

Denitrification in Sediments of a Lake Erie Coastal Wetland (Old Woman Creek, Huron, Ohio, USA)

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ABSTRACT. Denitrification in Old Woman Creek estuary (Lake Erie) sediments was measured by an *in vitro* N_2 -flux method with intact cores and by an *in situ* chamber method. In both methods, nitrogen gas, the end product of denitrification, was measured directly by gas chromatography. The *in situ* approach allowed measurement of denitrification directly over short time intervals but its use was limited to shallow depths. Denitrification rates measured with *in situ* chambers agreed well with those from *in vitro* intact cores when temperatures in the estuary remained constant. However, the two methods could not be accurately compared during the spring when temperature increased rapidly, because of the 4-day pre-incubation time needed for sparging for the *in vitro* method. *In vitro* denitrification rates ranged from ca 40 to 135 $\mu\text{mole } N_2 \text{ m}^{-2} \text{ h}^{-1}$ in October 1993 and from 66 to 428 $\mu\text{mole } N_2 \text{ m}^{-2} \text{ h}^{-1}$ in May and July 1994. Oxygen consumption rates in these experiments ranged from 0.71 to 3.0 $\text{mmole } O_2 \text{ m}^{-2} \text{ h}^{-1}$. Denitrification rates tended to decrease along the flow axis but differences among stations were usually not significant. *In situ* N_2 accumulation rates ranged from 45 $\mu\text{mole } N_2 \text{ m}^{-2} \text{ h}^{-1}$ in dark chambers during October 1993 up to apparent values of 2,100 $\mu\text{mole } N_2 \text{ m}^{-2} \text{ h}^{-1}$ in May 1994, immediately after the water temperature had rapidly increased to 27°C. These calculated values included gas-solubility corrections due to the water-temperature increases. *In situ* measurements of denitrification rates in transparent chambers were 76–79% higher than rates measured in a similar dark chamber. The results suggest that denitrification is an important sink for nitrogen in Old Woman Creek estuary and that environmental conditions such as temperature, light, and available substrate affect denitrification rates.

INDEX WORDS: Denitrification, sediment, nitrogen, estuary, Lake Erie, Old Woman Creek.

INTRODUCTION

Many estuaries and tributaries are able to assimilate large quantities of nutrients which can be stored, incorporated into organisms, released, or cycled. Bottom and suspended sediments may be a sink or source of nutrients and can influence estuarine productivity and water quality (Matisoff and

Eaker 1992). Nutrient concentrations in estuaries/tributaries often do not reflect the large quantities of nutrients introduced through river outlets, agricultural sources, or other inputs (Webb and Walling 1985). Depletion of nitrate between the upper reaches and outlets of some rivers has been reported (Jorgensen and Sorenson 1988). Denitrification removes nitrate and exports nitrogen to the atmosphere and thereby serves as an important sink for nitrogen (Seitzinger 1988, Jorgensen 1989). Deni-

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trification is especially evident in estuaries where a significant portion of the nutrient supply comes from mineralization of organic matter in the sediments (Nixon 1981). Abundant supplies of organic matter and low concentrations of oxygen in estuarine and coastal sediments present favorable conditions for denitrification (Seitzinger *et al.* 1984).

The Old Woman Creek tributary or fresh-water "estuary" (OWC) is a coastal wetland on the shoreline of western Lake Erie, where multidisciplinary environmental research has been conducted for more than a decade (Krieger *et al.* 1992). This wetland serves as an interface between agricultural runoff and Lake Erie and appears to be a nutrient sink, storing P in sediments and, presumably, removing available N by denitrification (Wickstrom 1988, Heath 1992).

We measured sediment denitrification, oxygen consumption rates, and nutrient fluxes in OWC during 1993 and 1994 to examine the hypothesis that denitrification is a major sink for nitrogen in this coastal ecosystem. Our specific objectives were to determine dissolved inorganic nitrogen (DIN) concentrations and sediment-water fluxes and to determine denitrification rates, using both *in vivo* and *in situ* measurement techniques. We also examined short-term changes in *in situ* denitrification rates during the spring warm-up period and compared denitrification rates in light vs dark *in situ* incubation chambers in two of the *in situ* experiments.

METHODS

Study Site

Old Woman Creek wetland is a small (ca 0.3 km²), shallow (40 cm depth) tributary located on the southern margin of Lake Erie, about 5 km east of Huron, Ohio (Fig. 1). It is situated along the 2.1 km drowned mouth of OWC and drains a 69 km² watershed with agriculture as the predominant land use (Heath 1992). Old Woman Creek is usually separated from Lake Erie by a narrow barrier beach, which occurs at the stream mouth during approximately May through September. The mouth is generally open during storm activity on Lake Erie and during seasonal high flows that commonly occur during November through April (Heath 1992). The wetland is mostly non-wooded, with some patches of the rooted aquatic macrophyte *Nelumbo lutea* that are surrounded by open water. A mixed hardwood forest borders the wetland. This natural area was designated as the only National Estuarine Re-

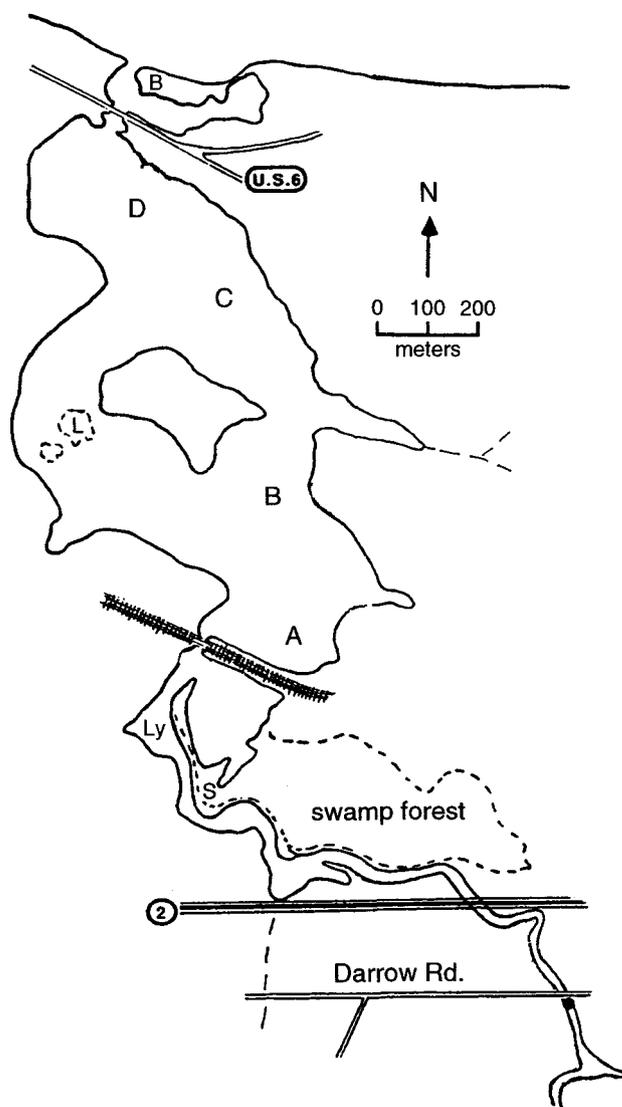


FIG. 1. Location of the sampling sites in Old Woman Creek estuary.

search Reserve on the Great Lakes in 1977 (Herdendorf 1992).

Sediment deposition, mostly silts and clays, has increased at the mouth of OWC during the past 100 years due to agricultural activities in the watershed (Heath 1992). Dissolved inorganic nitrogen enters the estuary primarily as nitrate, with concentrations ranging from 0.5 to > 200 μM (Wickstrom 1988). Except for some information on suspended sediments and selected chemical parameters (Matisoff and Eaker 1992), very little information has been published on sediment chemistry and on the rates of accumulation or depletion of nutrients in OWC. A

study of chemical characteristics for Great Lakes coastal wetlands indicated that the average concentration of organic matter in OWC (10.2%) was lower than that for eight other diked and undiked wetlands (Mitsch 1992).

Sediment and overlying water samples were collected in OWC (Fig. 1) in October 1993 and in May and July 1994. Temperatures ranged from 12°C to 28°C. Four sites along the flow axis (A, B, C, and D; Fig. 1) were sampled for nutrient measurements, whereas three (B, C, and D) and one station (C), respectively, were sampled for the *in vitro* and *in situ* denitrification measurements. Sediments were mostly silts and clays with some sand, decomposing leaves, and detritus from plants, and were gray and dark in color. The surface layers (< 1 cm depth) of our sediment cores were brown and very soft. The deeper layers of the cores (> 1.5 cm depth) contained sand, small branches, and roots of plants.

Denitrification Measurements

Two methods were used to measure denitrification rates at the sediment-water interface: the *in vitro* N₂ flux technique (modified from Seitzinger *et al.* 1980, 1984) and direct measurements with an *in situ* chamber (Tomaszek 1991).

In vitro N₂ Flux Technique

Duplicate sediment cores, approximately 20 cm deep, were collected from a boat, within a few m of each other, by twisting a plastic cylindrical corer (76-mm i.d.) into the sediments, closing the top with a rubber stopper, and removing the sediment core with overlying water. The water above the sediments was carefully poured off. After the lower part of the core was extruded by gravity and discarded, the upper 6–7 cm of the sediment cores were transferred to the lower sections of glass incubation chambers (75-mm i.d.; Seitzinger *et al.* 1980, Gardner *et al.* 1987) and transferred to the laboratory. The upper chamber section was attached via a joint sealed with a greased O-ring, and the components were locked together with a metal clamp. The assembled chambers were weighed, filled with overlying water up to the stopcock, and weighed again to measure the volume of overlying water. Sixty mL of water were slowly withdrawn from each chamber via a canula inserted through the open stopcock at the top of the chamber to leave an exact volume for the overlying gas phase.

The water and the gas phase were repeatedly

sparged in the dark with a mixture of 80% He and 20% O₂ (Scott Specialty Gases, Inc., Plumsteadville, PA) to remove atmospheric N₂ (Seitzinger *et al.* 1980). When *in situ* temperatures were lower than room temperatures, the chambers were held in an ice bath during sparging. Between sparging intervals of about 1 hour, the chambers were placed in an incubator at *in situ* temperature for 30–70 min intervals (and overnight). The sparging procedure was repeated 7–8 times daily for 4 days to allow N₂ to diffuse from the sediments and be replaced with He. The repeated sparging with fresh He:O₂ gas during the initial incubation treatments maximizes the concentration gradient of the gases and thereby enhances the degassing flux. Although individual gas measurements are somewhat variable (Seitzinger *et al.* 1984), comparison of results from successive incubation intervals indicated that rates do not change predictably with time of incubation after the initial 4 days of intensive sparging (unpublished data).

After the sparging period, the chambers were closed and incubated in the dark for successive 2–3 d periods to allow gases to accumulate. During incubations, the water in each chamber was stirred continuously at a speed of 60 rpm, using suspended magnetic bars (Gardner *et al.* 1987) to enhance gas diffusion and facilitate the equilibration between the gas-water phases. Chambers were kept in the dark during incubation and sparging to minimize assimilation of inorganic nitrogen by phytoplankton. Nitrogen and oxygen gas fluxes were measured after each incubation by gas chromatography (Seitzinger *et al.* 1980, 1984) and the system was sparged again in preparation for the next incubation.

Gas samples were analyzed on a Shimadzu gas chromatograph (Model GC-8A) equipped with dual stainless steel columns (6 m × 0.32 cm O.D.) packed with molecular sieve 5 A (60/80 mesh) having a carrier gas flow rate of 25 cm³ He min⁻¹ and operated at 50°C. The thermal conductivity detector was maintained at 60°C.

Gas samples (0.5 mL) were taken with a 1-mL gas-tight syringe that had been flushed six times with N₂-free He. Precautions to prevent atmospheric contamination with N₂ were similar to those described by Seitzinger *et al.* (1984). Results usually replicated well and showed no contamination with ambient N₂. Oxygen and nitrogen concentrations were estimated by comparing sample peak

sizes to those obtained from standard gases containing either 2.0% N₂ or 20.8% O₂ in a He mixture.

After the gas phase was sampled, 5 mL of water was withdrawn from each chamber with a cannula, filtered, and analyzed for NO₃⁻ and NH₄⁺. Nitrate (+ nitrite) was determined on a Technicon Auto Analyzer II using the cadmium reduction method; ammonium was measured either by the Bertholet reaction (USEPA 1974, APHA 1990) or by HPLC (Gardner and St. John 1991). When gas and water sampling were completed, each chamber was again sparged for ca 1 h with He + O₂ to remove remaining reaction gases and prepare it for the next measurement. The incubations and measurements were repeated sequentially for replication.

After a series of 3–4 successive denitrification measurements was completed, the conditions in all chambers were changed from oxic to anoxic by sparging with He and allowing any remaining O₂ to be depleted. The chambers were then held in the incubator for three successive 2–3 d periods to determine background N₂ fluxes. After O₂ and NO₃⁻ were depleted, nitrification and denitrification were assumed to have stopped and any accumulation of N₂ was assumed to result from background N₂ flux. It is possible that the background flux could have been slightly greater during some of the experiments than when actually measured (Nowicki 1994). However, the lack of a consistent trend among sequential measurements and the observation that the *in vitro* method produced similar or lower rates than the *in situ* method (see results) indicates that this potential error was probably not large.

Calculation of Denitrification Rates and Sediment Oxygen Consumption

The areal rate of denitrification or oxygen consumption was calculated by dividing the changes in N₂ or O₂ content (concentration changes of N₂ or O₂ times the total gas volume) per unit time by the surface area of the core. Volume changes in the gas phase due to removal of water samples were considered in the flux calculations. Background fluxes, described above, were subtracted from the measured fluxes to estimate denitrification rates. To prevent pseudo-replication and bias from repeated measures, the results from three or four sequential measurements from each chamber were averaged to obtain a representative single rate for the chamber. Precision estimates among duplicate chambers were then obtained by calculating the means and SE

(= one-half of the range for duplicate measurements) from the mean composite rates that had been calculated for the individual chambers. Using this approach, relative SE for duplicate chambers ranged from 2.5 to 35% (see results).

In situ Chamber Measurements

In situ denitrification rates were measured at Station C (ca 40 cm water depth; Fig. 1) in October 1993 and in May and July 1994. In 1994, denitrification and oxygen consumption rates and nutrient fluxes were measured in transparent chambers as well as in the dark chamber.

For the *in situ* measurements of denitrification (Tomaszek 1991), we assumed that the water in the shallow estuary was saturated with N₂ and that any N₂ produced by denitrification in the sediments would collect in a burette placed at the domed top of the chamber (Fig. 2). *In situ* denitrification rates were calculated in the same way as described above for the *in vitro* N₂ flux measurements except that the rates were corrected for changes in nitrogen gas solubility (Gas Solubility Table from Handbook of Chemistry and Physics; Hodgman *et al.* 1960) when the water temperature changed during the experimental incubations. This method is useful for shallow (e.g., < 1.0 m depth), non-tidal, nutrient-rich wetland environments, where the water can be assumed to be saturated with N₂ and measureable volumes of gas accumulate in the burette.

The dark chamber (Fig. 2) was attached to an "anchor pipe," using a polyvinyl tube and supports, and penetrated ca 10 cm into the sediments. The chamber enclosed 21.5 L of overlying water and had a surface area of 0.073 m². A rubber stopper in the top of the chamber contained two sealed holes, for a manual stirrer and a gas burette, respectively. Water was sampled through a tube that had been inserted into the chamber through the stirrer pipe. The upper part of the burette was closed with a rubber septum, so that gases could be sampled by syringe without contamination from the atmosphere. At the beginning of the experiment, air was pulled out of the burette by syringe through a needle and displaced by water drawn up from the chamber. After the system was closed and filled with water, the chamber was left in place for measured time intervals, ranging from several h to a few days, to allow gases to accumulate in the burette.

In 1994, modified *in situ* chambers for measuring denitrification under natural light conditions were made from 20-L transparent polycarbonate carboys.

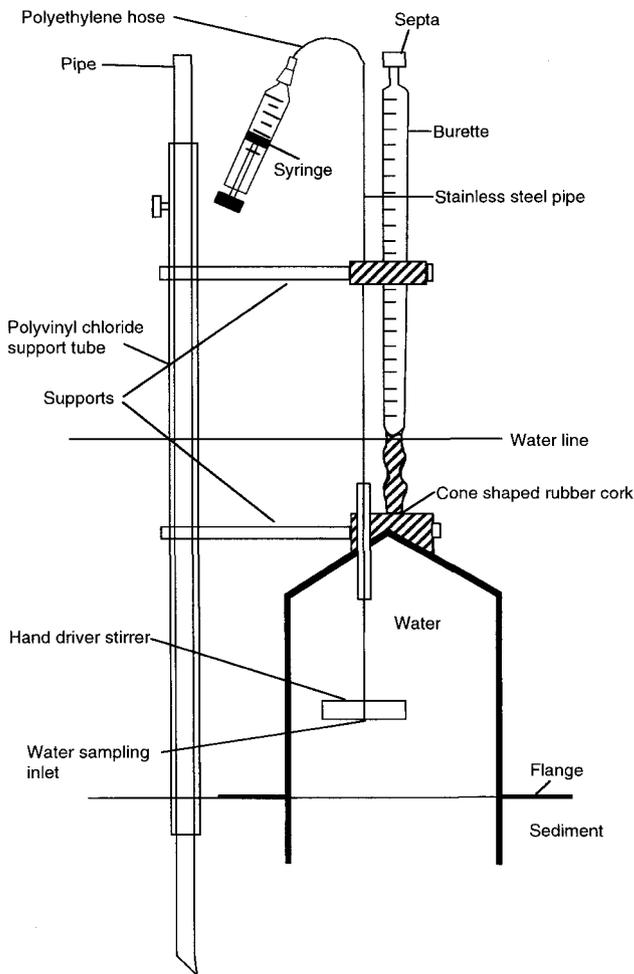


FIG. 2. Schematic diagram of denitrification in situ measurement apparatus.

These chambers enclosed 16 L of water and a surface area of 0.063 m² and were not equipped with manual stirrers. The water in one chamber was mixed with a battery-operated circulating pump system that was activated for 5 minutes per hour; the second chamber was not equipped with a mechanical mixing system. The two chambers yielded almost identical results when compared (see results).

Before gases were sampled, the total volume of accumulated gases in the burette was recorded. Gas samples were collected with 10-mL glass gas-tight syringes equipped with valves and needles. Before transportation to the site, the syringes were flushed and filled with He and the tips of the needles were plugged by sticking them into rubber septa. Immediately before sampling, each septum was removed,

the valve was opened to the needle, and the He was expelled. The needle was immediately inserted through the burette septum and a gas sample was pulled out. The valve was closed, the needle was removed from the burette, and the tip of the needle was sealed by inserting it into a septum for transportation to the laboratory.

To take a subsample of gas for chromatographic analysis, the syringe was placed on a stand, the needle was removed, the opening of the valve was flushed with He, and a septum was placed over the end of the valve. The septum prevented gas exchange during sampling. The gas sampling and analysis procedures were identical to those used for the *in vitro* N₂ flux determinations. The volume of gas collected in the burette was generally sufficient to allow multiple samples of gas (0.5 mL each) to be collected for replicate measurements.

RESULTS

Dissolved Inorganic Nitrogen (DIN) Concentrations

Dissolved inorganic nitrogen concentrations varied significantly over time and reflected changes in both external loading and internal fluxes. The observed variation in mean nitrate concentrations (1.1–313 μM) was greater than that for ammonium concentrations (0.5–58.2 μM) (Table 1). In October 1993, mean nitrate and ammonium concentrations were similar and ranged between 0.5 and 13 μM. In May 1994, concentrations were somewhat higher and average ammonium concentrations were about four times greater than nitrate concentrations (overall mean = 28 versus 6.8 μM, respectively). In contrast, during July 1994, there was significant streamflow into the estuary and nitrate concentrations were more than 50 times higher than those for ammonium (overall mean = 280 versus 5.5 μM, respectively).

In late October, nitrate concentrations were generally near detection at all stations. In May and July, nitrate concentrations tended to be highest at the upstream Station A and to decrease downstream, whereas, ammonium concentrations were sometimes highest at Stations B or C (Table 1).

Denitrification and Oxygen Consumption Rates

In vitro denitrification rates ranged from ca 40 to 428 μmole N₂ m⁻² h⁻¹ during the study (Table 2). Rates sometimes decreased in the downstream direction, but differences among stations were fre-

TABLE 1. Dissolved inorganic nitrogen concentrations (μM) along a four-station transect within the Old Woman Creek estuary (Lake Erie), Huron, Ohio.

Date	Parameter	Stations				Mean (SE)
		A	B	C	D	
1 Oct. 1993	NO ₃	—	12.1	12.3	16.2	13.5 (1.3)
	NH ₄	—	9.5	11.6	12.8	11.3 (1.0)
23 Oct. 1993	NO ₃	1.07	1.30	1.07	1.30	1.19 (.07)
	NH ₄	0.43	0.38	0.43	0.59	0.46 (.05)
27 Oct. 1993	NO ₃	1.07	1.30	1.07	1.07	1.13 (.06)
	NH ₄	0.95	1.32	1.16	1.65	1.27 (.15)
29 Oct. 1993	NO ₃	0.84	1.19	0.84	0.84	0.93 (.09)
	NH ₄	0.47	0.47	0.55	0.47	0.49 (.02)
17 May 1994	NO ₃	8.44	6.88	8.79	8.44	8.14 (.43)
	NH ₄	26.4	21.8	24.2	24.5	24.2 (.9)
19 May 1994	NO ₃	16.1	12.4	9.83	6.88	11.3 (2.0)
	NH ₄	8.36	3.44	2.05	1.90	3.94 (.61)
23 May 1994	NO ₃	2.55	1.34	1.25	1.34	1.62 (.31)
	NH ₄	13.8	21.3	2.82	6.51	11.1 (4.1)
25 May 1994	NO ₃	4.80	4.28	3.59	3.76	4.11 (.27)
	NH ₄	38.2	76.7	28.8	26.8	42.6 (11.6)
26 May 1994	NO ₃	19.5	7.92	4.28	4.11	8.95 (3.62)
	NH ₄	64.4	76.7	53.6	38.2	58.2 (8.2)
7 July 1994	NO ₃	361	302	309	279	313 (17)
	NH ₄	0.78	1.20	0.07	0.06	0.53 (.28)
8 July 1994	NO ₃	264	250	241	224	245 (8.4)
	NH ₄	9.30	10.1	12.7	10.3	10.6 (0.7)

TABLE 2. Mean denitrification and oxygen uptake rates observed by the in vitro method on intact cores from Old Woman Creek estuary. A single rate estimate for each chamber was obtained by averaging the rates calculated from successive measurements. The means and SE (= 0.5 of the range) were then calculated from results obtained from duplicate chambers. Cores collected on 25 October and incubated at the in situ temperature of 12°C were subsequently further incubated at 17°C to examine the effects of temperature on denitrification and oxygen uptake rates.

Sampling Date	Temperature (°C)	Denitrification Rate ($\mu\text{mole N}_2 \text{ m}^{-2} \text{ h}^{-1}$)			Oxygen Consumption Rate ($\text{mmole O}_2 \text{ m}^{-2} \text{ h}^{-1}$)		
		Sta B	Sta. C	Sta. D	Sta B	Sta. C	Sta. D
1 Oct. 1993	12	46 ± 8	40 ± 1	43 ± 7	1.04 ± 0.02	0.88 ± 0.03	0.9 ± 0.05
25 Oct. 1993	12	135 ± 6	76 ± 15	66 ± 10	1.04 ± 0.17	0.94 ± 0.18	0.71 ± 0.06
25 Oct. 1993	17	121 ± 7	104 ± 36	55 ± 9	0.92 ± 0.11	1.16 ± 0.55	0.78 ± 0.28
17 May 1994	13	118 ± 23	66 ± 4	76 ± 21	1.61 ± 0.02	1.02 ± 0.06	0.90 ± 0.02
8 July 1994	28	428 ± 72	363 ± 10	278 ± 6	3.04 ± 0.45	2.51 ± 0.57	2.11 ± 0.01

quently not significant (overlapping SE; Table 2). Rates of *in vitro* oxygen consumption ranged from 0.71 to 3.0 mmole O₂ m⁻² h⁻¹ (Table 2). Oxygen consumption rates were higher in July than in May and October, but other trends were not obvious. Differences among stations were not large (Table 2). A high correlation ($r = 0.964$) was observed between *in vitro* denitrification rates and oxygen consumption rates in OWC. Likewise *in vitro* denitrification rates were highly correlated with concentrations of nitrate in the overlying water ($r = 0.966$, based on data from the three dates when both measurements were made; 1 October 1993, 17 May and 8 July 1994; Tables 1 and 2).

In situ denitrification rates at Station C generally compared well with results from the *in vitro* method on dates when they were measured simultaneously. Average denitrification rates for two sets of measurements at 12°C were 45 μmole N₂ m⁻² h⁻¹ and 89 μmole N₂ m⁻² h⁻¹ for the *in situ* method as compared to 40 mole N₂ m⁻² h⁻¹ and 76 μmole N₂ m⁻² h⁻¹ for the *in vitro* method in October 1993. In July 1994 at 28°C, the *in vitro* and *in situ* methods yielded respective rates of 363 and 502 μmole N₂ m⁻² h⁻¹ in dark incubations (Tables 2 and 3).

In situ chamber results were higher in spring and summer of 1994 than in autumn of 1993 (Table 3). *In situ* N₂ accumulation rates increased dramatically with increasing temperature in May. However, denitrification rates were much lower in July at 28°C than in May at 27°C. Denitrification rates did not increase when the temperature of *in vitro* chambers was increased from 12°C to 17°C in October 1993 (Table 2).

TABLE 3. Denitrification rates observed by the *in situ* method at Station C in Old Woman Creek estuary. All data were obtained from single chambers, except for the 8 July light chamber results that were obtained from duplicate chambers.

Sampling Date	Temperature (°C)	Denitrification Rate (mole N ₂ m ⁻² h ⁻¹)	
		Dark	Light
<i>In situ</i>			
6 Oct. 1993	13	45	—
25 Oct. 1993	12	89	—
17 May 1994	19	296	—
23 May 1994	27	1,200	2,100
8 July 1994	28	504	902 ± 3

Sediment-water Fluxes in the *in situ* Chambers

With the unexplained exception of 25 May, nitrate fluxes in the *in situ* chambers showed net removal from the water, and ranged from 0–822 μM m⁻² h⁻¹ (Table 4). Fluxes appeared to be dependent upon initial concentrations; however, there was not a broad range of concentrations during the study to examine this relationship closely. In May, nitrate concentrations were around 1 μM and fluxes were not measurable. In July, nitrate concentrations increased to around 300 μM and fluxes increased correspondingly to between 700–800 μM m⁻² h⁻¹. Small, concurrent changes in nitrate concentrations and fluxes in October support the positive correlation observed between these two variables.

In contrast to nitrate, net ammonium fluxes were always into the water with the exception of 23 May, when the sediments appeared to have been disturbed during chamber placement and the water had artificially high initial concentrations (115 μM versus 3 μM for ambient levels). Ammonium fluxes ranged from 60–1,033 μM m⁻² h⁻¹ for other experiments (Table 4).

In July, light and dark chambers were incubated in parallel. Nitrate fluxes were similar in both chamber types (Table 4). In contrast, the net increase in ammonium concentration was nearly four times greater in the dark than in the light chamber. Changes in oxygen concentrations inside the light chambers substantiate the conclusion that phytoplankton or benthic epiflora were active, and presumably assimilating a large fraction of the dissolved ammonium. In one light chamber dissolved oxygen actually increased during the incubation and in the other light chamber the decrease was only 20% of that observed for the dark chamber.

Short-term Changes in Denitrification Rates During Spring Warm-up

The temperature increased dramatically during the period of our 1994 springtime measurements. When we sampled on 17 May 1994 for *in vitro* measurements and placed the *in situ* chambers in the sediments, the water temperature was 13°C. However, by the end of the first *in situ* incubation period of 48 h, the water temperature had risen to 19°C. The temperature continued to increase during the next few days to 27°C on 23 May. Because of the long time necessary for sparging in the *in vitro* N₂⁻ flux method, it was not possible to accurately compare the results of *in situ* and *in vitro* methods during this period of rapid warm-up. However, the

TABLE 4. Nutrient concentrations and fluxes for *in situ* chamber incubations in Old Woman Creek estuary during October 1993, May 1994, and July 1994. Oxygen fluxes were not calculated because initial time-point samples for dissolved oxygen were taken from outside the chambers and may not reflect true initial conditions inside the chamber.

Date	Chamber Type	Sampling Time point	Interval (h)	Concentrations			Fluxes	
				NO	NH ₄ (μM)	O ₂	NO ₃ (μM m ⁻² h ⁻¹)	NH ₄ (μM m ⁻² h ⁻¹)
1 Oct. 1993	Dark	T0		12.3	11.6	460		
6 Oct. 1993		Tf	120	5.9	57.8	33	-15.7	113
6 Oct. 1993	Dark	T0		2.4	1.0	460		
8 Oct. 1993		Tf	45.0	2.4	24.9	65	0.0	156
25 Oct. 1993	Dark	T0		2.7	0.5	244		
27 Oct. 1993		T1	47.7	1.2	12.6	71	-9.3	74.7
29 Oct. 1993		T2	46.7	0.8	22.1	25	-2.5	59.9
23 May 1994	Dark	T0		1.3	115*	294		
		Tf	18.5	1.3	20.4	75	0.0	-1,506*
24 May 1994	Dark	T0		1.3	0.7	100		
		Tf	28.5	1.3	58.8	13	0.0	600
25 May 1994	Dark	T0		1.7	28.4	213		
		Tf	14.5	3.4	47.0	69	34.5	378
7 July 1994	Dark	T0		316	1.8	294		
		Tf	21.5	256	77.2	200	-822	1,033
7 July 1994	Light-1	T0		315	0.8	294		
		Tf	21.8	252	20.9	300	-742	245
7 July 1994	Light-2	T0		313	0.8	294		
		Tf	22.3	252	20.9	275	-702	231

*Initial sample was artificially elevated due to disturbing of the sediments during chamber placement. See ambient concentrations, Table 1.

large temperature increase over this short period allowed us to obtain unique preliminary data on the effects of the initial temperature increase in spring on apparent denitrification rates by the *in situ* method (after corrections were made for changes in gas solubility at the different temperatures; May results, Table 3). Temperature-corrected dark denitrification rates increased from about 300 μmole N₂ m⁻² h⁻¹, at 19°C (at end of incubation interval), up to about 1,200 μmole N₂ m⁻² h⁻¹ on 23 May, at 27°C.

Effects of Light on Denitrification Rates

In May and July 1994, direct comparisons were made between *in situ* denitrification rates measured under dark and natural light conditions (Table 3). In

May, comparisons were made between single light and dark chambers, whereas in July rates from two light chambers were compared to the results from one dark chamber. Although experimental replication was minimal in these experiments, a consistent pattern was demonstrated from these light/dark comparisons. In both experiments, higher *in situ* denitrification rates were observed in the transparent chambers than in the dark chamber used for the other measurements (Table 3). Rates were 76% and 79%, respectively, higher in the light as compared to those measured in the dark in the two experiments. The duplicate light chambers measured in July gave results that were virtually identical to each other (905 vs 899 μmole N₂ m⁻²h⁻¹) despite the fact that one was artificially mixed and

the other depended on natural mixing through gas production.

DISCUSSION

Denitrification Methodology

Both of the denitrification methods used in our experiments were based on direct gas chromatographic measurements of N_2 production either from intact cores in sealed chambers (Seitzinger *et al.* 1984) or from *in situ* chambers (Tomaszek 1991). These methods avoid inhibitors that can decrease measured denitrification rates by preventing nitrification and do not require the use of isotopes. Additional advantages are that the sediments remain vertically intact, and O_2 consumption rates can be estimated simultaneously with N_2 production rates. Both methods require special precautions to avoid contamination with atmospheric N_2 . Laboratory-measured denitrification rates obtained from the N_2 flux method in October 1993 (Table 2) were verified with ambient field fluxes of N_2 (Table 3). The agreement in results between the *in vitro* N_2 flux method and the *in situ* measurements in October supports the assumption of Seitzinger *et al.* (1980) that the pre-incubation of the sediments for sparging does not appreciably change the denitrification rate if temperatures remain constant. However, *in situ* results were about 40% higher than *in vitro* results in July. This difference could be due either to spatial or methodological heterogeneity or could possibly reflect decreases in the *in vitro* denitrification rates during the sparging period.

A disadvantage of the *in vitro* N_2^- flux method is the long incubation time required to sparge the samples of atmospheric N_2 . Changes in denitrification rates over periods of changing temperature, therefore, cannot be readily measured by the *in vitro* method. *In situ* methods require less time and have the advantage of including natural factors that may influence denitrification rates in aquatic sediments. Changes in gas solubility with changing temperatures must be accounted for to make reasonable estimates under conditions of changing temperatures. However, if the sediment temperature changes less rapidly than that of the overlying water, it is also possible that part of the measured N_2 could be due to concentration-mediated diffusion from the sediment pore water, rather than only from instantaneous denitrification (personal communication, B. Nowicki, University of Rhode Island). Other limitations of the *in situ* method are the necessity for spaced intermit-

tent field measurements and its restriction to relatively shallow non-tidal aquatic environments.

Denitrification Rates in OWC

Results from the denitrification and nitrate flux measurements agree with our hypothesis that denitrification is a sink for nitrate in OWC. Although our *in vitro* denitrification rates were relatively low compared to some reported values (Jorgensen and Sorensen 1988, Law *et al.* 1991), they resemble those reported for many estuaries (Jenkins and Kemp 1984, Seitzinger *et al.* 1984, Seitzinger 1990, Yoon and Benner 1992, Nowicki 1994). However, they are high relative to those reported for Lake Michigan (Gardner *et al.* 1987) or Narragansett Bay (Nowicki 1994).

The relatively large seasonal differences in denitrification rates by both measurement methods (Tables 2 and 3) suggest that *in situ* conditions such as temperature, redox, and available substrate may affect denitrification. *In situ* rates were comparable to the *in vitro* rates when temperatures were stable, but were much higher than other measured rates during the period of rapid spring warm-up. The data presented in Table 3 indicate that the spring warm-up period may be a dynamic period for N cycling in OWC. The *in situ* rates measured after the rapid spring warm-up in May are among the highest that have been reported (Seitzinger 1988). Denitrification rates increased by a factor of about 4 over a period when the temperature of the water overlying the sediments increased from 13 to 27°C. These values could be conservative if the water became supersaturated with N_2 during the period of increasing temperature and did not release as much N_2 as predicted from gas-solubility tables. However, as mentioned above, the rates could be potentially high if the sediment pore water had warmed at a slower rate than the overlying water and released substantial N_2 due to a temperature-mediated concentration gradient.

Although more data are needed to verify this trend, the results suggest that in temperate environments such as OWC, rates of N cycling and denitrification may increase quite dramatically over short periods when the temperature suddenly increases in the spring. It is possible that N from organic substrates, which are not completely degraded at cold winter temperatures, may be mineralized, nitrified, and denitrified rapidly when temperatures increase. The dramatic changes in denitrification rates with increasing temperature in May suggest that tempera-

ture may be an important factor controlling denitrification. However, the observation that denitrification rates were much lower in July at 28°C than in May at 27°C suggests that the controlling factor may have changed from temperature to another controlling factor, such as substrate or oxygen availability, as the season progressed. This conclusion was also supported by the fact that denitrification rates did not increase when the temperature of *in vivo* chambers was increased from 12°C to 17°C in the October 1993 experiment (Table 2). Nitrate supply from overlying water could have been restricted in the laboratory chambers. The quantity and composition of available organic nitrogen in the autumn may also have limited nitrate supply from ammonification/nitrification. A more extensive temporal coverage of *in situ* measurements of denitrification, particularly during spring transition periods of rapid temperature increases, is needed to evaluate the relative importance of these two environmental factors. New membrane inlet mass spectrometric methods that measure N₂:Ar ratios over short time intervals (Kana *et al.* 1994) may be useful for these measurements.

There are two main sources of nitrate for sediment denitrification: nitrate produced in the sediments from nitrification of ammonium, and nitrate diffusing into sediments from overlying water. Both sources appear to be significant in OWC but their relative importance varies temporally depending on nitrate concentrations in the water, that in turn relate to rainfall events that bring nitrate into the system from agricultural sources (Wickstrom 1988). For example, on 8 July 1994, when nitrate levels were exceptionally high, nitrate influx from overlying water accounted for most of the denitrification that was measured by the *in situ* method. Some nitrate can also be supplied by the advection of ground water through the sediments (Seitzinger *et al.* 1984). Nitrification of ammonium, that is produced by bacterial mineralization or benthic animal excretion, is likely a more dominant source of nitrate substrate for denitrification during periods when concentrations of nitrate in overlying waters are relatively low, such as for our October and May measurements.

Effects of Light on Nitrogen Fluxes and Denitrification Rates

The higher net production rates of ammonium in the dark vs the light chamber most probably reflects increased assimilation of ammonium by phyto-

plankton in the light chamber more than a large difference in production rates within the sediments. Alternatively, in situations where oxygen was depleted to low levels, the difference may reflect less nitrification/denitrification due to less production of oxygen in the dark than in the light chambers.

In situ denitrification rates in transparent chambers were about 75% higher than simultaneous results for the dark chamber when comparisons were made in May and July 1994. A reasonable explanation for this observation is that photosynthetic processes provided increased production rates of oxygen that in turn improved conditions for nitrification in the sediments that supplied the nitrate for denitrification (Rysgaard *et al.* 1994). In the transparent chambers, when N assimilation by phytoplankton is high and the oxic zone extends in depth due to phyto-benthic O₂ production, denitrification of water-phase NO₃⁻ can be expected to decrease, whereas coupled nitrification-denitrification at the sediment-water interface may increase (Rysgaard *et al.* 1994). In the dark chambers, where there is less photosynthetic N assimilation, the oxic zone may become relatively thin due to high rates of O₂ consumption in the sediments. Under these circumstances, relative rates of denitrification of NO₃⁻ from the water column should increase, while denitrification of NO₃⁻ from nitrification should decrease in the dark relative to results for the lighted chambers (Risgaard-Petersen *et al.* 1994). The close agreement between relative changes in the two experiments, and in the duplicate light measurements in the July experiment, supports the idea that total denitrification rates in OWC were enhanced under natural light conditions.

Denitrification and Oxygen Consumption

Measured rates of oxygen consumption were similar to those reported by Seitzinger (1990) and Tomaszek (1991) and those observed in sediments from the Neuces and Guadalupe Estuaries (Yoon and Benner 1992). Although oxygen was continuously consumed, conditions inside the chambers remained aerobic after 3 days of incubation. These results indicate that, during this time period, conditions inside the chambers were still suitable for nitrification.

Oxygen regulation of nitrification and denitrification in sediments was investigated by Rysgaard *et al.* (1994). Their results suggest that increasing O₂ concentration will decrease denitrification derived from NO₃⁻ in the overlying water, but stimulate

denitrification of NO_3^- produced endogenously by nitrification. According to Blackburn (1990), who explored this relationship with a denitrification model for marine sediments, organic mineralization rates do not necessarily always correlate well with denitrification rates. The coupling between these two processes is complex, and mostly related to coupled nitrification and denitrification in sediments. Denitrification is generally considered to be an anaerobic process. However, denitrification rates can increase with increased organic loading, due to oxygen consumption resulting from increased carbon availability (Billen and Lancelot 1988). It is possible that when the process depends on a supply of NO_3^- from the overlying water (Blackburn 1990), the two processes are independent. However, when a high percentage of the benthic oxygen consumption is due to nitrification, which supplies the NO_3^- for the denitrification process in sediments, the two processes should be related. Because all these pathways of organic matter decomposition depend on the availability of organic carbon, a significant correlation is not surprising. Our observed correlation between the *in vitro* denitrification rates and oxygen consumption rates agree with observations made by Seitzinger (1990) and seem to support this relationship for OWC sediments.

Dissolved Inorganic Nitrogen Concentrations and Sediment-water Fluxes

Previous studies showed that dissolved inorganic nitrogen entered the OWC estuary primarily as very high levels of nitrate, but low levels of nitrate were observed in August and September 1986 (Wickstrom 1988) and in October 1993 and May 1994 (this study). High levels of nitrate ($> 250 \mu\text{M}$) were observed after a rain storm in July 1994. These elevated nitrate concentrations are similar to those reported by Wickstrom (1988), but lower than those observed by Klarer and Millie (1989), who reported concentrations greater than $1,000 \mu\text{M}$ after periods of intense storms. Nitrate transformations in OWC are undoubtedly affected by flow rate and by whether the tributary is open to Lake Erie. When the wetland is open, most of the nitrate entering the system may be transported into Lake Erie quite rapidly and be relatively unaffected by estuarine processes. In this case, substantial decreases in nitrate concentrations may not be observed. However, during the period when the wetland is closed (as for all our samplings), biological processes have more influence and help maintain low concentrations of

ammonium and nitrate. Low levels for dissolved ammonium and nitrate can be explained by the coupling of nitrification and denitrification at the sediment water interface (Jenkins and Kemp 1984, Gardner *et al.* 1987). The variable ammonium and nitrate concentrations that we observed, along with measured denitrification and nitrate removal rates (see below), reflect the dynamic nature of this hypereutrophic wetland. In general, our results substantiate previous studies indicating that nitrogen primarily enters the wetland in the form of nitrate, and that net production of ammonium occurs in the sediments or water of OWC (Wickstrom 1988, Klarer and Millie 1989).

As expected, sediment-water fluxes of ammonium were not related to initial concentrations, and the primary source of ammonium was most likely remineralization of sedimentary organic material and excretion by aquatic animals. Old Woman Creek has a rich abundance of zooplankton (Kreiger and Klarer 1991) and protists (Kepner and Pratt 1996). Net production rates of ammonium were approximately four times higher in May than October and most likely reflect the increase in water temperature from 12°C to 27°C . However, fluxes in the dark chamber in July were twice as high as in May, despite similar temperatures. These results indicate that both temperature and organic substrate availability could be important determinants of ammonium production rates in the sediment. Additionally, oxygen concentrations in the sediments or other factors, such as cascading trophic control of the abundance or activity of nitrifying bacteria (Lavrentyev *et al.* 1997), could change net ammonium fluxes by affecting nitrification rates.

CONCLUSIONS

Denitrification rates in OWC sediments resemble rates that have been reported for many estuaries, except those immediately following the spring warm-up, when rates were exceptionally high. Large seasonal differences were observed, suggesting that factors such as temperature and available substrate measurably affected the process. Although denitrification rates seemed to decrease somewhat in the downstream direction, rates among stations were often not significantly different. Nitrate from outside agricultural sources, or produced in the sediments by nitrification, were both important sources of N-substrate for denitrification in OWC. *In situ* measurements of denitrification compared favorably with *in*

vitro results if the temperature remained constant over the same period but could not be accurately compared during the spring when the estuarine water temperature was rapidly increasing. Denitrification rates correlated quite well with oxygen consumption rates in the OWC system. The relatively high rates of measured denitrification and nitrate removal in OWC, and the low buildup of nitrogen ions in the water suggests that denitrification is an important sink for nitrogen in wetland regions such as OWC that process nutrients from agricultural regions before they enter the Great Lakes.

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