2+NO_3 in March followed by a sharp decline in April and May, followed by an increase in June. The increases may be attributed to increased nitrifying activity. The same trend in nitrate/nitrite concentrations in overlying water (Fig. 9) is evident where there is an increase through the winter months and a more gradual decline in the spring followed by an increased concentration once again in May. While porewater

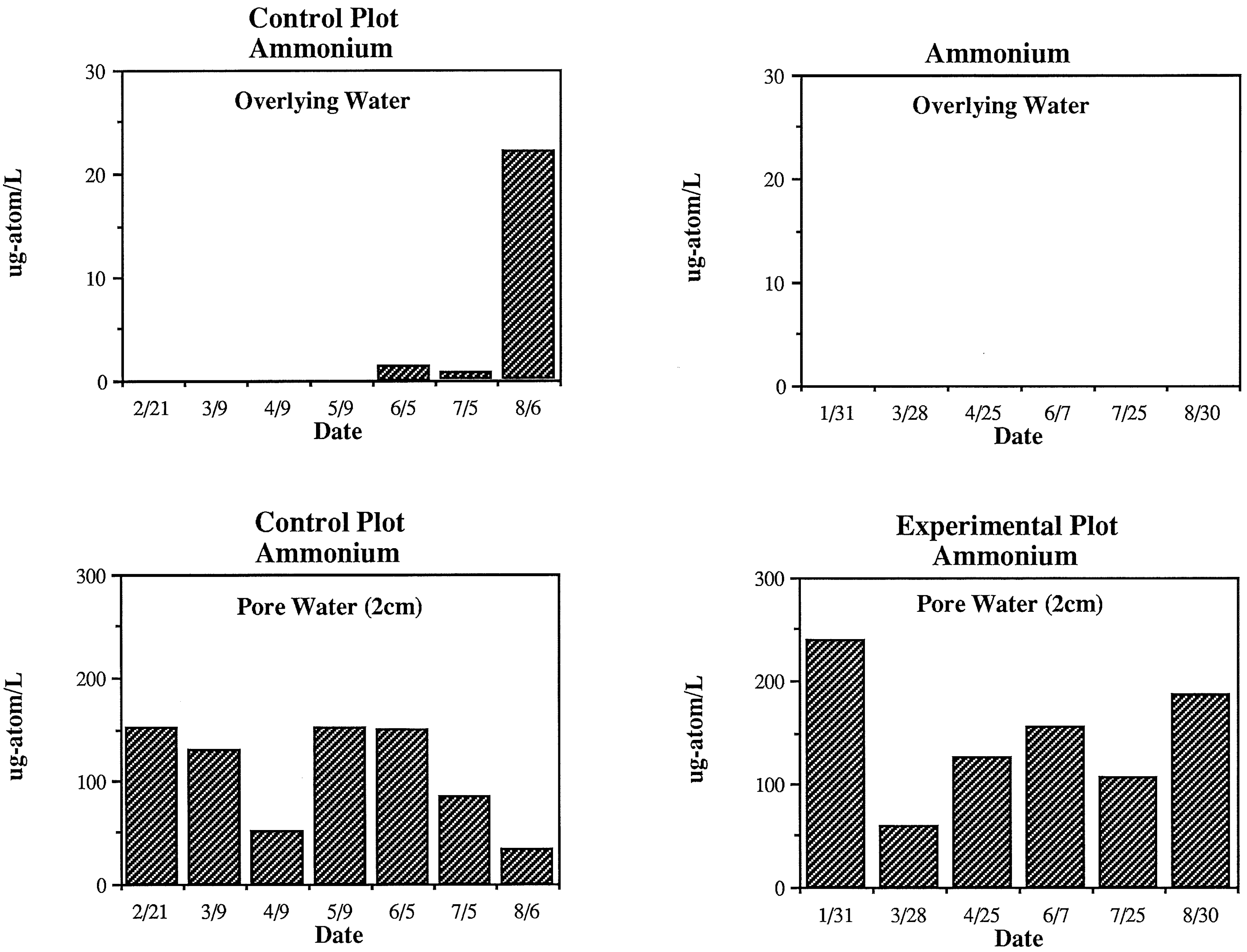

Figure 7. Monthly measurements of ammonium concentrations in the overlying water and porewater (2cm) of the control and experimental plots.

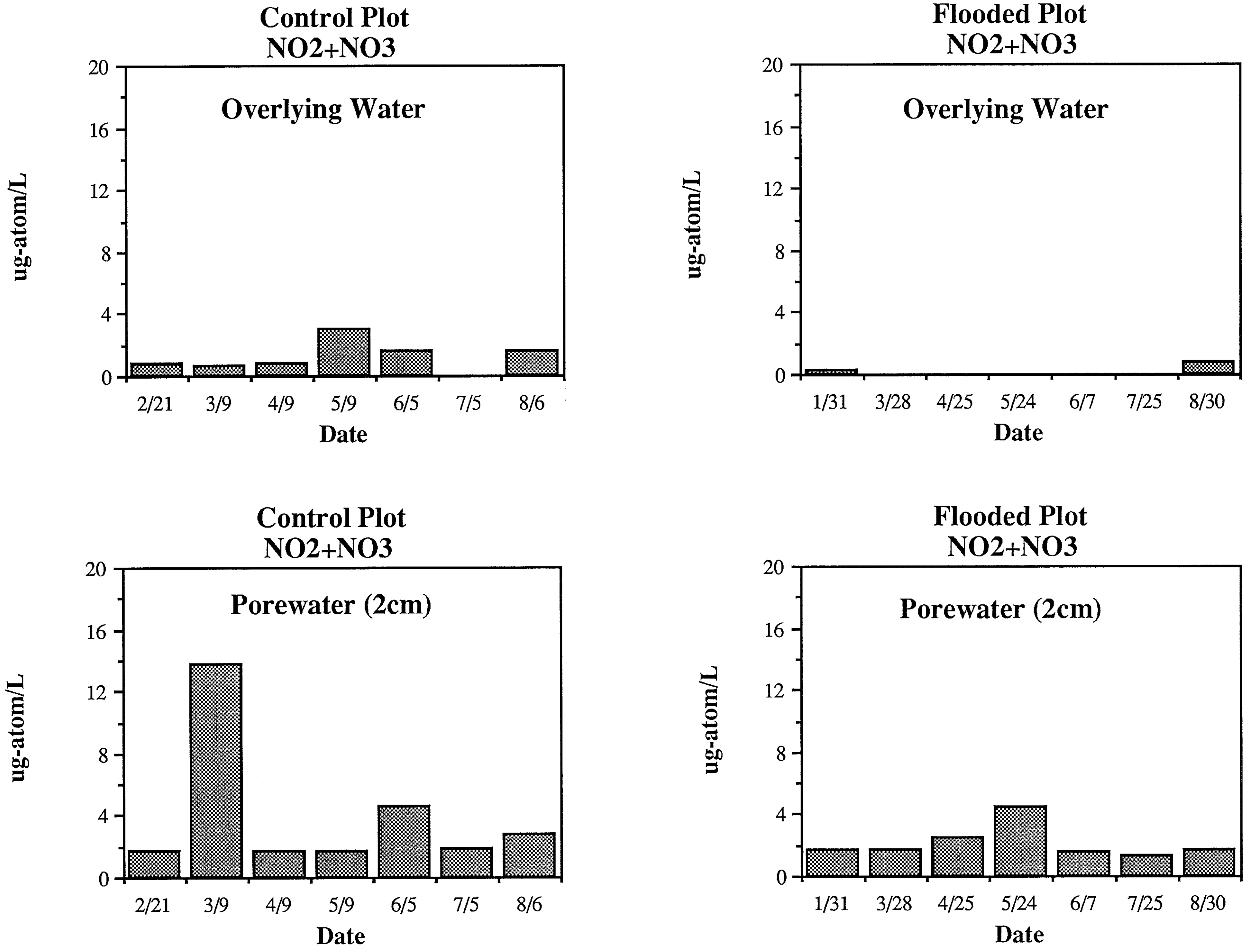

Figure 8. Monthly measurements of nitrite/nitrate concentrations in the overlying water and porewater (2cm) of the control and experimental (flooded) plots.

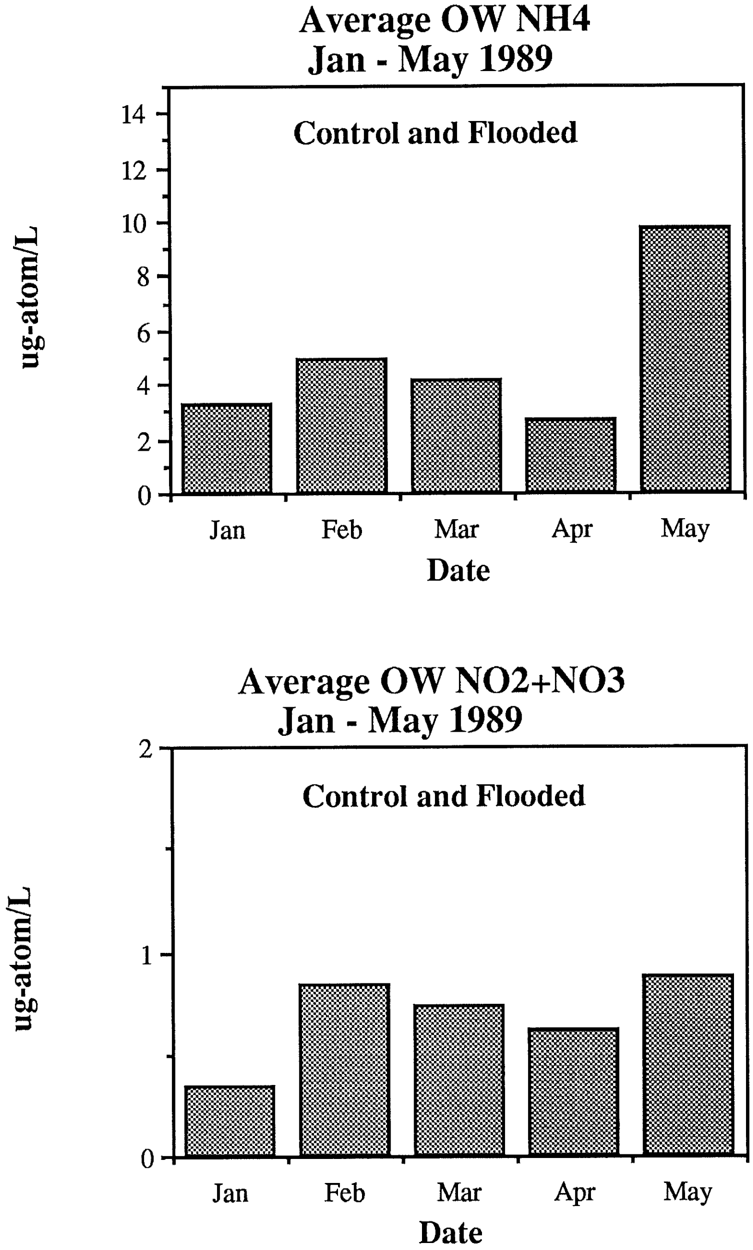

Figure 9. Average overlying water concentrations of ammonium and nitrite/nitrate taken throughout various points in both the control and experimental plots during Jan - May 1989. (data from Conner and and Day 1989).

concentrations from the flooded plot do not increase in the early spring, concentrations of NO_2+NO_3 increase in summer in the control plot overlying and porewater data (Fig. 8 and 9).

Denitrification Potentials:

The denitrification rates observed in Fig 10 represent those generated from the high concentration amendments (3.0 mM) over a duration of 3 hours. Monthly nitrous oxide production rates are found in Appendix A for each plot including ambient, 0.05mM, 1.0mM and 3.0mM amendments. Denitrification rates tend to be relatively high in both plots during the winter and spring months. The highest rates are observed in March in the flooded plot and rates decreased in activity throughout the remainder of the sampling period. In the control plot the highest rate occurred in May and rates were similar throughout June and July with a significant reduction in August. There tends to be a reduction in denitrifying activity in the warmer months in both plots reaching lowest rates in August.

Coupled Nitrification/Denitrification:

Initial concentrations of dissolved oxygen (Fig. 11) in the hypoxic core and the aerobic core are similar on day 1, but there is a significant reduction in the unaerated core. Concentrations remain below 2 mg/L throughout the remainder of the experiment in the hypoxic cores. The initial dissolved oxygen concentrations in the aerated cores, refered to as the aerobic core, ranged from 5 to 7 mg/L. When the cores were sealed, a significant reduction in dissolved oxygen was observed during 6 h incubation to concentrations less than 4 mg/L. Nitrate concentrations in the hypoxic core are negligible throughout the experiment. In the aerobic core, nitrate concentrations are less than 1 umol/L during the first two days and increase to about 2.5 umol/L on days 4 and 6 (Fig. 11). On day 9 there is a significant increase in initial nitrate concentration greater than 10 umol/L. During these latter days when nitrate was observed at initial sampling, there was a significant reduction in nitrate concentration during the 6 hour period when the cores were sealed and aeration ceased. The drop in initial dissolved oxygen concentrations

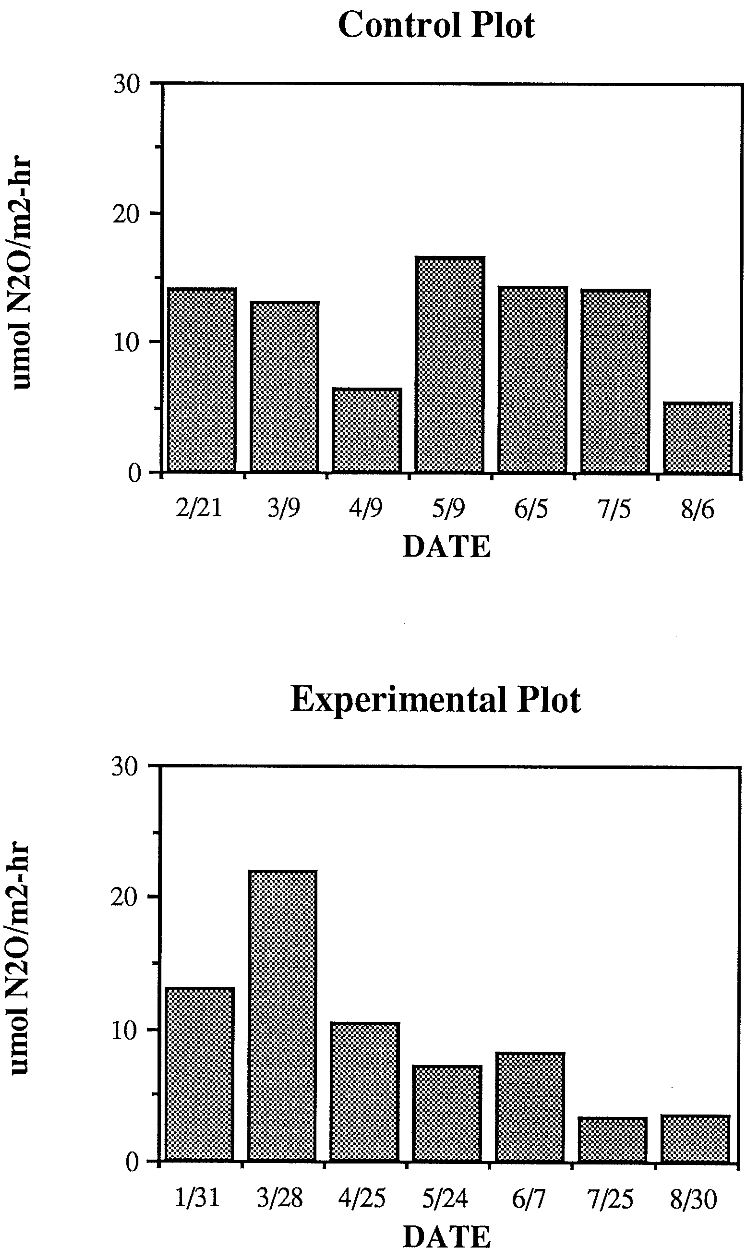

Figure 10. Monthly potential rates of denitrification as determined by the acetylene block technique for the control and experimental (flooded) plots.

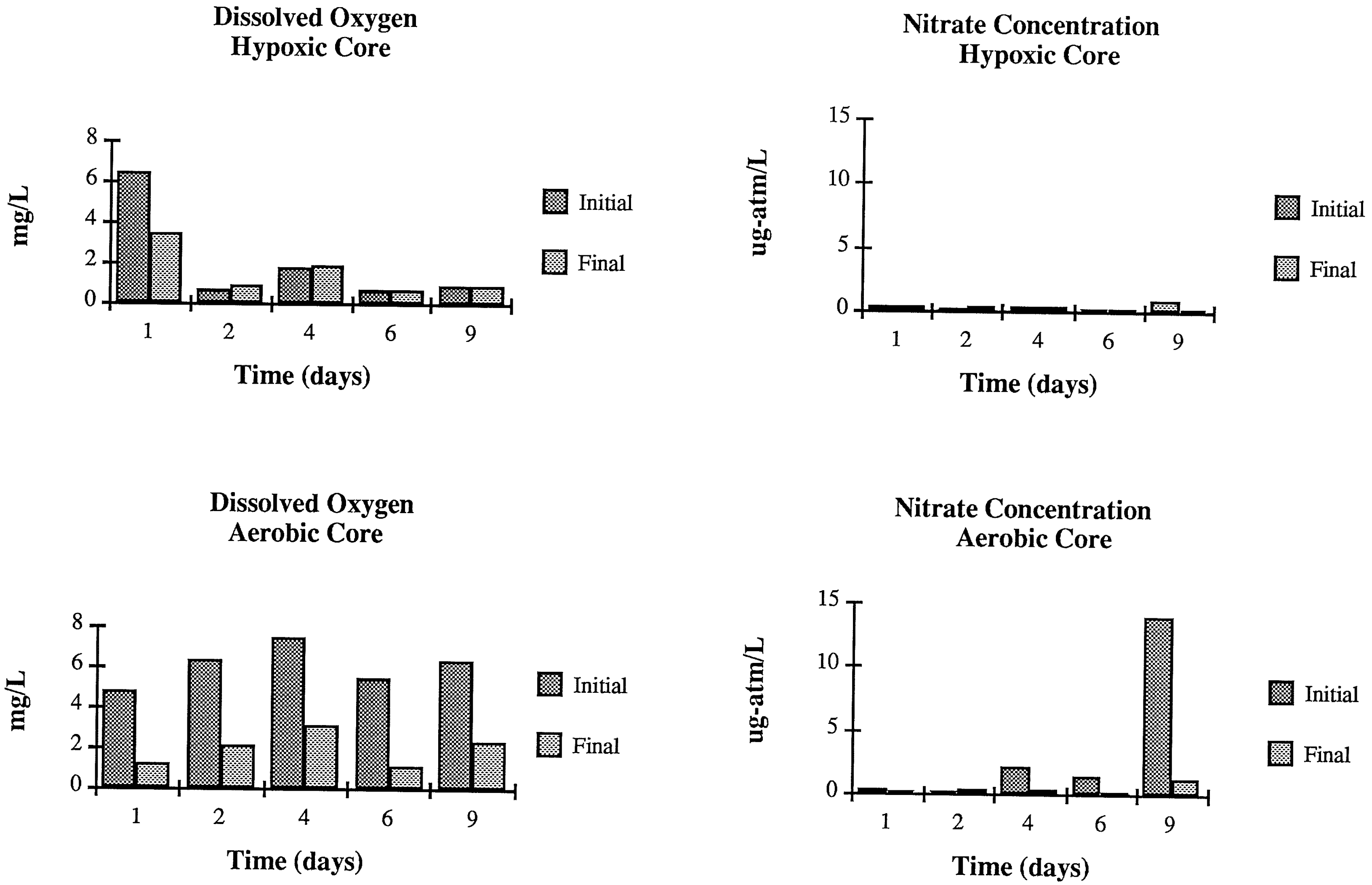

Figure 11. Initial and final concentrations of dissolved oxygen and nitrate in aerated (aerobic) and unaerated (hypoxic) cores.

following the 6 hour incubation time and the sharp reduction in nitrate suggests an increase in denitrifying activity with the decline of oxygen availability.

Conclusions

Sediment Characteristics

Denitrification proceeds slowly at a temperature of 2 ^{o}C and increases to a maximum at temperatures ranging up to 60 to 65 ^{o}C (Nichols 1983). The optimal temperature for nitrification ranges between 30 and 36 ^{o}C (Focht and Chang 1975). The sediment temperatures observed throughout the sampling period were fairly moderate even in the winter months ranging from 17 to 27 ^{o}C. In the low-temperature range, soil denitrification decreases markedly, but is nevertheless measurable even at 0 to 5 ^{o}C, where relatively larger mole fractions of N_2O and NO tend to occur (Knowles 1982). While Louisiana is subtropical region and subject to short periods of extreme cold, lower sediment temperatures can be expected in the study site; however, denitrification is expected to continue at a moderate rate within the wetland. Increased rates of denitrification were expected with increased temperature during the summer, yet there was a decline in activity in both plots. These observations suggest that temperature is not an important factor influencing temporal rates of denitrification in the wetland.

Similarly, no significant changes in pH were recorded during the sampling period suggesting that the system is well buffered with measurements rarely deviating from neutral. Optimal rates of nitrification and denitrification occur at pH ranges from 7.0 to 8.0, and denitrification occurs much more slowly under acidic conditions than at neutral or moderately alkaline pH (Nichols 1983). At low pH values, the reduction of N_2O is progressively inhibited such that the overall rate of denitrification decreases but the mole fraction of N_2O produced is increased, and at pH 4.0 N_2O may be the major product (Knowles 1982). Even under these conditions moderate rates of denitrification can be expected in the wetland. Nevertheless, data indicate that pH has little effect on the temporal rates of denitrification.

Redox potential and dissolved oxygen appear to be most significant to the nitrogen dynamics of the system. Redox potential is a measure of the degree of reduction in soils which can be attributed to the oxygen availability to the sediments. Redox potentials above 300 mV are favorable to nitrification since it is an aerobic process, whereas denitrification, an anaerobic process, occurs at redox potentials nearer to 200 mV and below. In general, redox potentials remain high in the surface water even though dissolved oxygen may drop to 1 or 2 mg/L. However, in the porewater redox potentials are low as expected due to the rapid uptake of oxygen in organic decomposition. In both plots the redox potentials are high (200 to 300 mV) in the cooler months where nitrification may be prevalent, but subsequently drop in the warmer months. The high redox potentials early on may be attributed to the combined high dissolved oxygen seen in the spring and slow organic decomposition due to the cooler temperatures, whereas with increasingly warm temperatures follows reduced dissolved oxygen by quicker diffusion to the atmosphere and increased decomposition, which subsequently lower redox potentials. In comparing measurements in the two plots, there is a significant drop in redox potentials in the months of July and August (below 0 mV), whereas in the flooded plot this drop is not observed and readings remain above 100 mV. This difference may be attributed to a more dominant rhizosphere of an aquatic macrophyte, *Saururus cernuus* (lizard's tail), present in the flooded plot. Large numbers of these plants were observed in the experimental (flooded) plot, especially in the spring, whereas they appeared to be less dominant in the control plot. De la Cruz et.al. (1989) found a significant difference between vegetated and unvegetated plots where the vegetated plots always contained less reduced soils. They concluded in there study that living plants tend to raise redox potentials significantly by possibly oxidizing the rhizosphere. Additionally, Reddy et.al. (1989) suggest that oxygen transport through the air spaces of the stem and roots of aquatic macrophytes into the root zone may support nitrification of NH_4^+, with the NO_3^- formed diffusing into the adjacent anaerobic zone where it subsequently undergoes denitrification. Therefore, the presence of aquatic macrophytes may enhance the coupling of nitrification to denitrification by providing microsites of oxygen within reduced sediments, especially in the warmer parts of the year.

Overlying water ammonium and nitrite/nitrate concentrations are very low in both plots never exceeding 10 ug-atm/L; however, there is a significant difference in concentrations when considering porewater data in figures 4, 5, and 6. Porewater nitrite/nitrate concentrations are extremely low, averaging less than 5 ug-atm/L, relative to ammonium where concentrations average closer to 100 ug-atm/L. These observations indicate that nitrification is the more limited process since there is an apparent buildup of ammonium. Furthermore, when nitrification does occur, the resulting nitrate rapidly undergoes denitrification, thus maintaining extremely low concentrations. In monthly observations, there tends to be a drop in ammonium concentrations in the spring in both plots where dissolved oxygen and redox potentials are at a seasonal high, again suggesting a triggering of increased nitrifying activity. Nitrite/nitrate porewater concentrations remain low with the exception of the March reading in the control plot where there is a slight increase correlated with peak nitrifying activity.

Nitrification/Denitrification Potentials

While the rates of denitrification in the control plot remain fairly consistent throughout the sampling period, there tends to be a gradual decline in potentials in both plots, particularly the flooded plot, towards the warmer months. This decline appears to be due to the lack of nitrifying activity and the low availability of nitrate in the system as previously discussed in observing sediment nitrogen characteristics. Table 1 consists of the annual rates of denitrification calculated on a monthly basis for an average annual rate of 3.69 g N_2O/m^2-yr in the flooded plot and 4.59 g N_2O/m^2-yr in the control plot. Although these rates appear relatively low, it is difficult to make comparisons to previous studies due to the differences in methodology where rates range from 2 to 100 g N/m^2-yr. The most important factors tend to be differences in the time of incubations, amendment concentrations, methods and the type of wetland sediments analyzed. Presumably, when the available nitrogen is in the form of ammonium and nitrification is not occuring at a rate comparable to denitrification, there tends to be a shortage of nitrate in the sediments. With a lack of nitrate availability in the sediments there may be a subsequent population reduction in the denitrifying microbial community. This in turn reduces the denitrifying potential of the sediments.

	Plot		Plot
Date	Flooded	Date	Control
Jan	4.97	Feb	5.37
Mar	8.46	Mar	5.02
Apr	3.98	Apr	2.48
May	2.74	May	6.37
Jun	3.16	Jun	5.44
Jly	1.24	Jly	5.35
Aug	1.31	Aug	2.09
Average	3.69		4.59

Table 1. Potential rates of denitrification from cores amended with 3mM KNO_3 equivalent to the projected loading rate of total nitrogen of 20 mg/L or 44 g N/m^2-yr. All values in g N_2O/m^2-yr. (Acetylene Block)

Since the acetylene block method utilized in this study involved intact short-term sediment cores incubated for a period of only 3 hours, the rates observed represented the present condition of the sediments relative to the facultative anaerobic microbial community at the time of sampling. Therefore, the rates achieved in Table 1 may be a gross underestimate of the denitrifying potential of the sediments, particularly following effluent application where nitrogen concentrations will be significantly increased throughout the area.

The coupled nitrification/denitrification analysis utilizing $^{15}NH_4^+$ suggest that this may in fact be the case. While these were long-term incubations carried out for several days, nitrifying activity was not evident until the 4th day where it was moderate and significant rates were not observed until the 9th day (Fig. 8). These data suggest that there is a lag period from the time of initial application to the time of significant nitrifying activity. Lindau et al. (1988) observed this same lag in a study performed in a similar Louisiana swamp forest where significant nitrifying activity was not recorded until 15 days following application. They also ran cores amended with KNO_3 and did not observe significant denitrifying activity until two days after application. Nevertheless, the applied nitrate underwent denitrification much more readily than the nitrification of ammonium.

These observations suggest that these wetland sediments are nitrogen deficient bearing the capacity to handle high concentrations of nitrogen given the proper environmental conditions. Although rates tend to be low initially, the efficient treatment of nitrogen-rich municipal wastewater will largely depend on developing a sustainable microbial community, which tends to develop naturally with enrichment. The availability of oxygen is a key factor in determining the overall efficiency of the treatment process. While temperature and pH cannot be controlled to a large extent, dissolved oxygen may be manipulated to some degree by experimenting with different methods of wastewater application. By achieving an adequate application regime managers may enhance rates of nitrification and optimize the coupling of nitrification to denitrification. Finally, while much attention has been placed in aquatic vegetation involved in the uptake of nitrogen in wastewater treatment, various indices in this study indicate that some aquatic vegetation which develop significant root/rhizoid mats may contribute significantly to the efficiency of gaseous nitrogen removal.

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Appendix

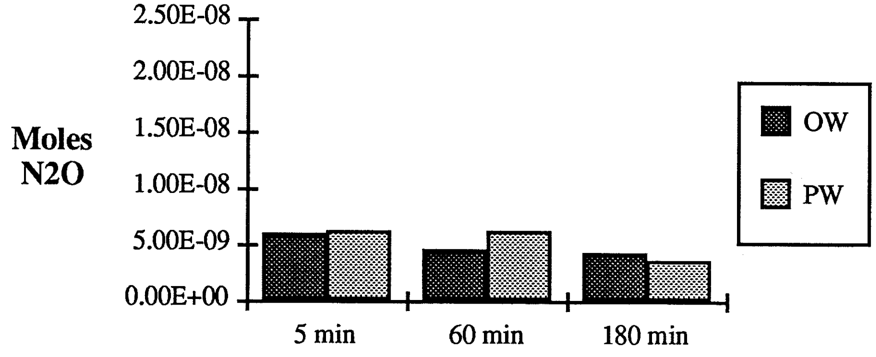
Monthly data sets including:

1. Ambient rates of N2O generation as well as in 0.05mM, 1.0mM and 3.0mM enrichments (Acetylene Block Technique)

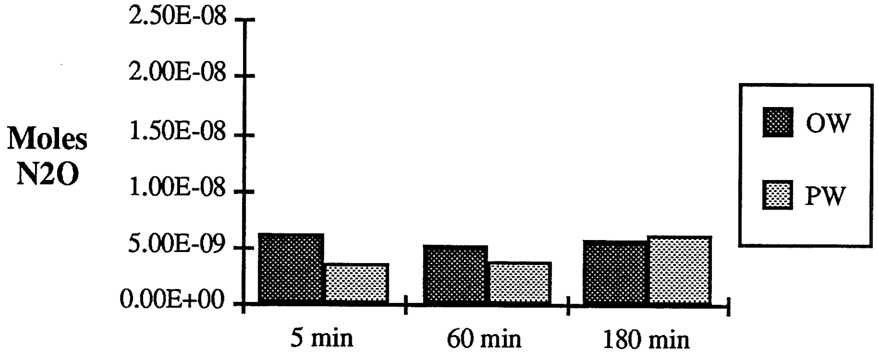
2. Porewater measurements including Redox potentials and pH measurements to a depth of 10 cm and Ammonium and Nitrite/Nitrate concentrations up to a depth of 8 cm.

January 1990
Flooded Plot

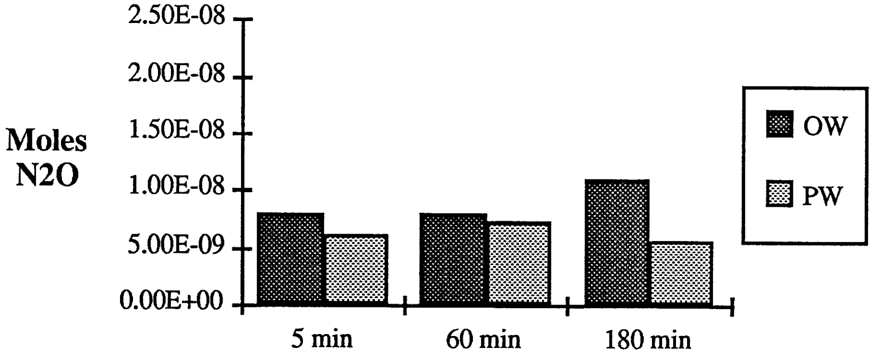
Ambient



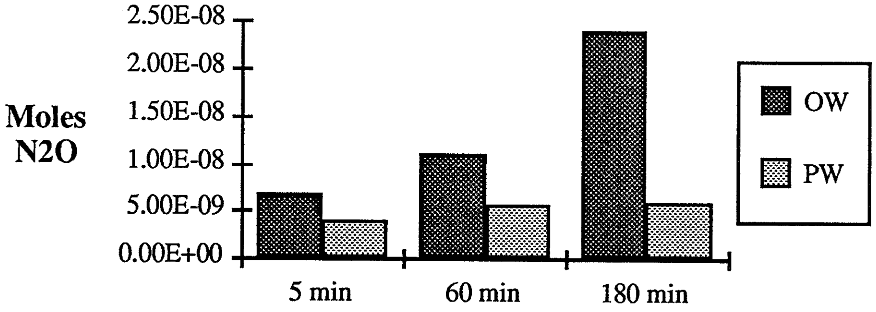
0.05 mM



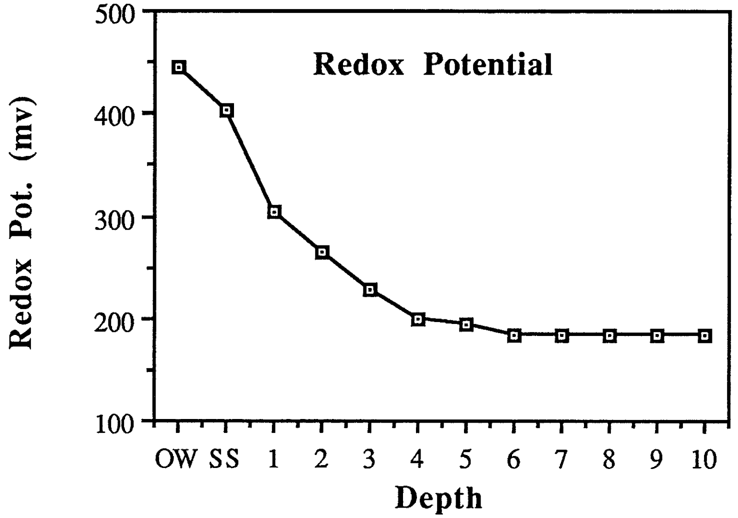
1.0 mM



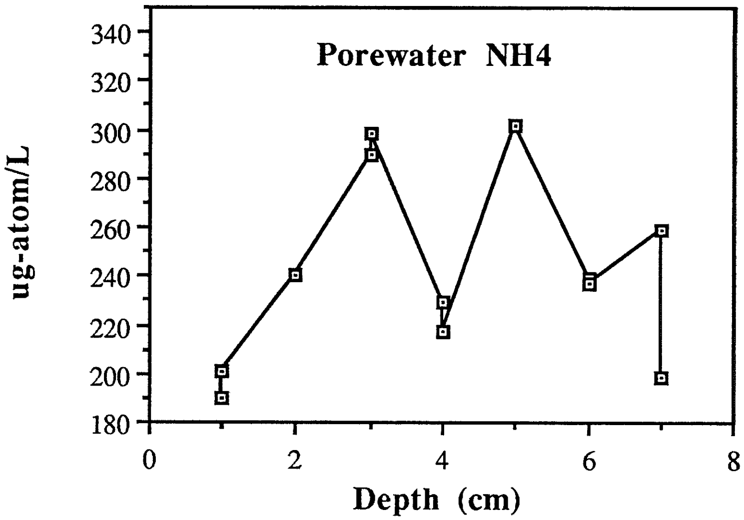
3.0 mM



January 1990
Flooded Plot



January 1990
Flooded Plot



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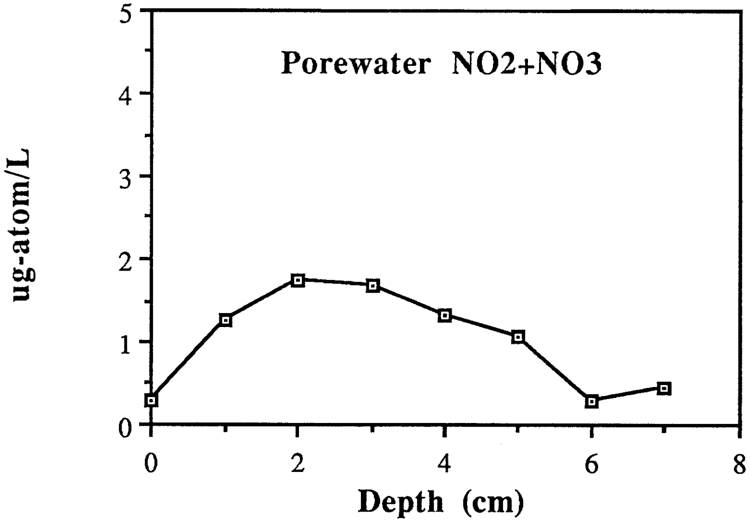
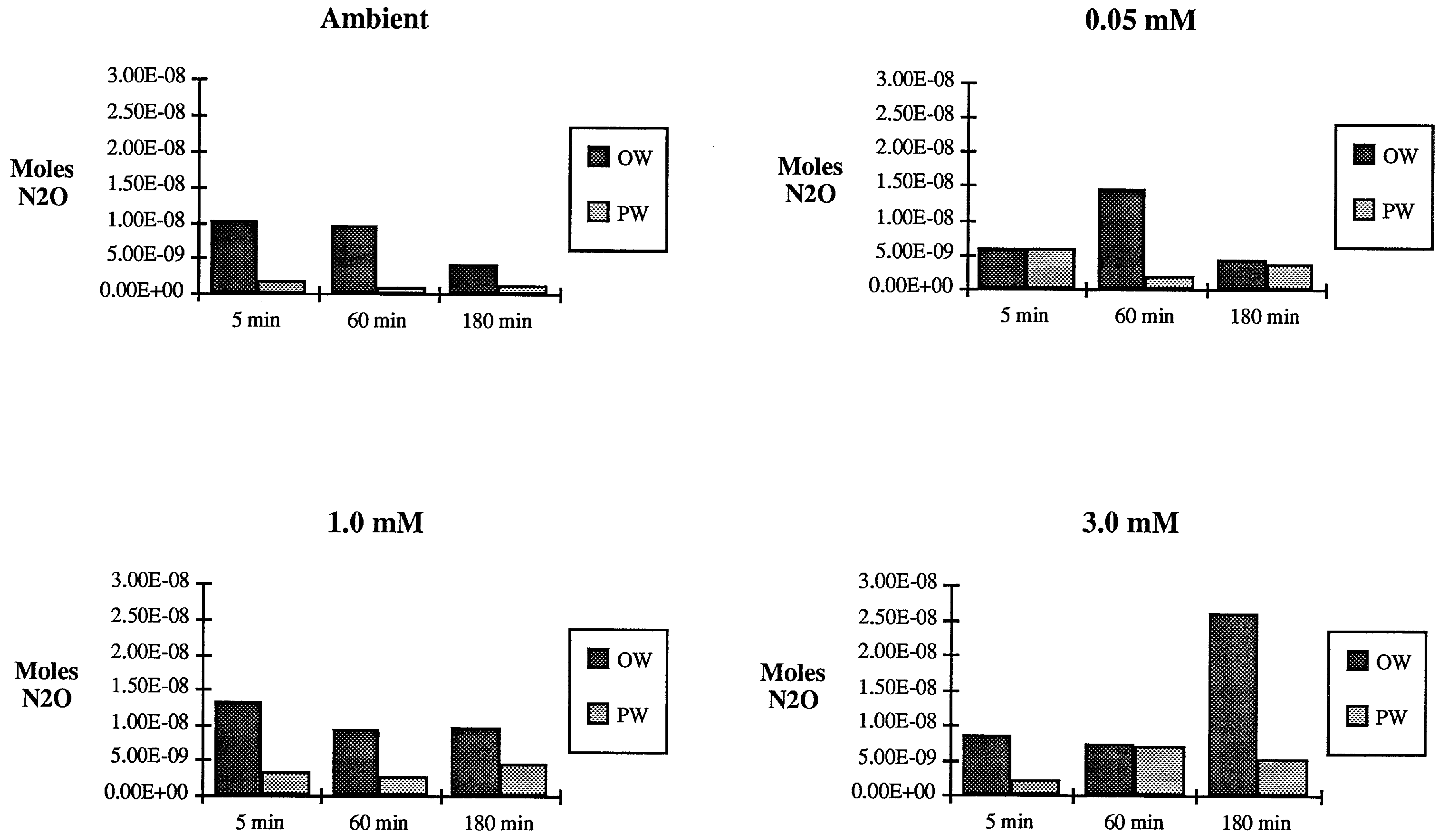
Flooded Plot



February 1990
Control Plot

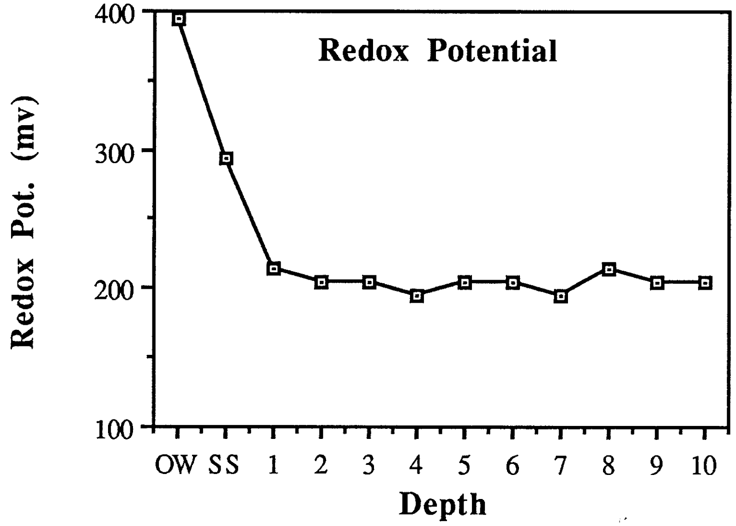


Ambient

0.05 mM

1.0 mM

3.0 mM



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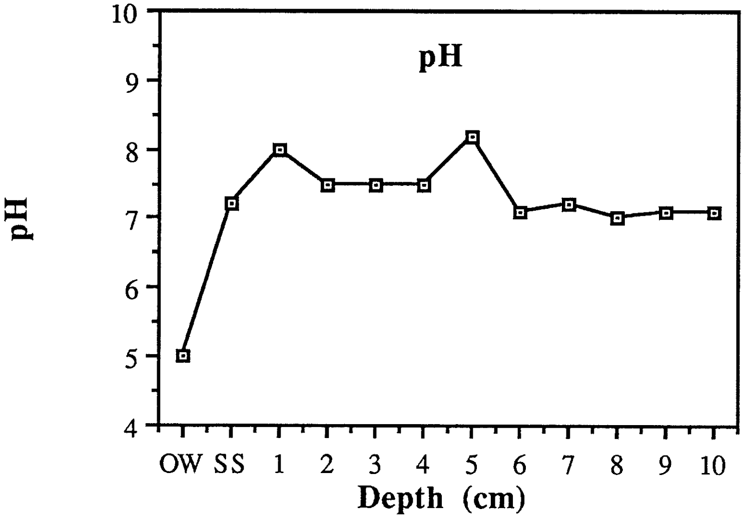

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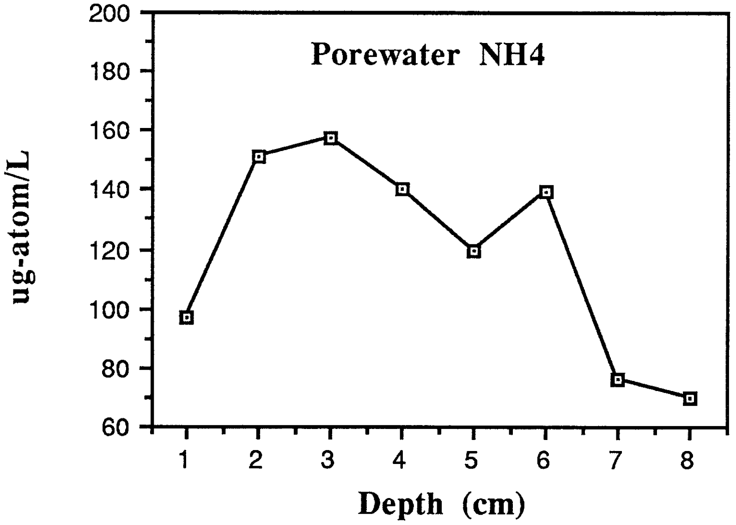
Control Plot



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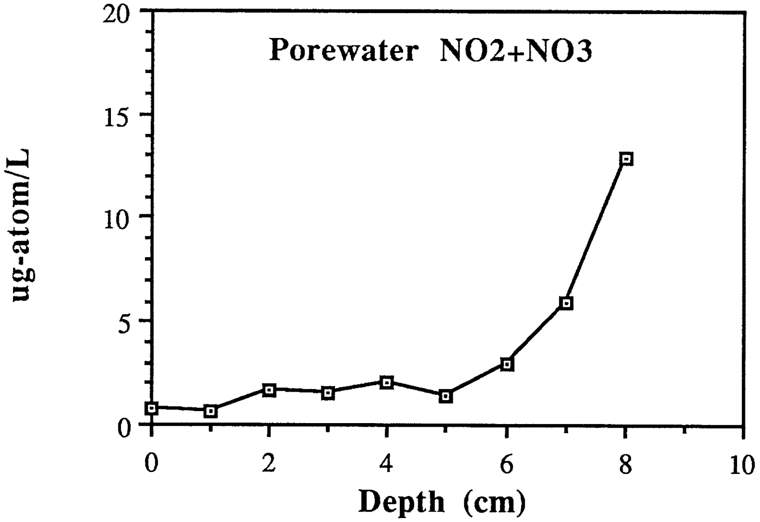
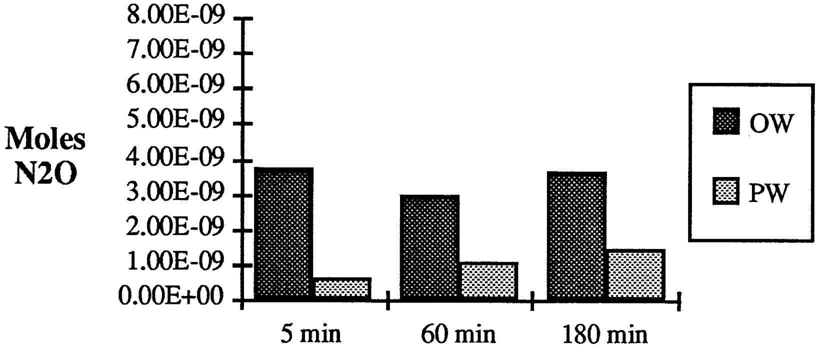


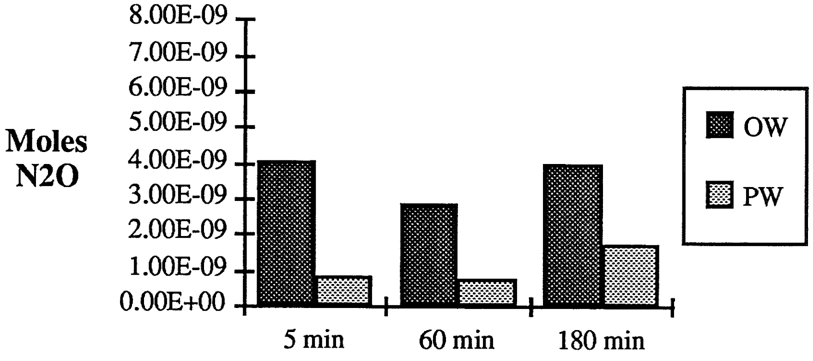
February 1990
Control Plot

March 1990
Control Plot

Ambient



0.05 mM



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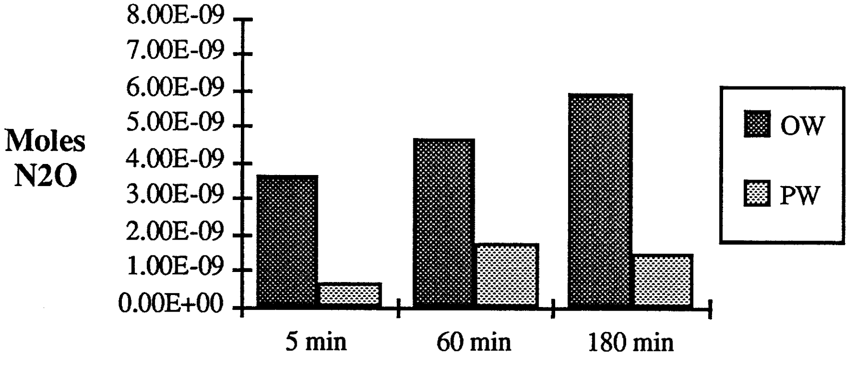
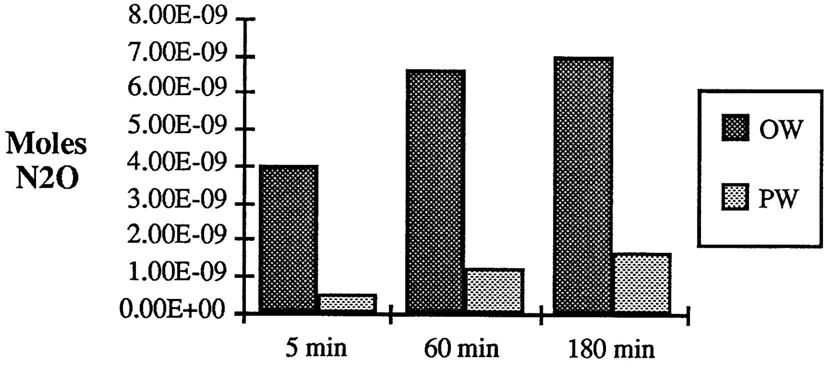


3.0 mM



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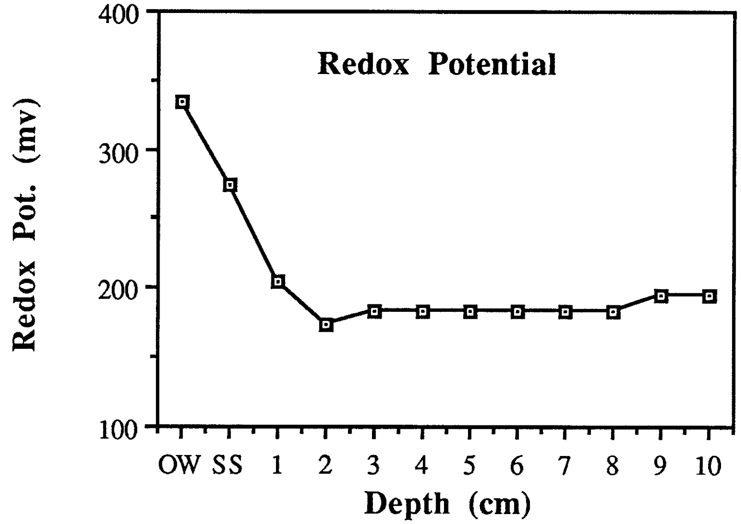
Control Plot**



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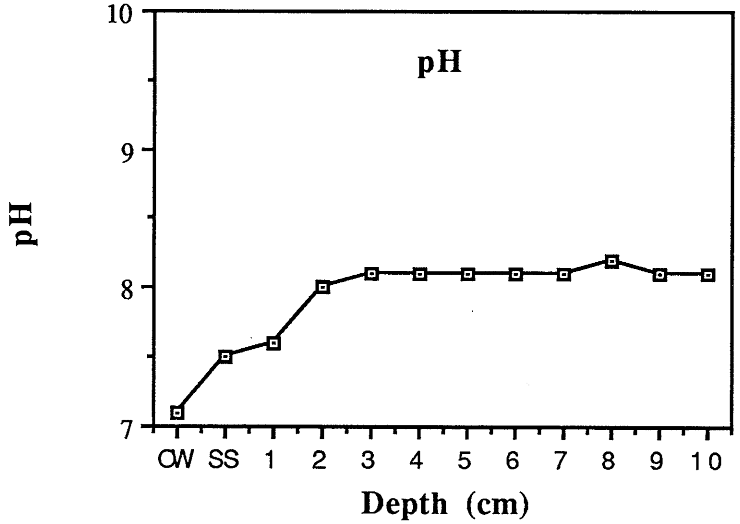
Control Plot**



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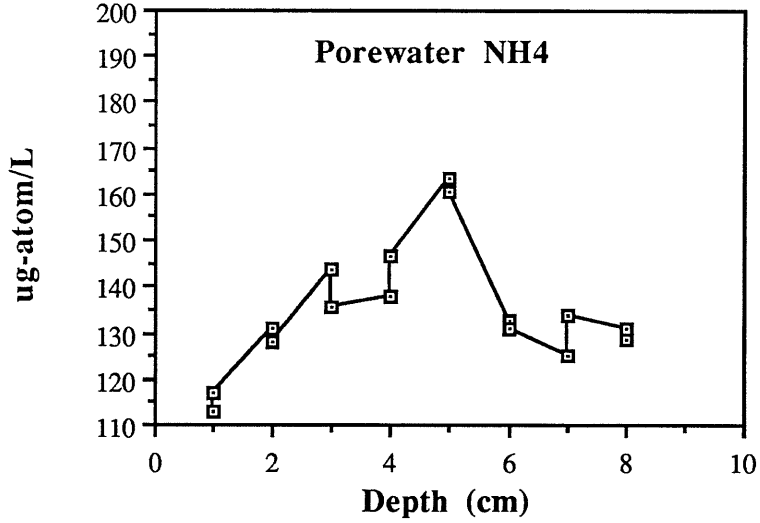

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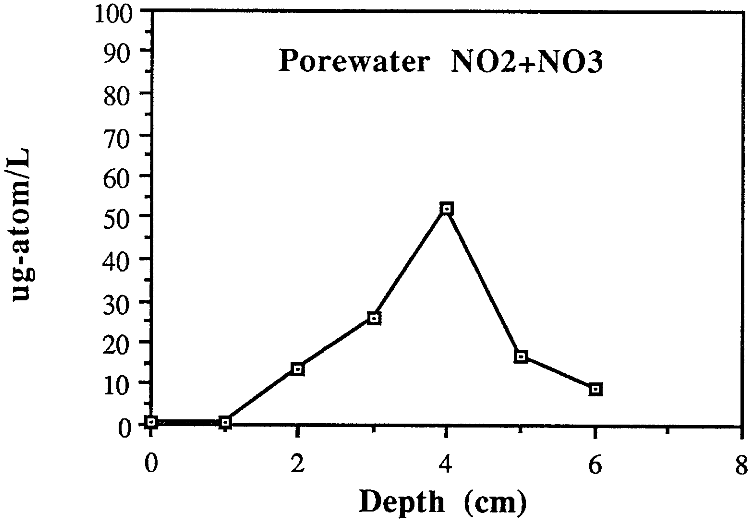
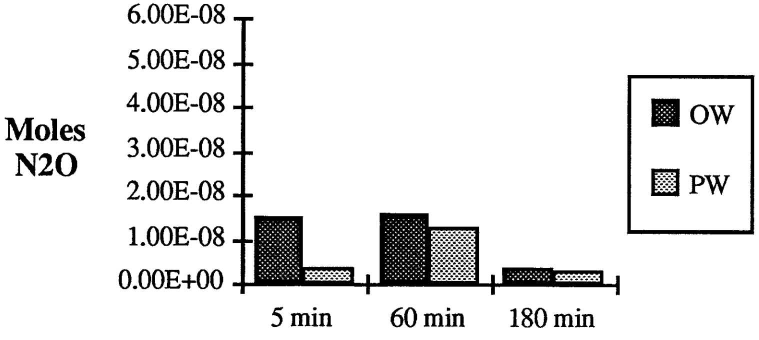
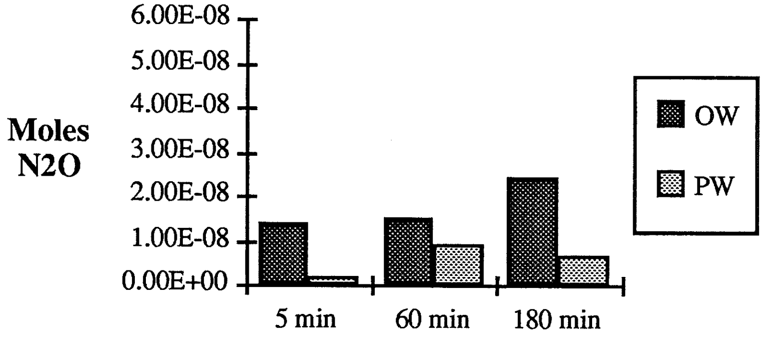
Control Plot**



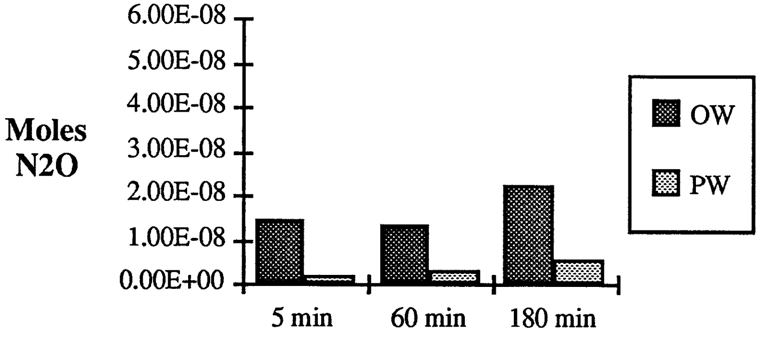
March 1990
Flooded Plot



Ambient



0.05 mM



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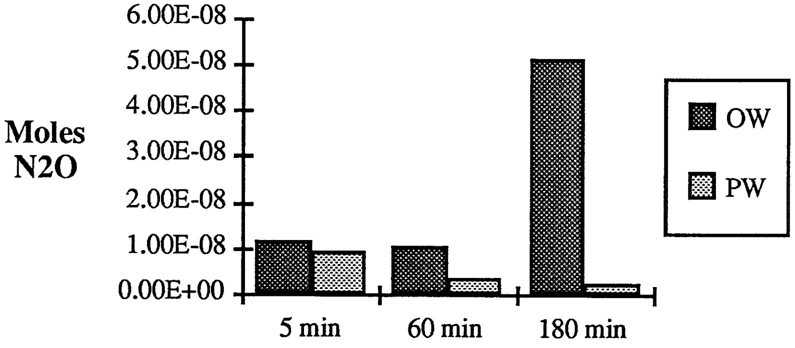

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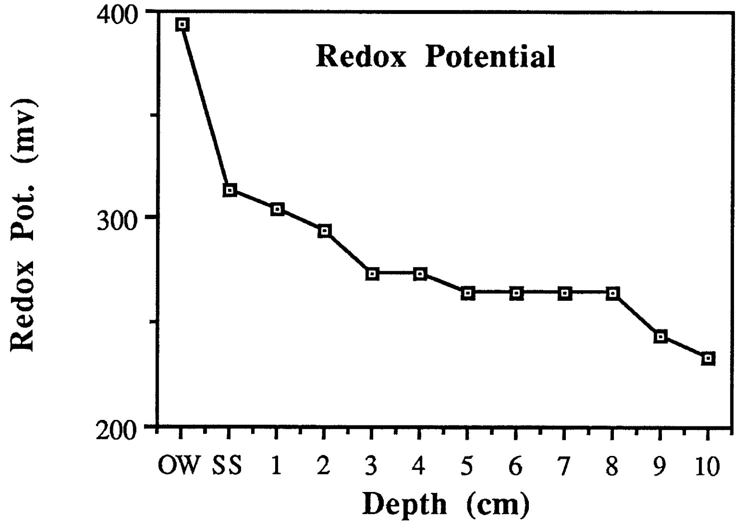
March 1990
Flooded Plot



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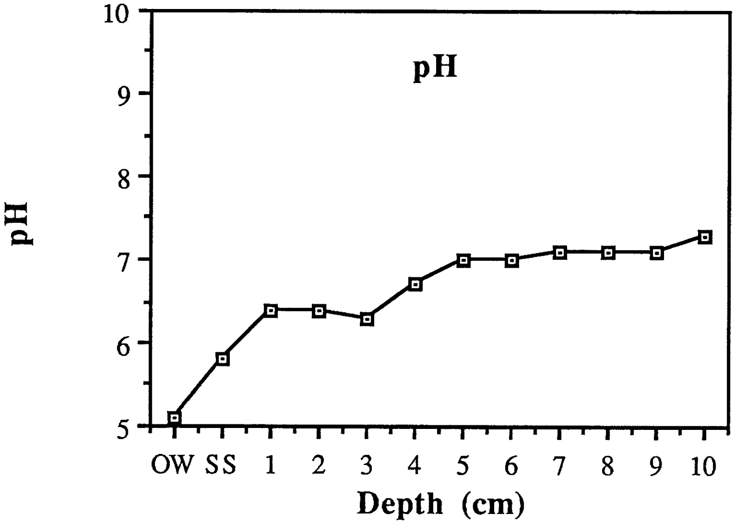

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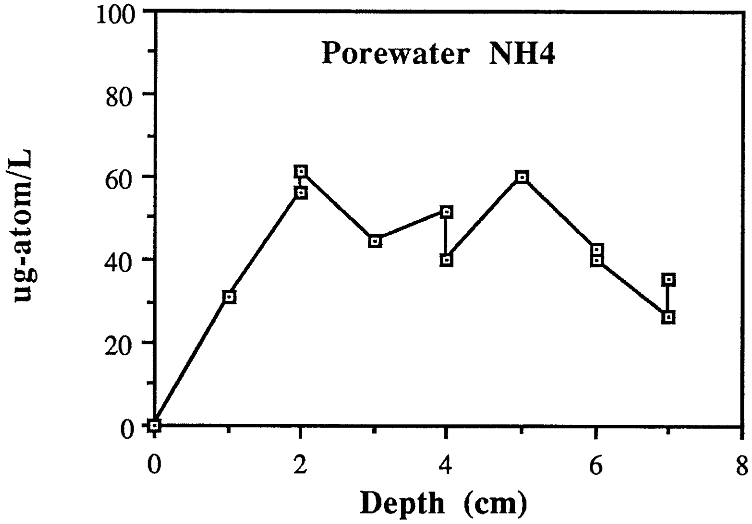

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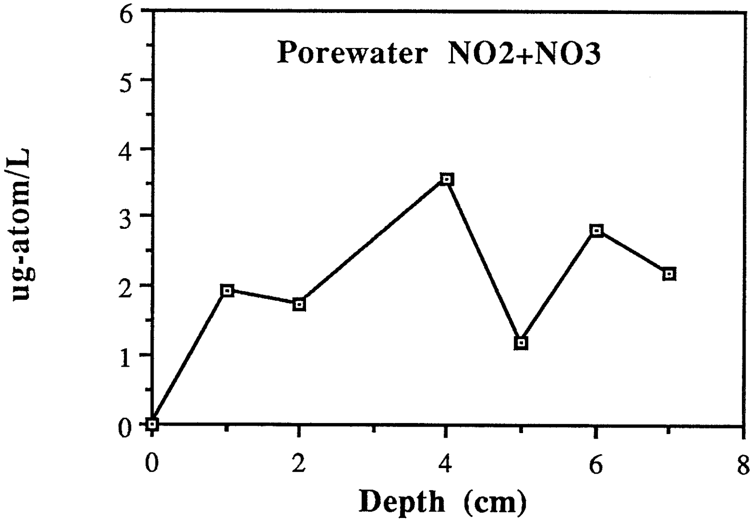
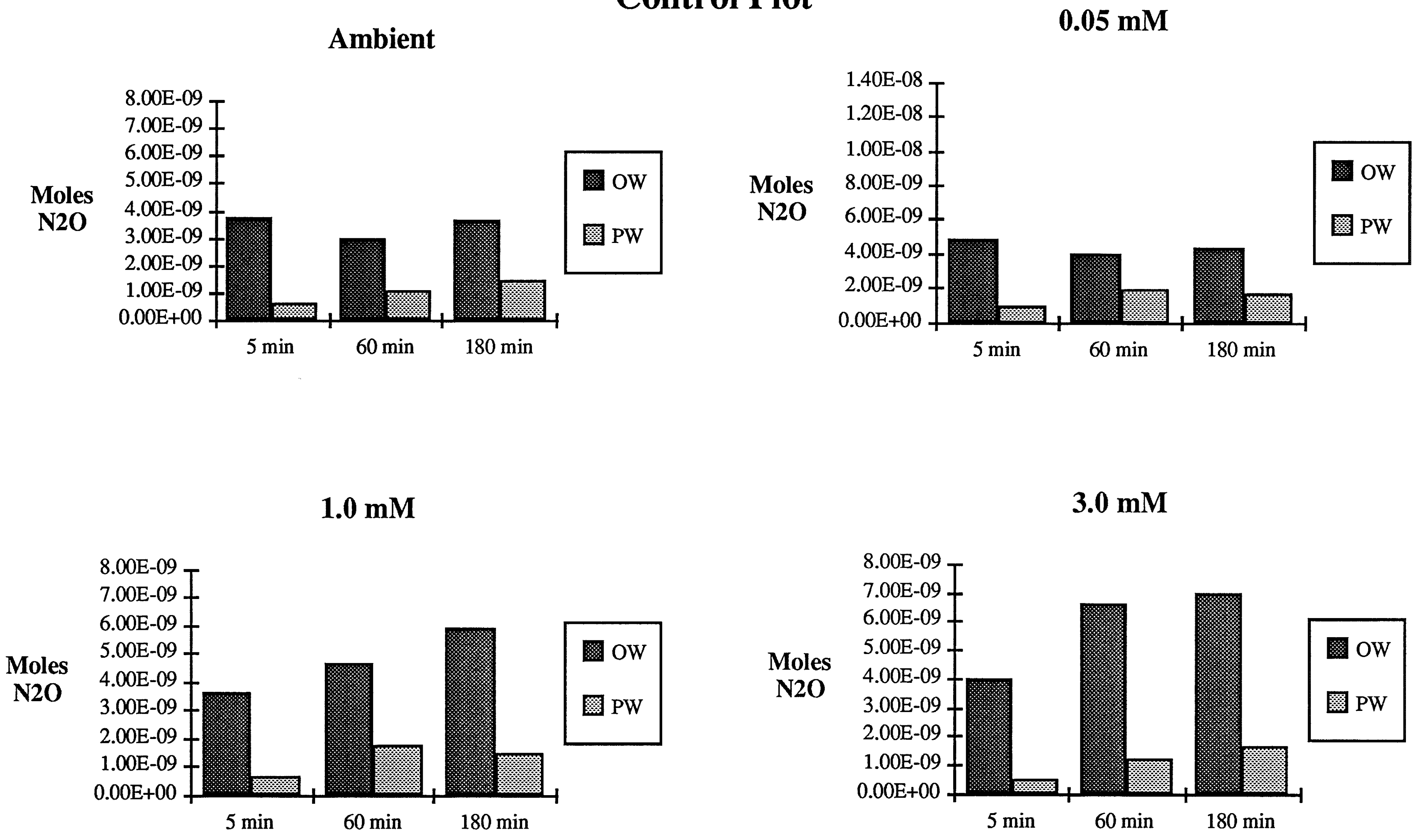


April 1990
Control Plot



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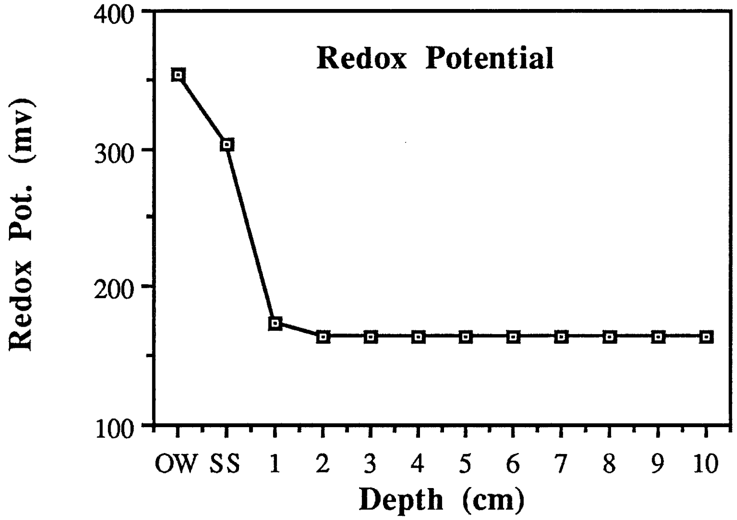
Control Plot



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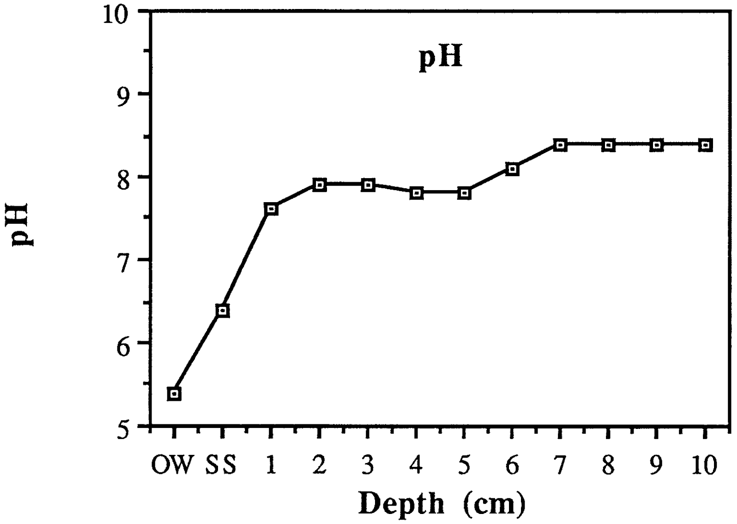
Control Plot



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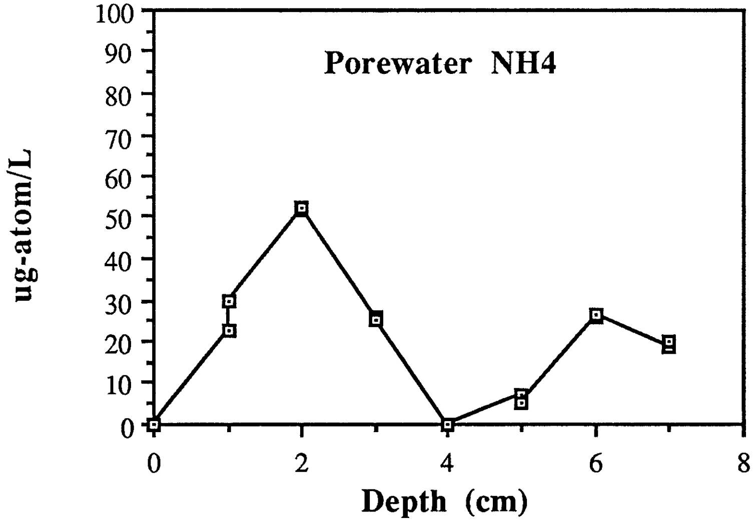
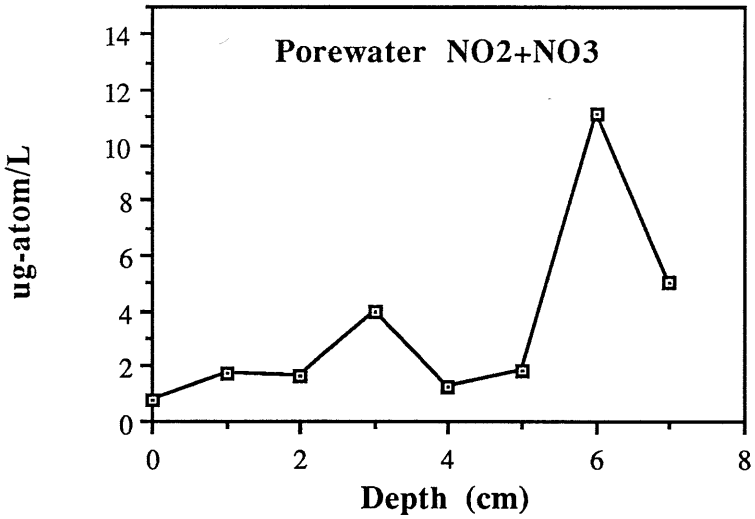
Control Plot

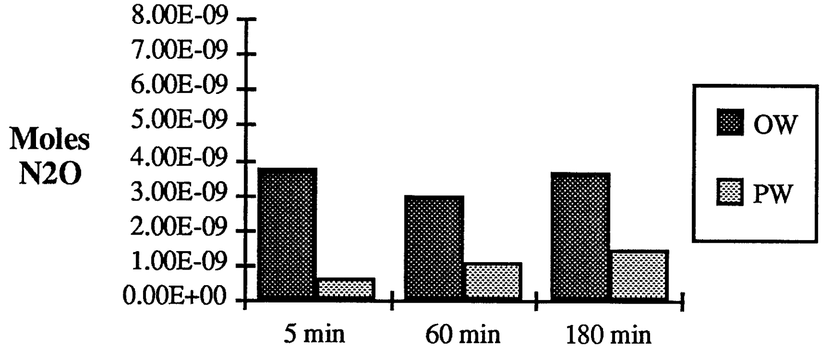


April 1990
Control Plot

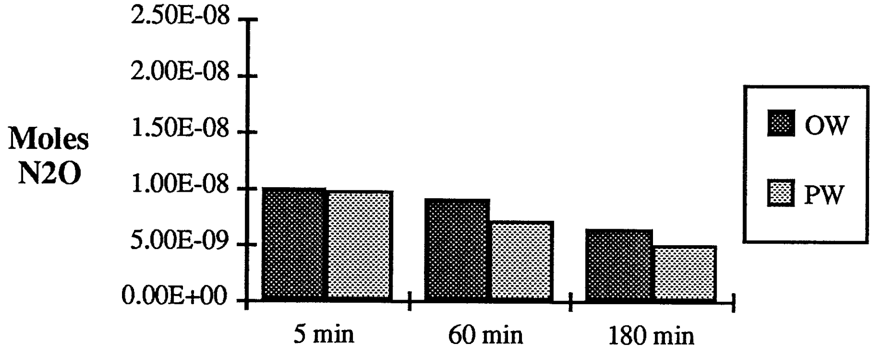


April 1990
Flooded Plot

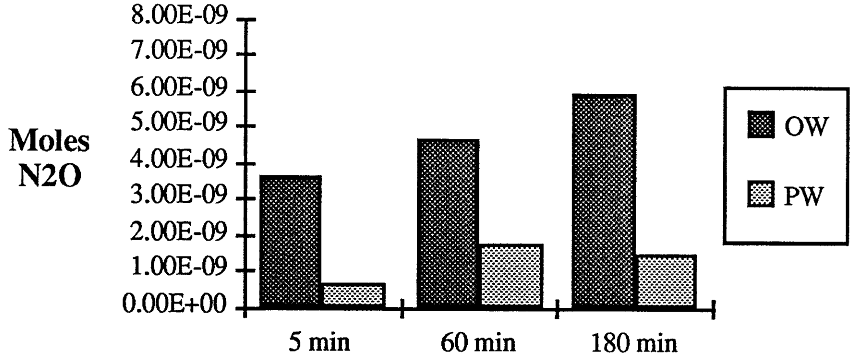
Ambient



0.05 mM



1.0 mM



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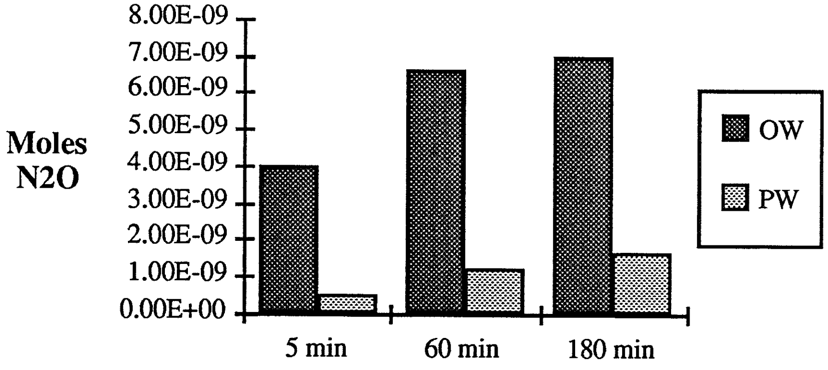
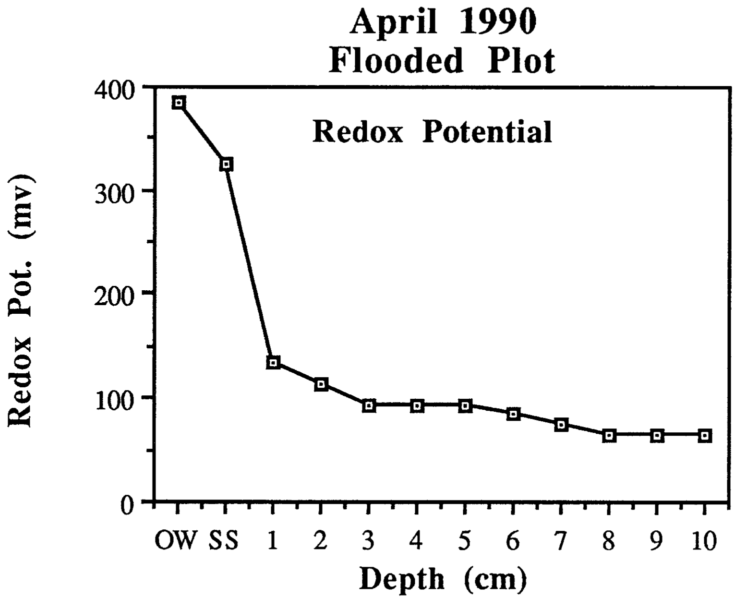
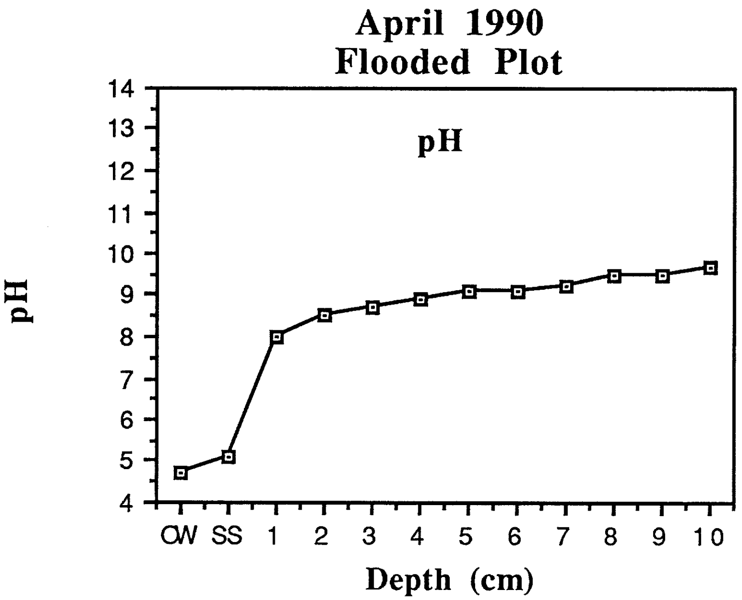
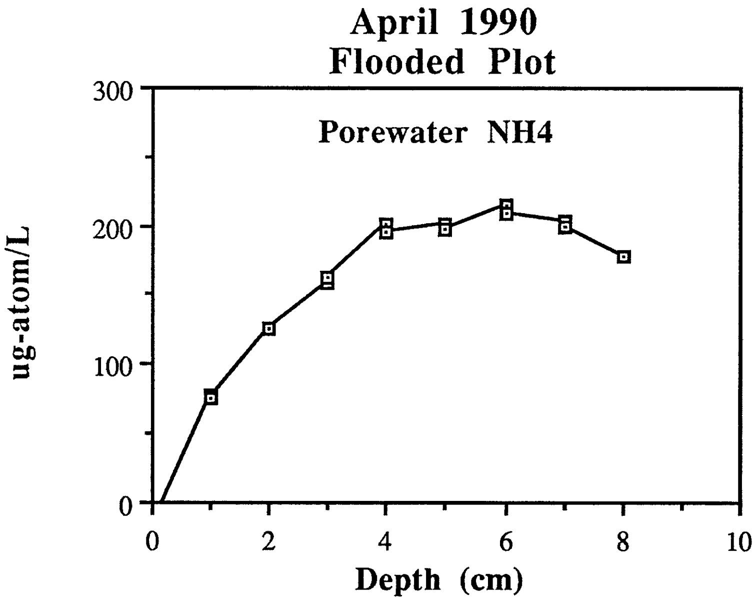
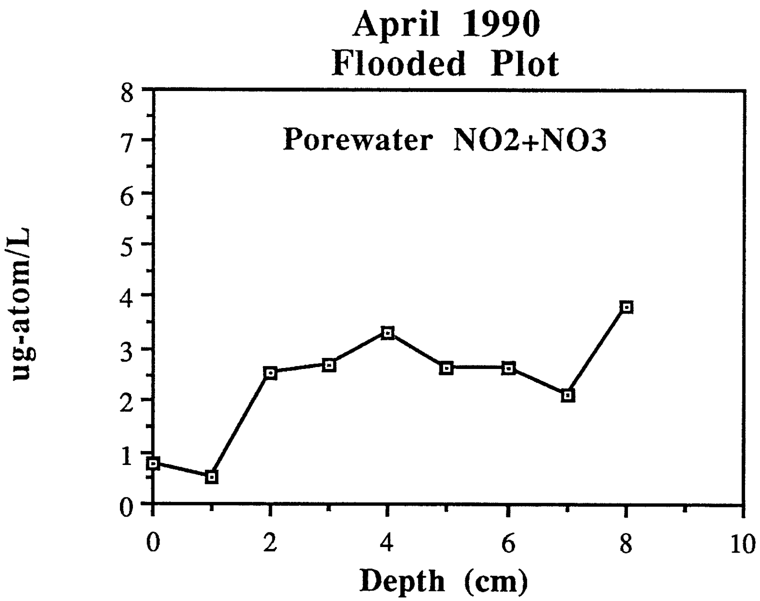



April 1990
Flooded Plot
Redox Potential
Redox Pot. (mv)
400
300
200
100
0
OW SS 1 2 3 4 5 6 7 8 9 10
Depth (cm)

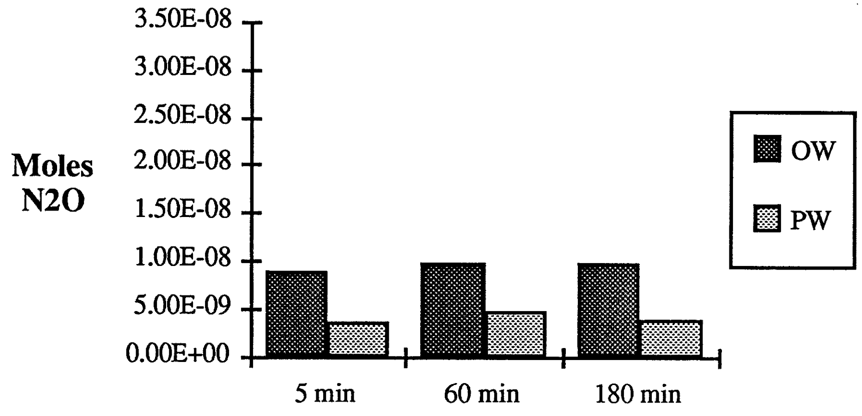

April 1990
Flooded Plot
pH
pH
14
13
12
11
10
9
8
7
6
5
4
OW SS 1 2 3 4 5 6 7 8 9 10
Depth (cm)


April 1990
Flooded Plot
Porewater NH4
ug-atom/L
300
200
100
0
0 2 4 6 8 10
Depth (cm)

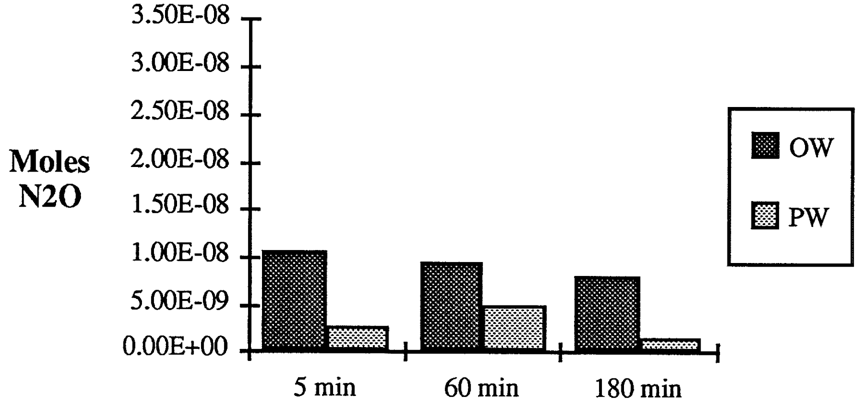

April 1990
Flooded Plot
Porewater NO2+NO3
ug-atom/L
8
7
6
5
4
3
2
1
0
0 2 4 6 8 10
Depth (cm)

May 1990
Control Plot

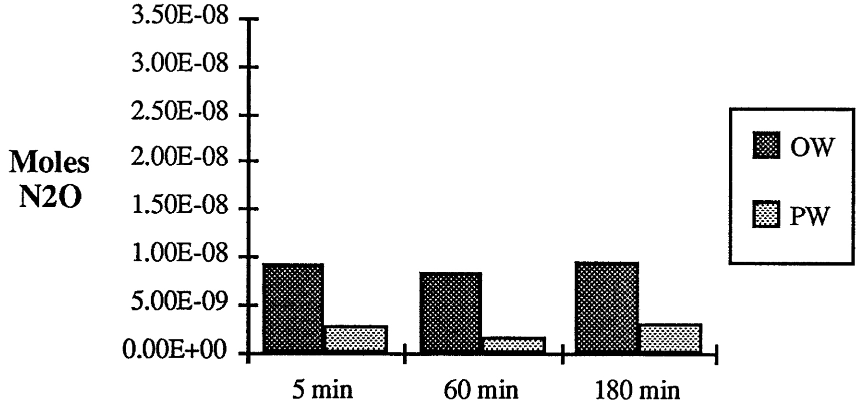
Ambient



0.05 mM



1.0 mM



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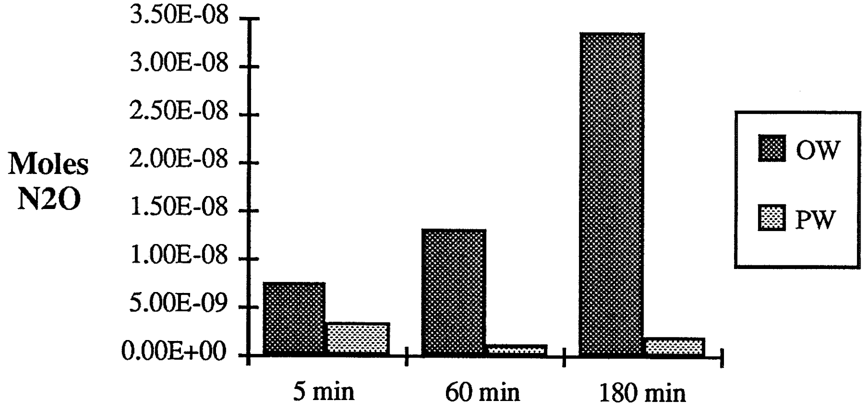
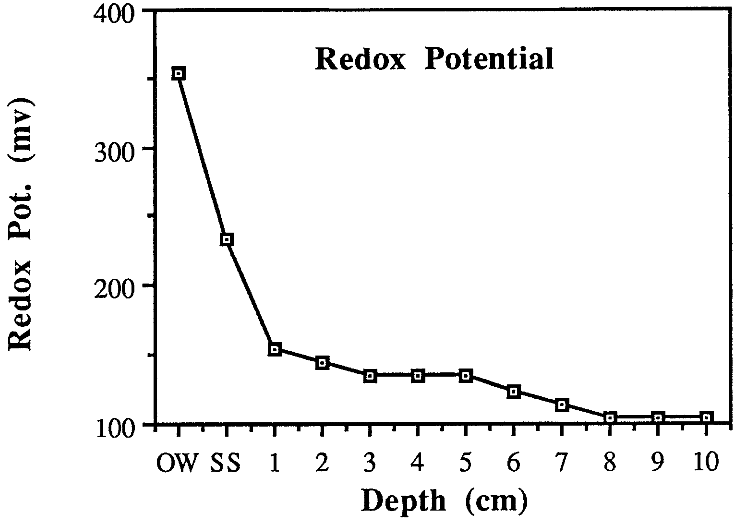


May 1990
Control Plot



May 1990
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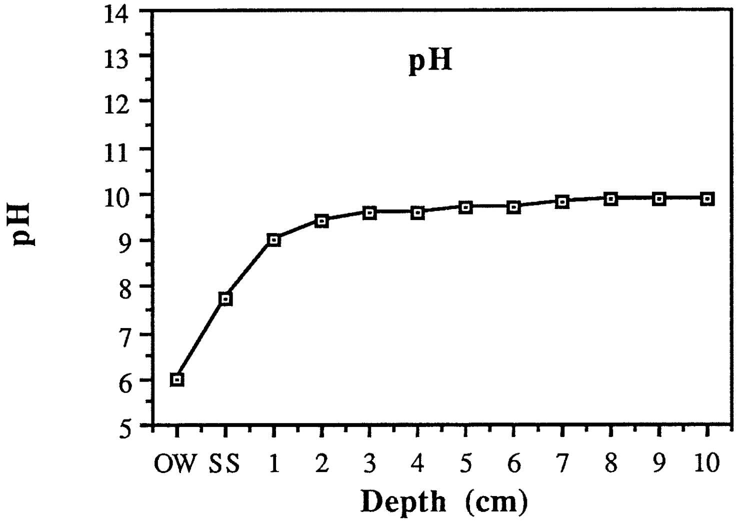
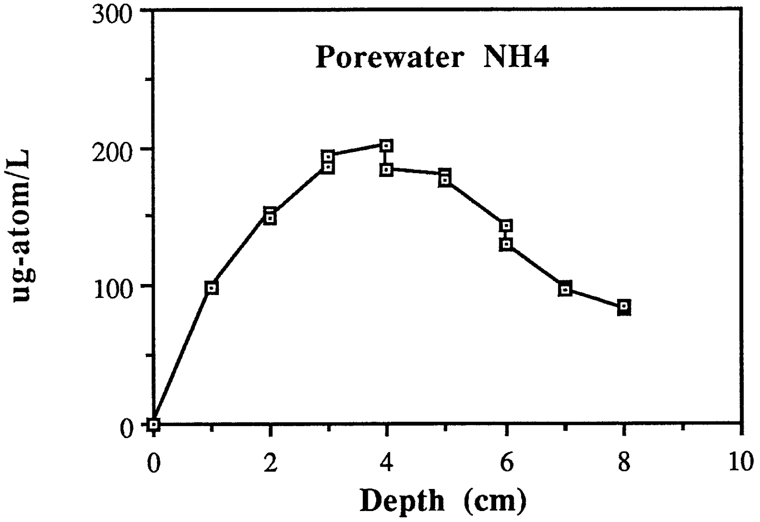
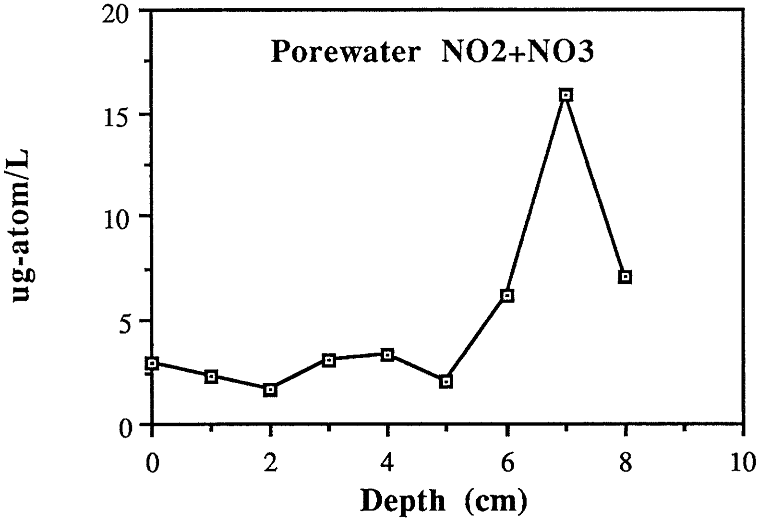


May 1990
Control Plot

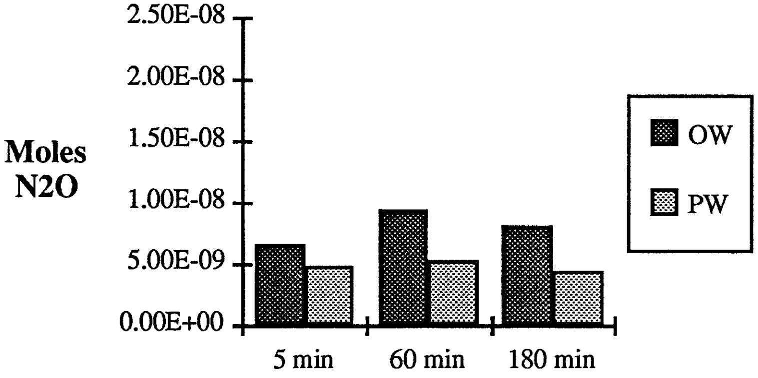


May 1990
Control Plot



May 1990
Flooded Plot

Ambient



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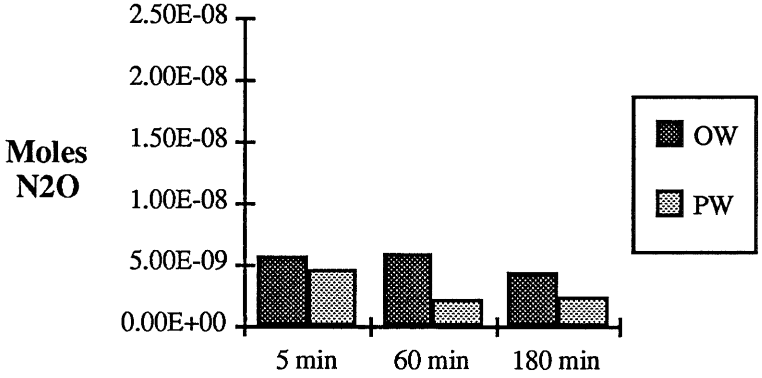

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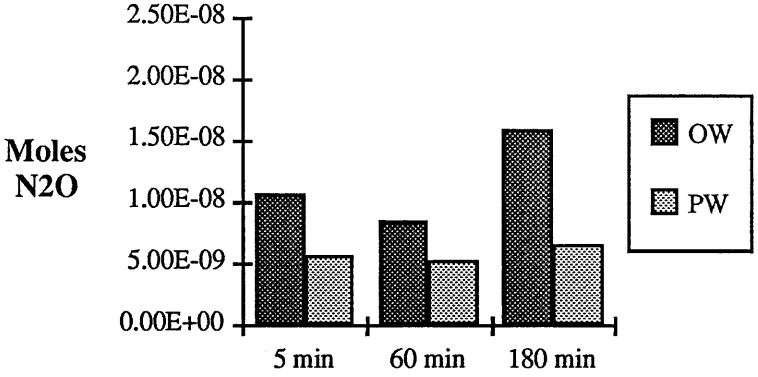

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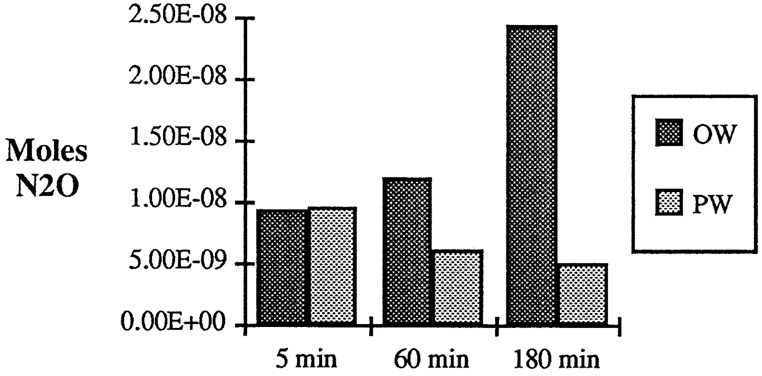
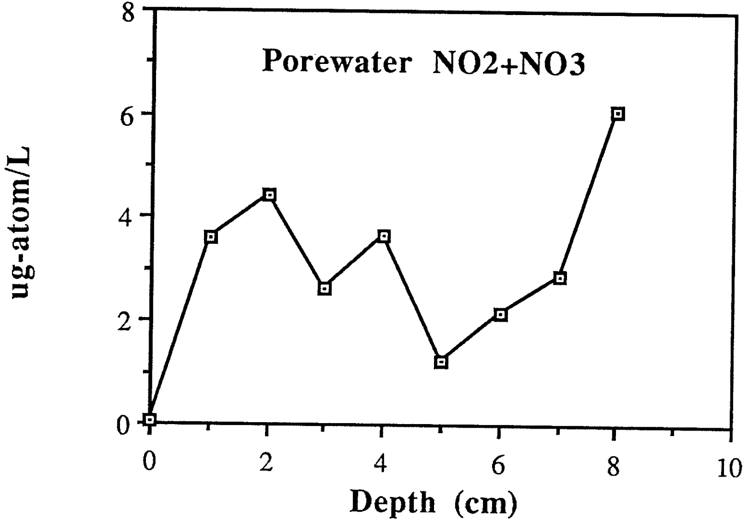
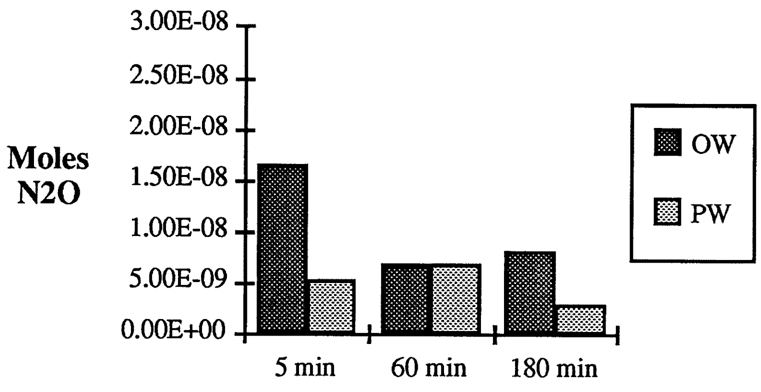


May 1990
Flooded Plot



June 1990
Control Plot

Ambient



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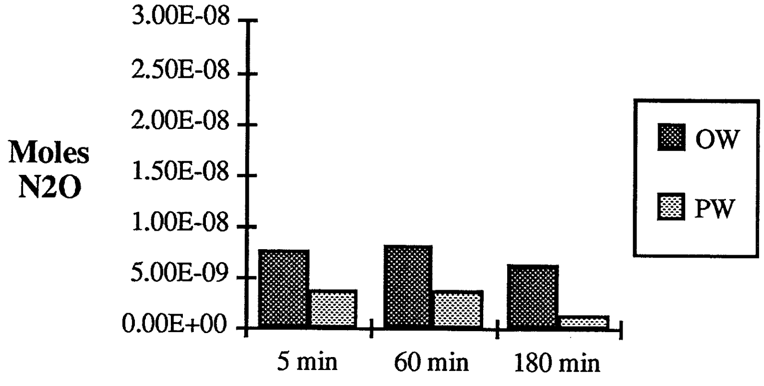

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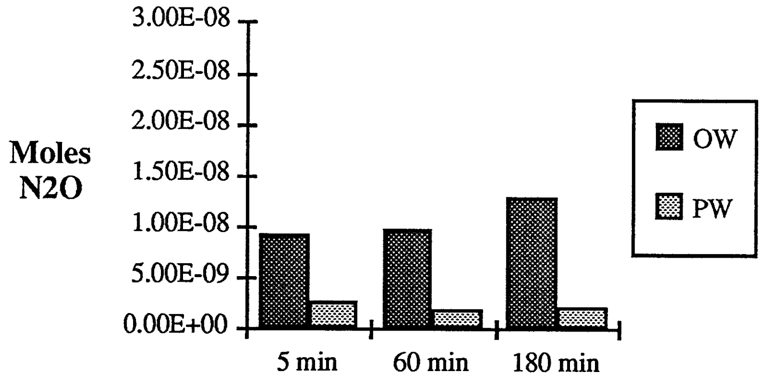

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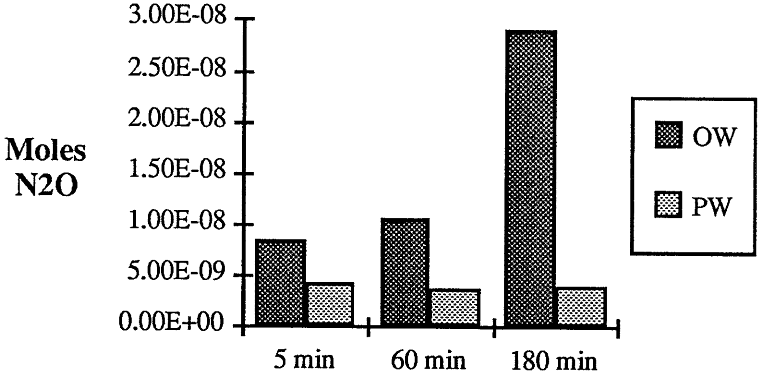
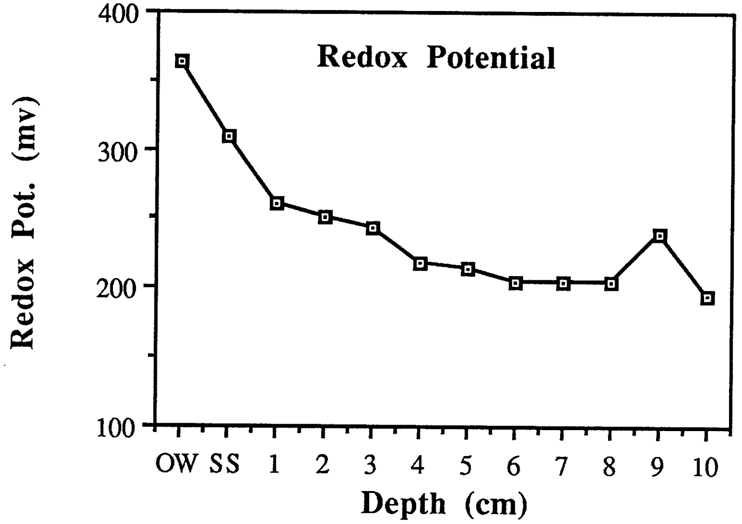
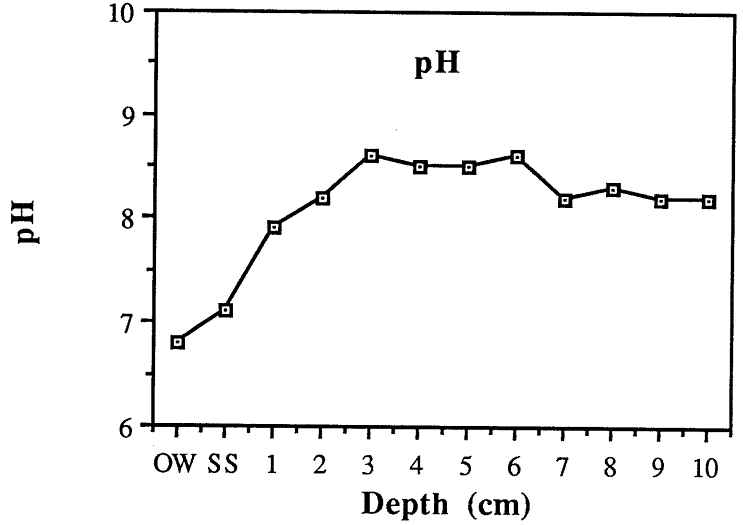
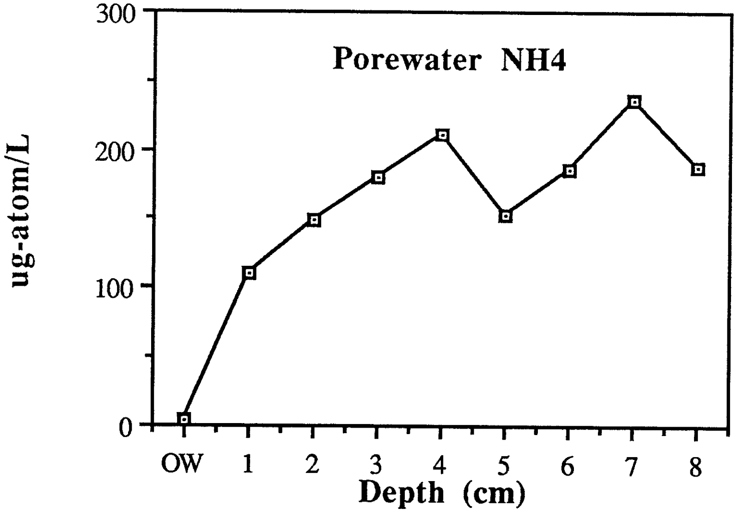
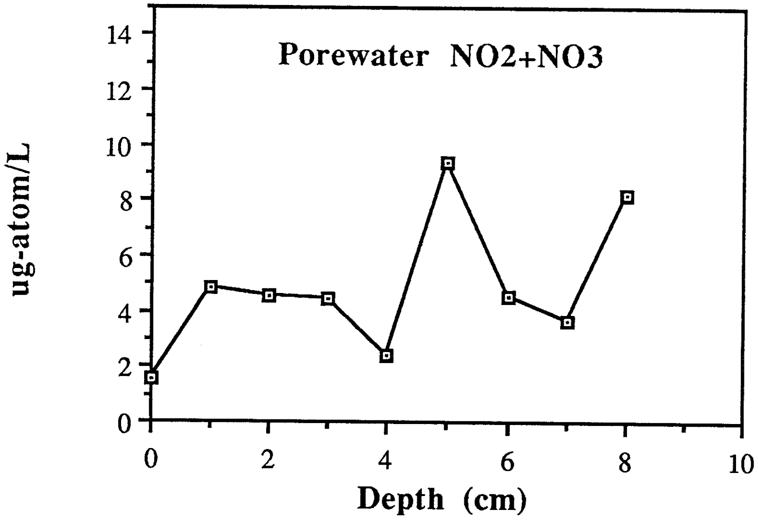



June 1990
Control Plot

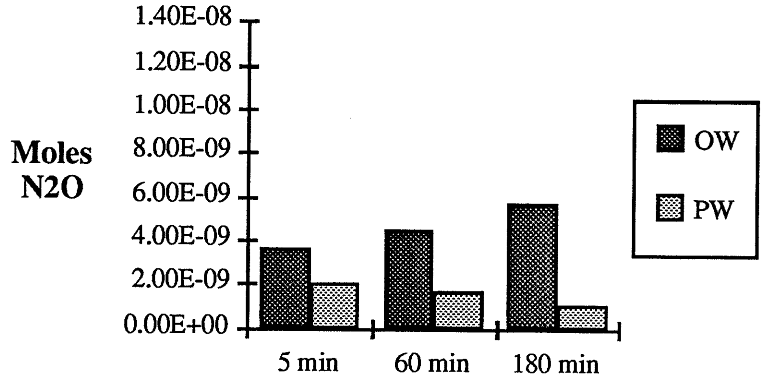

June 1990
Control Plot


June 1990
Control Plot


June 1990
Control Plot

June 1990
Flooded Plot

Ambient



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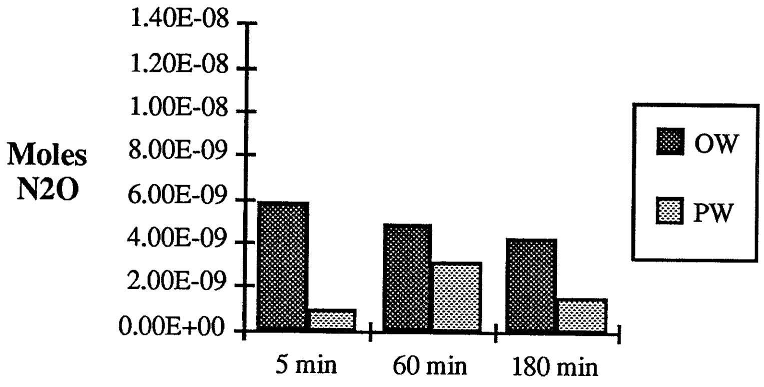

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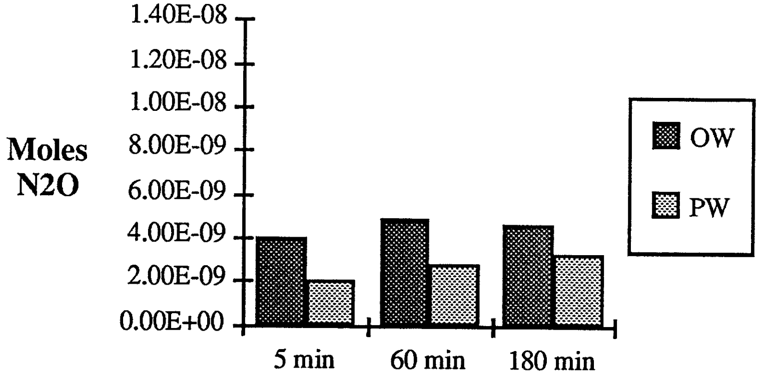

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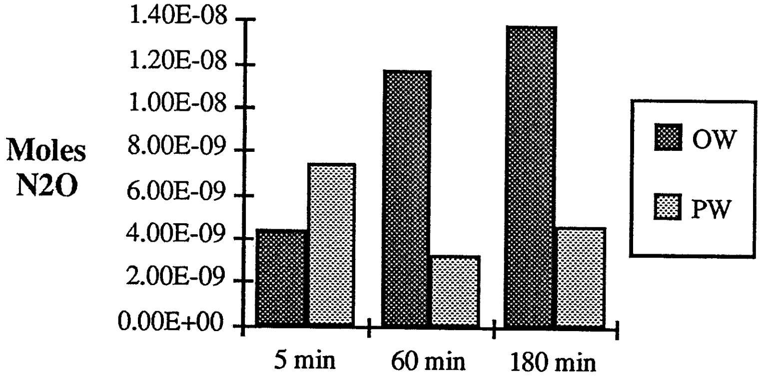
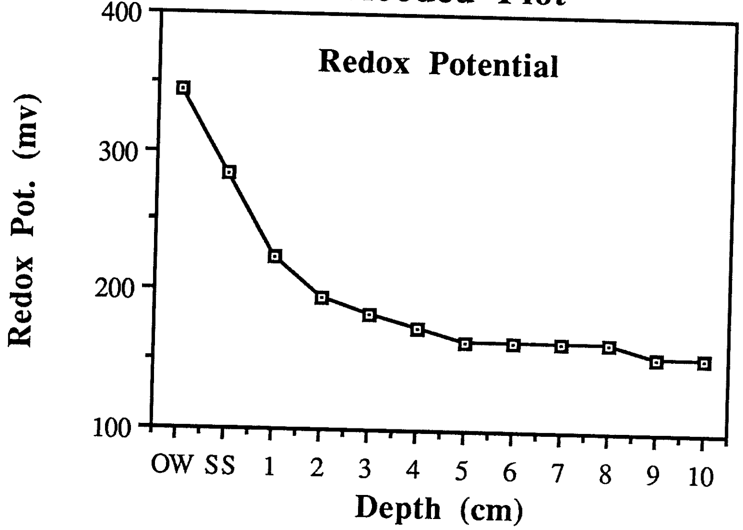
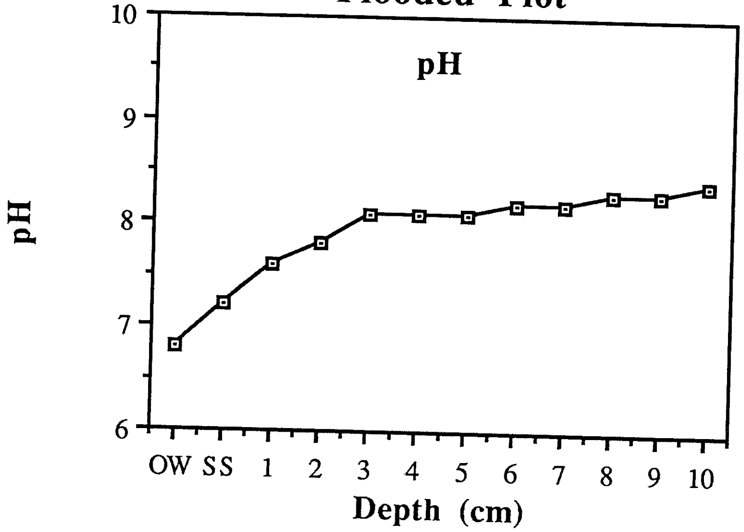


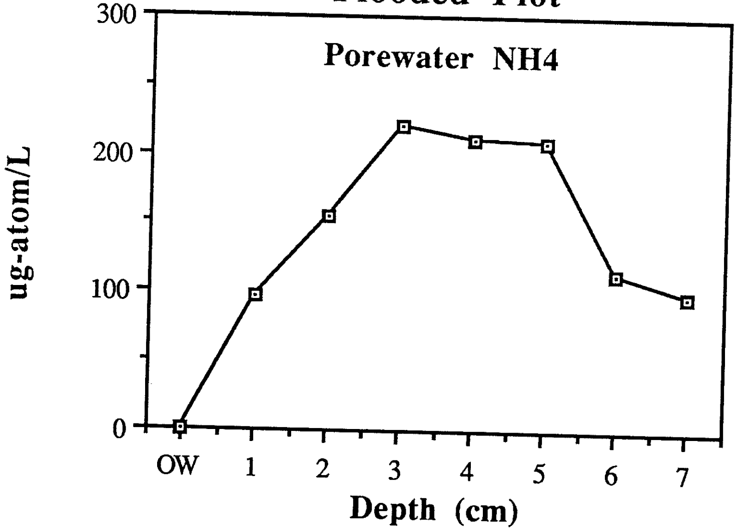
June 1990
Flooded Plot



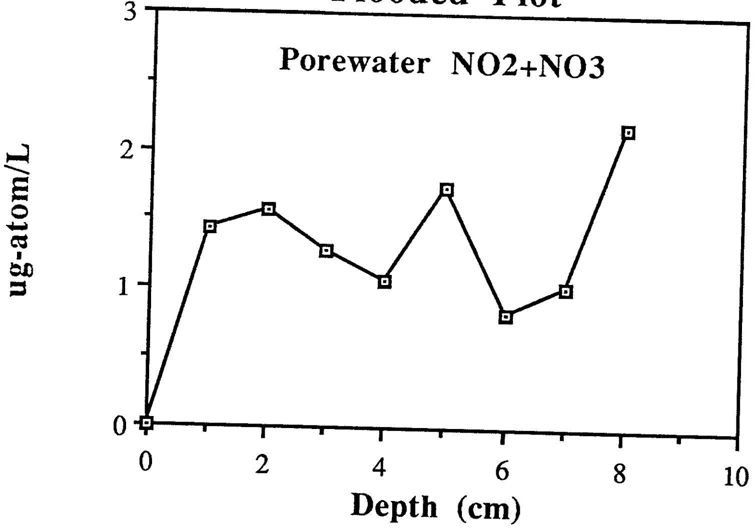
June 1990
Flooded Plot



June 1990
Flooded Plot

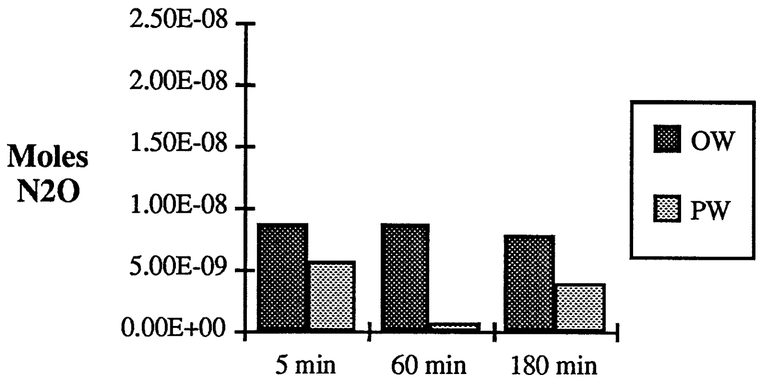


June 1990
Flooded Plot



July 1990
Control Plot

Ambient



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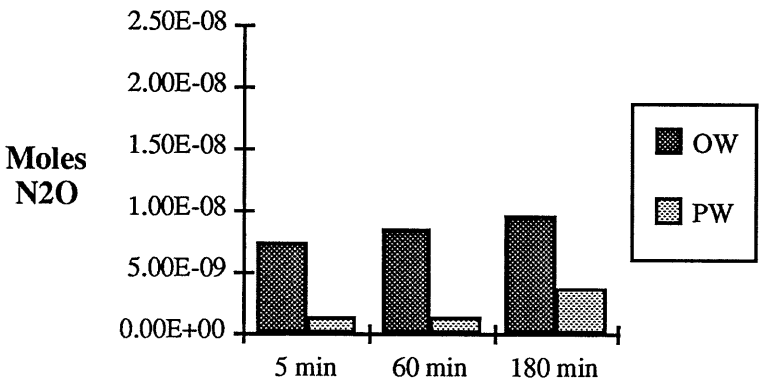

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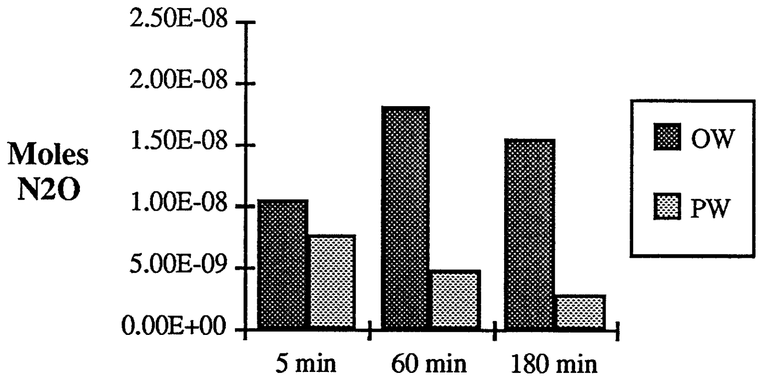

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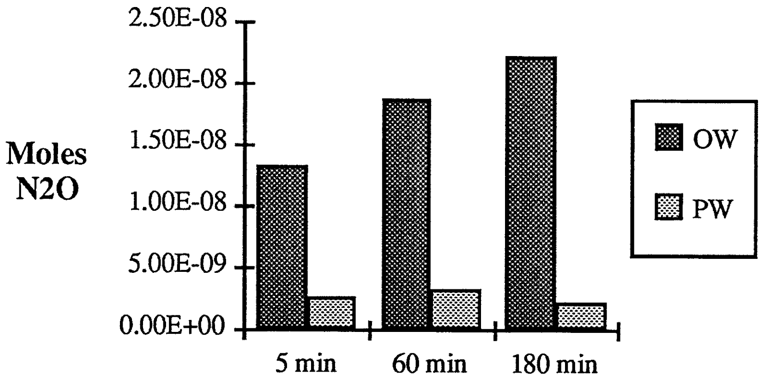
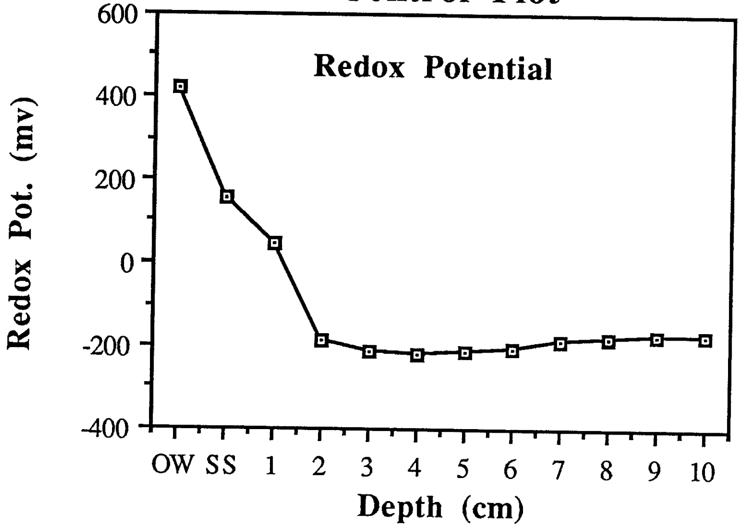
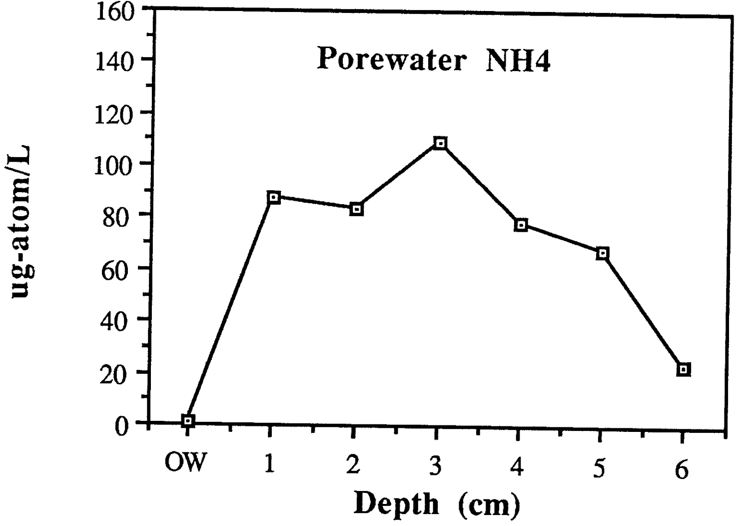
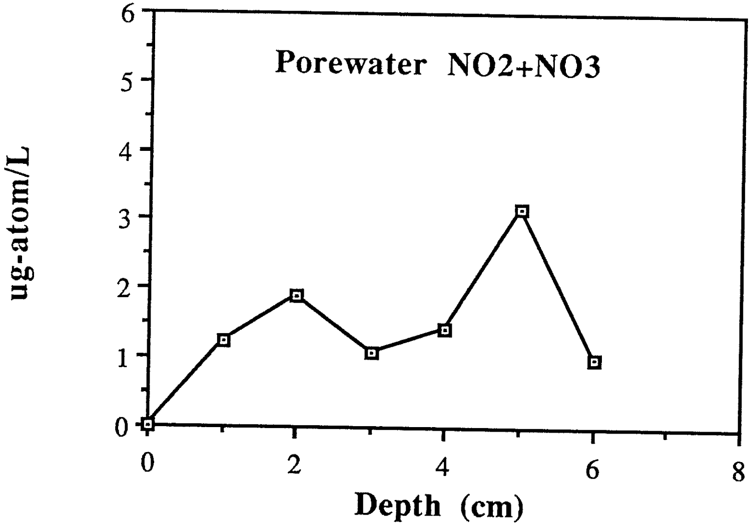



July 1990
Control Plot

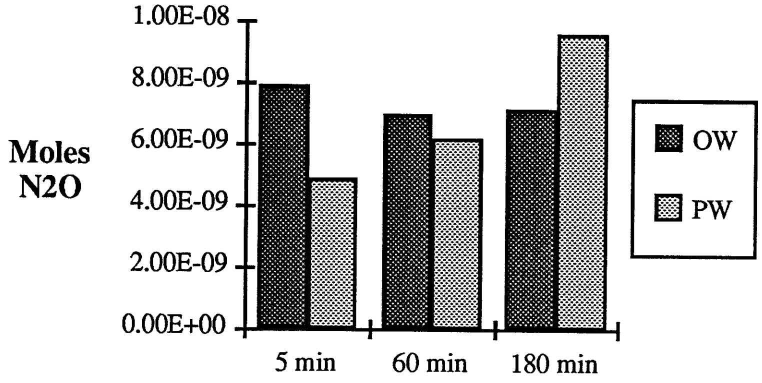
pH 6.68 (OW)


July 1990
Control Plot


July 1990
Control Plot

July 1990
Flooded Plot

Ambient



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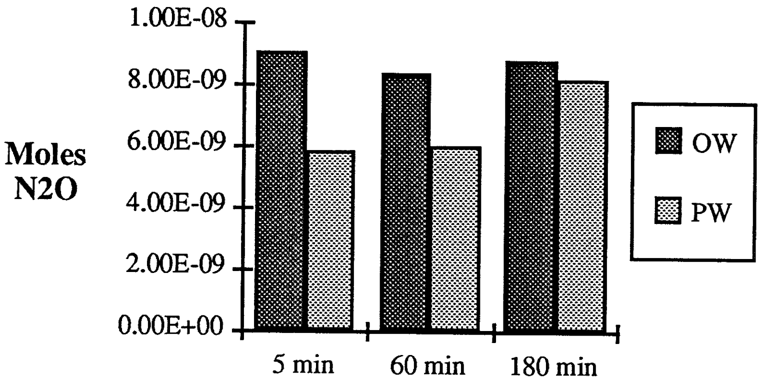

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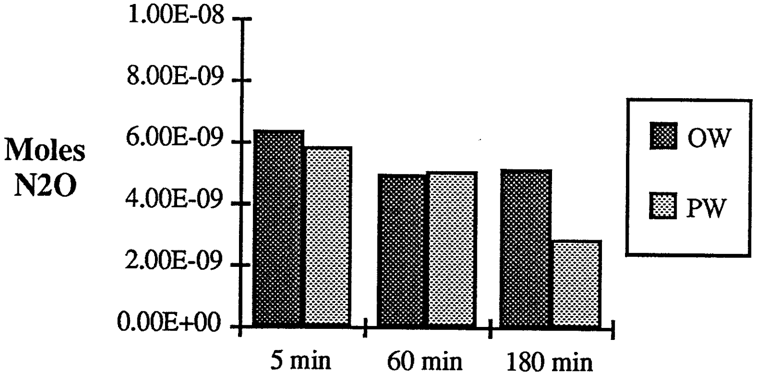

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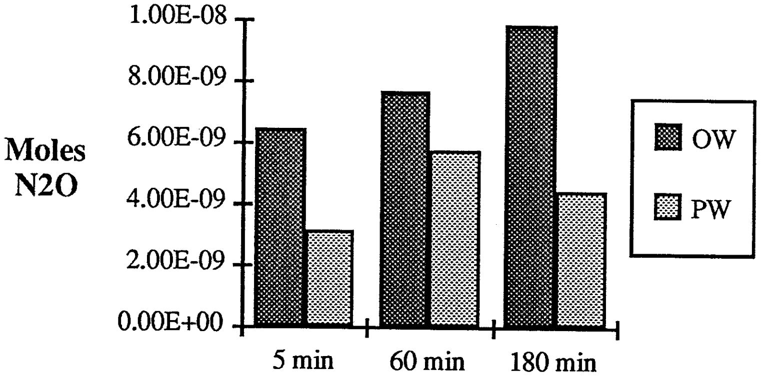
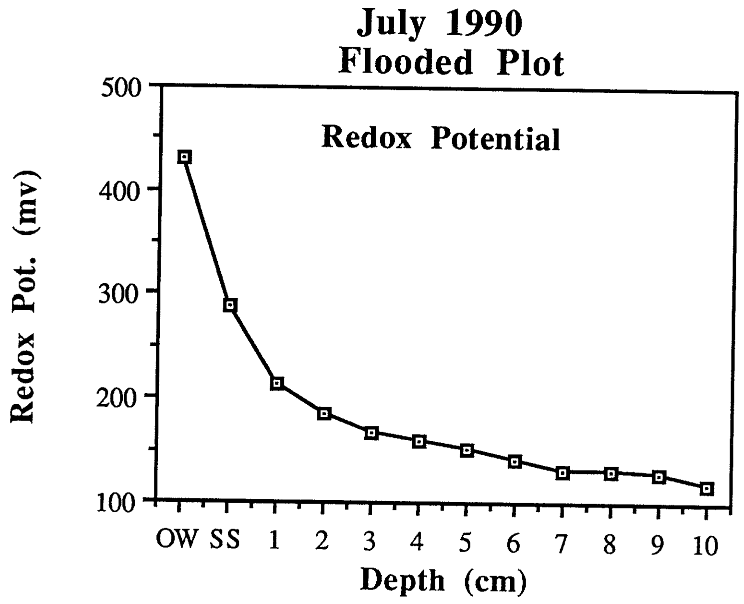
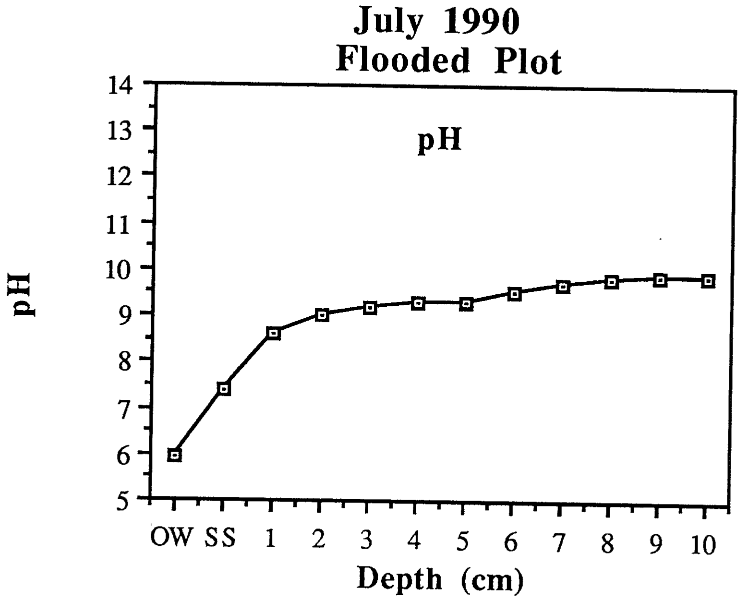
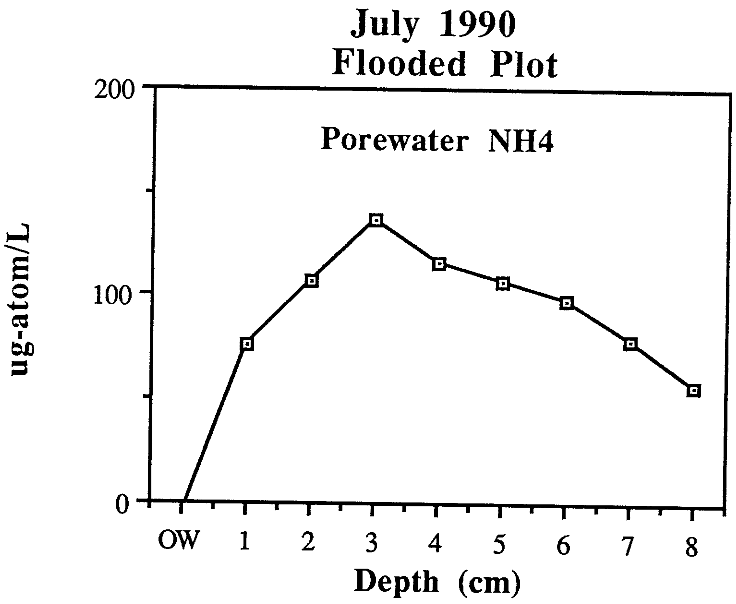
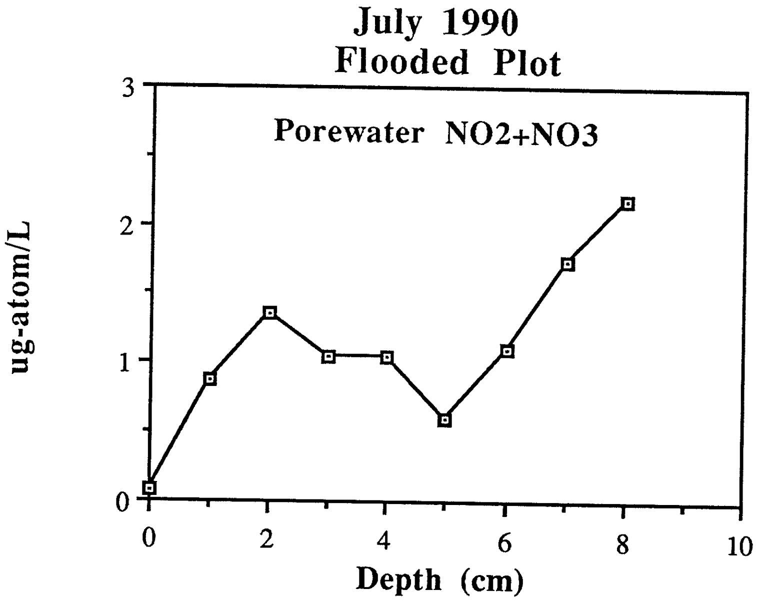



July 1990
Flooded Plot
Redox Potential
Redox Pot. (mv)
500
400
300
200
100
OW SS 1 2 3 4 5 6 7 8 9 10
Depth (cm)

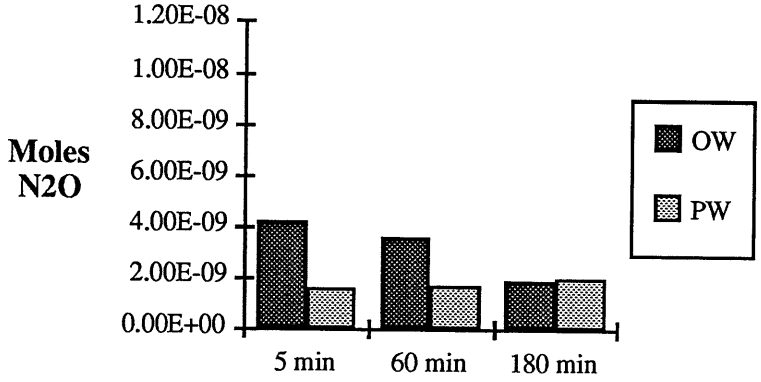

July 1990
Flooded Plot
pH
pH
14
13
12
11
10
9
8
7
6
5
OW SS 1 2 3 4 5 6 7 8 9 10
Depth (cm)


July 1990
Flooded Plot
Porewater NH4
ug-atom/L
200
100
0
OW 1 2 3 4 5 6 7 8
Depth (cm)


July 1990
Flooded Plot
Porewater NO2+NO3
ug-atom/L
3
2
1
0
0 2 4 6 8 10
Depth (cm)

August 1990
Control Plot

Ambient



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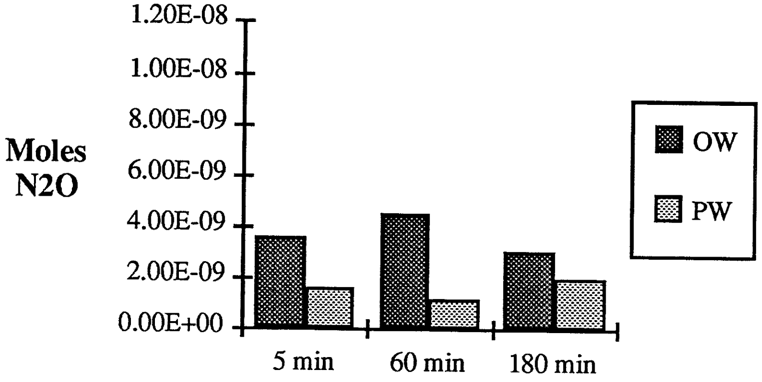

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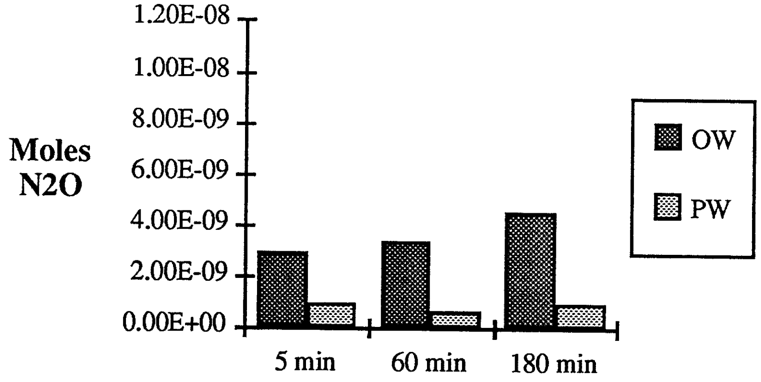

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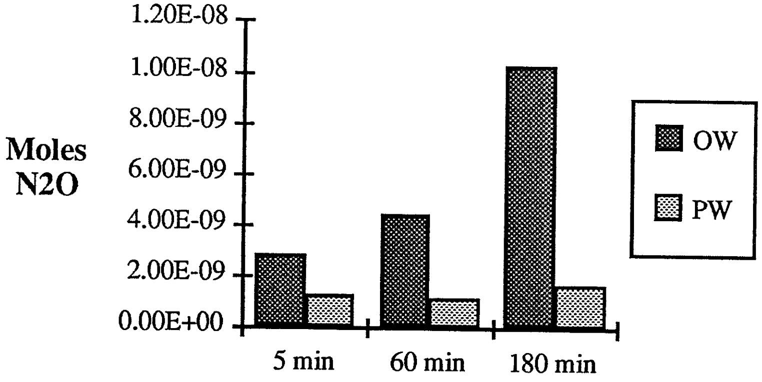
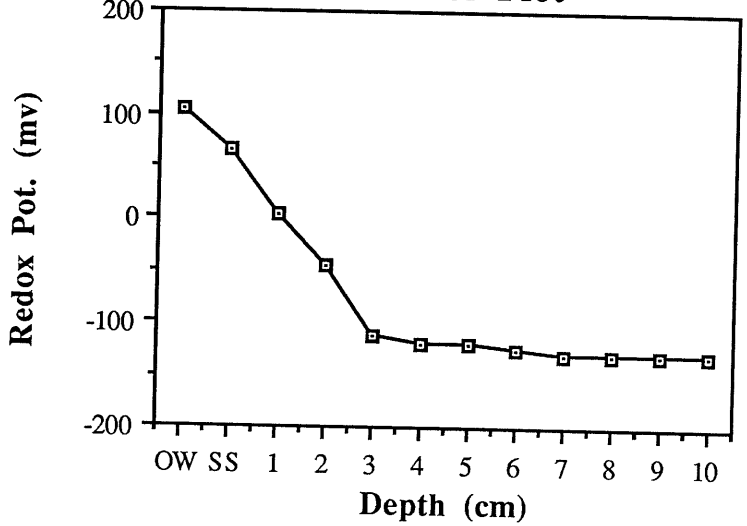
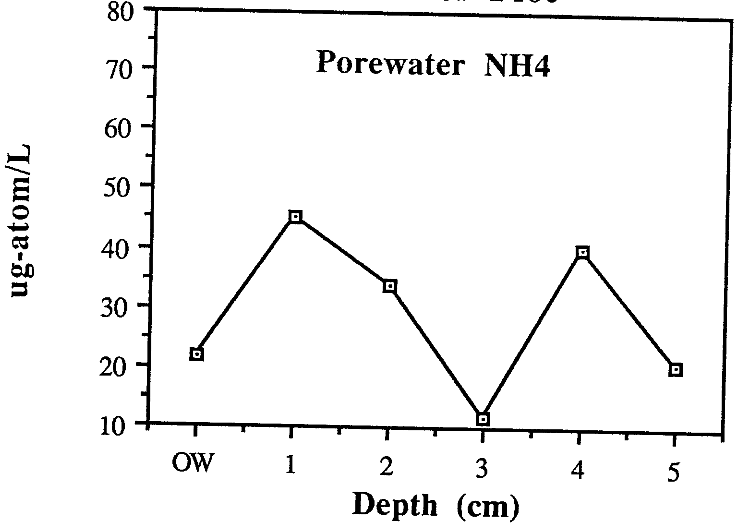


August 1990
Control Plot

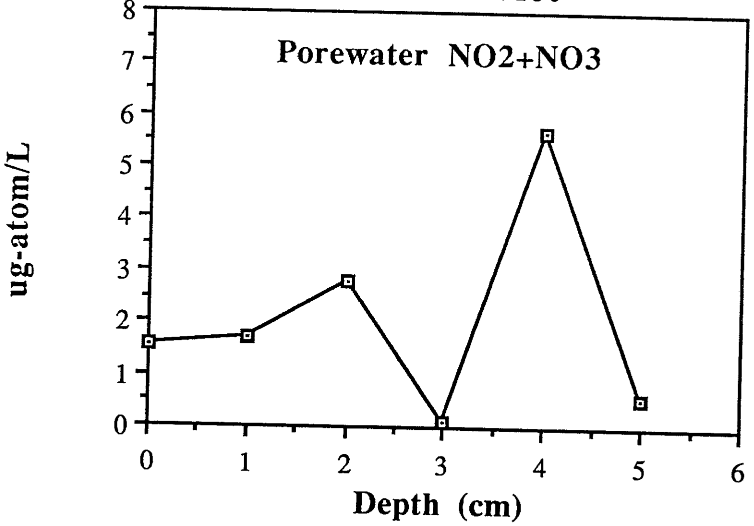


pH 6.4 (OW)

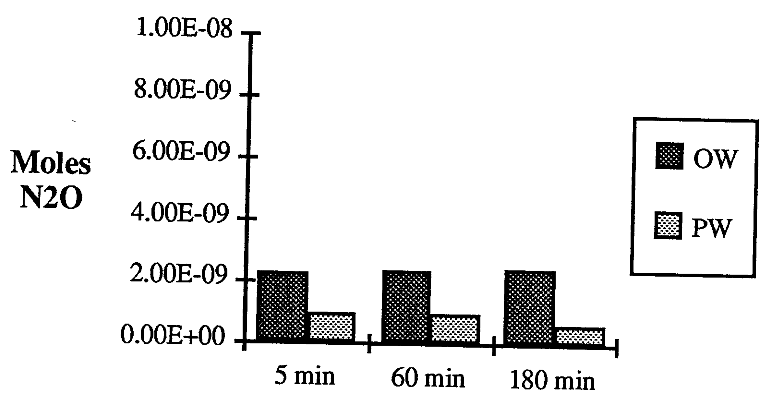
August 1990
Control Plot

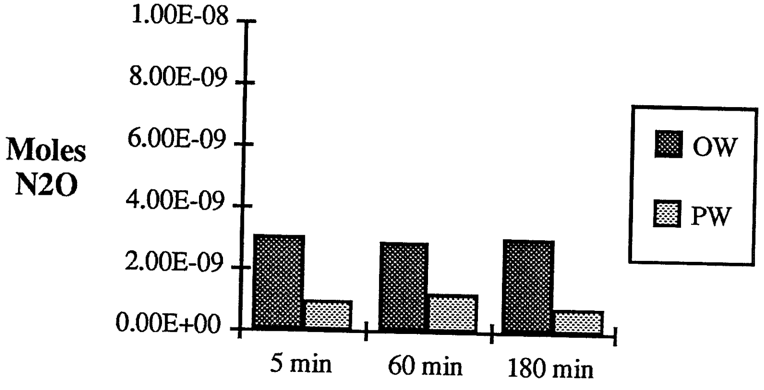


August 1990
Control Plot



August 1990
Flooded Plot


Ambient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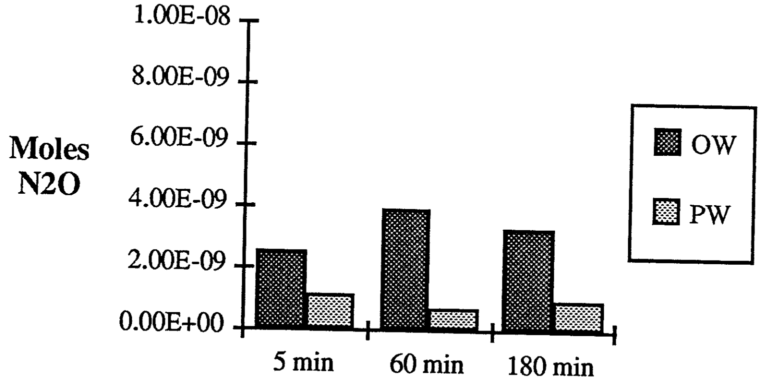
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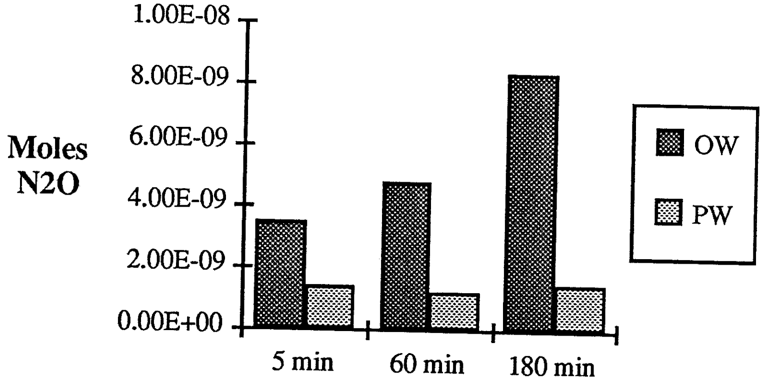
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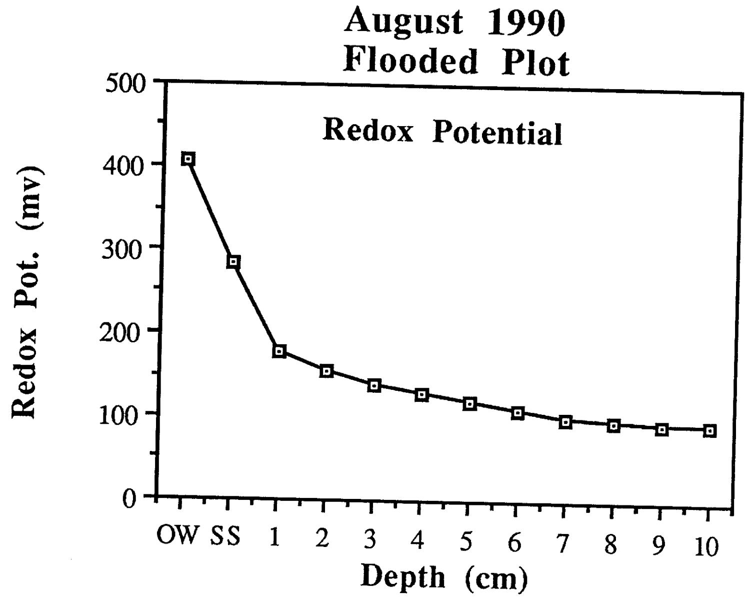
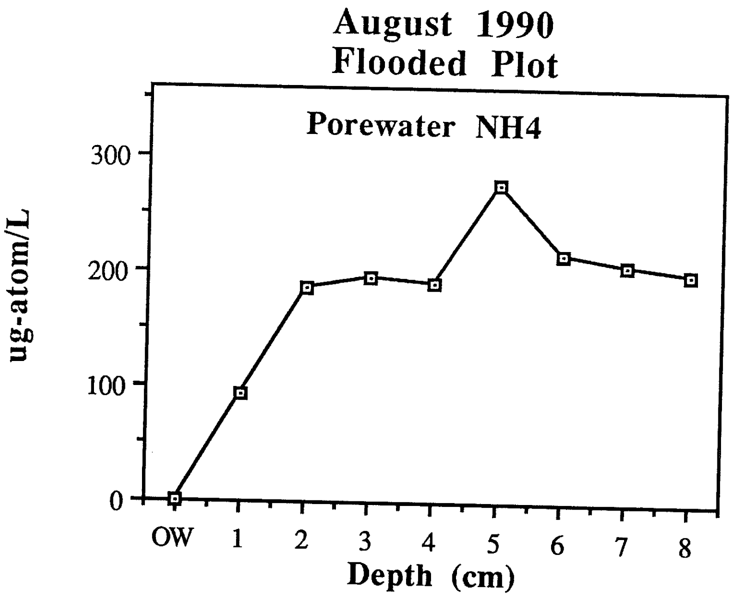
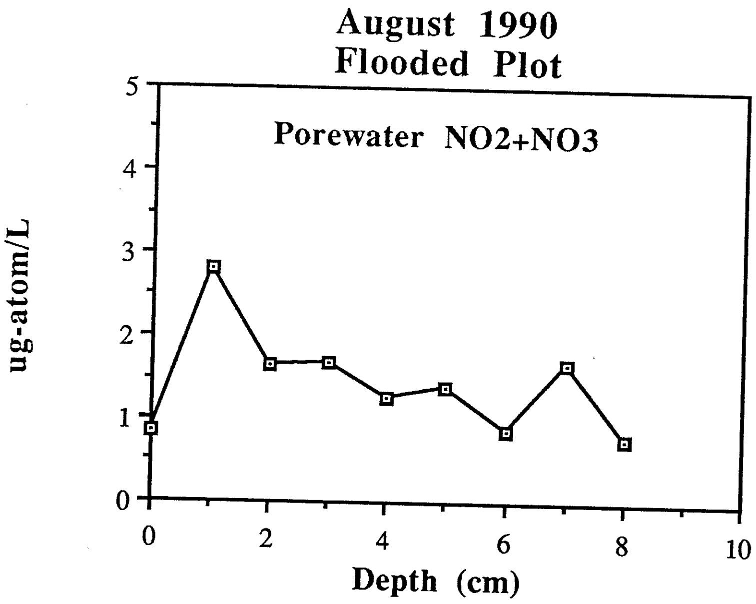

August 1990
Flooded Plot
Redox Potential
Redox Pot. (mv)
500
400
300
200
100
0
OW SS 1 2 3 4 5 6 7 8 9 10
Depth (cm)

pH 6.7 (OW)


August 1990
Flooded Plot
Porewater NH4
ug-atom/L
300
200
100
0
OW 1 2 3 4 5 6 7 8
Depth (cm)


August 1990
Flooded Plot
Porewater NO2+NO3
ug-atom/L
5
4
3
2
1
0
0 2 4 6 8 10
Depth (cm)