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Denitrification and dinitrogen fixation in two quaking fens in the Vechtplassen area, The Netherlands

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Abstract. This paper reports laboratory experiments on dinitrogen fixation and denitrification for two small quaking fens (discharge fen and recharge fen) using the acetylene reduction assay and the acetylene inhibition technique, respectively.

Nitrogenase activity was detected in peat muck and associated with *Alnus glutinosa* saplings throughout the study period (May–October 1987), whereas no activity was observed associated with *Sphagnum* species. The annual amount of dinitrogen fixed was estimated at 2.1 and 12.7 kg N/ha/y for the recharge fen and the discharge fen, respectively.

Denitrification at ambient nitrate levels (0.1 ppm NO_3) was absent in the discharge fen and very low in the recharge fen (0.1 μg N/g/d, or 0.3 kg N/ha/y). In nitrate-amended soil samples denitrification rates were 2 to 3 orders of magnitude higher. It is argued that *in situ* denitrification rates in the fens studied will depend almost entirely on the nitrate supply by precipitation. Denitrification rates associated with precipitation are estimated at 1.1 kg N/ha/y for both fens.

Introduction

Gaseous nitrogen fluxes such as dinitrogen fixation and denitrification may contribute considerably to the nitrogen budget of peatlands. Dinitrogen fixation has been found to occur in every mire complex studied in Europe, North America and Asia (Moore & Bellamy 1974; Waughman & Bellamy 1980; Dickinson 1983). Intrasite comparisons show that the activity is higher in minerotrophic than in ombrotrophic mires (Moore & Bellamy 1974; Waughman & Bellamy 1980).

The ability to incorporate atmospheric molecular dinitrogen into organic compounds in peatlands has been demonstrated in free-living bacteria, cyanobacteria (free-living as well as associated with *Sphagnum* species), and symbiotic *Actinomycetes* associated with *Alnus* spp. and *Myrica gale* (Dickinson 1983).

Denitrification has been less well studied. It is accepted that high denitrification rates occur in peatlands used for sewage treatment (Sloey et al. 1978; Nichols 1983; Verhoeven 1986) and in nitrate-amended soil cores from nutrient-poor peatlands (Müller et al. 1980; Hemond 1983; Westermann & Ahring 1987). Under ambient (low) nitrate concentrations, *in-situ* denitrification rates in undisturbed peatlands are very low (Martin & Holding 1978; Hemond 1983; Westermann & Ahring 1987; Urban et al. 1988).

This paper reports laboratory experiments on dinitrogen fixation and denitrification for two small quaking fens of different trophic status and pH. The fens are seriously threatened by nitrogen eutrophication caused by agricultural practices (Verhoeven et al. 1988). They are both scattered in a landscape used for intensive agriculture where nitrogen concentrations in surface and groundwater bodies are high and where gaseous ammonia volatilization from manure has caused a six to tenfold increase in nitrogen concentrations in precipitation. Currently, total nitrogen inputs on the fens exceed 40 kg N/ha/y (Koerselman et al. 1989a). One of the aims of the present study was to determine the significance of denitrification in reducing effects of eutrophication with nitrate.

This study is an integrated part of a research project on the hydrologic and nutrient (N, P and K) mass balances of fens in an agricultural landscape.

Study sites

The fens studied are located in the Vechtplassen area, the Netherlands (5°7′ E, 52°9′ N). The peat soil consists of floating root mats that support vegetation and move up and down with fluctuations of the water table. Therefore, water tables are near the soil surface during most of the year (Verhoeven et al. 1988).

One 0.32 ha discharge fen is dominated by *Carex diandra*, *Potentilla palustris*, *Menyanthes trifoliata*, *Caltha palustris* and *Equisetum fluviatile*. The plant community belongs to the Scorpidio-Caricetum diandrae (Westhoff & Den Held 1969), and can be classified as transitional rich fen vegetation (*sensu* Sjörs 1950).

The second fen (0.15 ha) is a recharge fen. At this site a sparse phanerogam vegetation is dominated by *Carex acutiformis* and *Phragmites australis*. Saplings of *Alnus glutinosa* (height < 0.5 m) occur scattered in the vegetation. A thick *Sphagnum* carpet (mainly *S. squarrosum*, *S. fimbriatum* and *S. fallax*) covers the soil completely. The plant community at this site is a transition between the Thelypterido-Phragmitetum and the Pallavicinio-Sphagnetum (Westhoff & Den Held 1969), and can be classified as a poor fen (*sensu* Sjörs 1950).

Table 1. Selected chemical and physical characteristics for the study sites.

	Recharge fen	Discharge fen
Soil parameters, top 10 cm (n = 8):		
Bulk density (g/L)	49 ± 15	69 ± 5
Water content (% by volume)	95 ± 1	93 ± 1
Fen water, surficial peat (n = 14):		
pH	5.3 ± 0.4	6.2 ± 0.4
EC_{20} (μS/cm)	110 ± 37	198 ± 41
NO_3 (ppm)	0.1 ± 0.1	0.1 ± 0.1
NH_4 (ppm)	0.5 ± 0.6	0.5 ± 0.6
PO_4 (ppm)	0.2 ± 0.2	0.1 ± 0.1
K (ppm)	6.5 ± 3.4	1.7 ± 1.3
Fe (ppm)	0.4 ± 0.2	0.7 ± 0.5
Al (ppm)	0.3 ± 0.2	0.0 ± 0.0
Ca (ppm)	6.4 ± 2.5	32.7 ± 7.4
Mg (ppm)	1.0 ± 0.6	2.9 ± 0.9
HCO_3 (ppm)	25.7 ± 22.6	92.3 ± 36.8
SO_4 (ppm)	15.3 ± 6.3	11.9 ± 9.2
Cl (ppm)	24.4 ± 12.6	13.1 ± 6.1
Na (ppm)	11.1 ± 7.9	20.2 ± 20.7

Selected soil characteristics of the two fens are given in Table 1. Differences in fen water chemistry of the two fens are strongly related to the contrasting hydrologic regime (see, Koerselman et al. 1989a, b).

Methods

Dinitrogen fixation assay

Nitrogenase activity was measured by the acetylene reduction assay (Hardy et al. 1973) using peat muck, living *Sphagnum* species and excised roots of *Alnus glutinosa* saplings, collected at the central part of the fens.

- Peat muck was collected 5 cm below the soil surface, and 20-ml samples were allowed to freely drain, before being placed in 40-ml serum vials. To half of the vials fen water was added to completely waterlog the samples.
- Fresh *Sphagnum* samples were collected from the recharge fen and placed in 40-ml serum vials.
- *Alnus glutinosa* saplings were carefully extracted from the recharge fen to avoid damage to the root system. Prior to the experiment and within

18 hours of sampling, all root material from each plant was excised and placed in 350 ml glass jars with lids fitted with a rubber septum.

Acetylene, produced from carbide pellets in water, was added by syringe to the vials and jars to create a concentration of 10% (v/v). All samples were incubated at ambient soil temperature at the time of sampling in the dark (peat muck and *Alnus*) or at a light intensity of 65 $\mu E/m^2/s$ (*Sphagnum*). During the incubation period 2-ml gas samples were withdrawn from the head space of vials with peat muck and *Sphagnum* after 3, 6 and 24 hours, and from the jars with *Alnus* after 1, 2 and 4 hours. Gas samples were injected into 10-ml serum vials until ethylene analysis the next day. The ethylene analyses were carried out on a Becker 407 gas chromatograph equipped with a flame-ionization detector. An aluminium-oxide column separated the ethylene from the acetylene. The carrier gas (N2) flow rate was 30 ml/min, column temperature was maintained at 80°C and the detector temperature at 120°C. Investigations of acetylene reduction activity were carried out from May to October 1987 on a monthly basis. All treatments were done with 5 replicates. Acetylene-free controls and ethylene-amended controls were run to measure endogeneous ethylene production and consumption, respectively. Activity in the controls appeared negligible.

For the conversion of acetylene reduction to dinitrogen fixation we used a 3.5:1 ratio for peat muck and *Sphagnum* that was determined by Chapman & Hemond (1982) for similar material. For *Alnus glutinosa* we used a 3.0:1 ratio (Akkermans 1971). Because nitrogenase activity in the peat muck and *Sphagnum* appeared very low, calculations are based on measurements over the entire 24-hour interval. For *Alnus glutinosa*, calculations are based on measurements from the 1-hour and 2-hour sampling times.

To estimate yearly dinitrogen fixation on an areal basis, determinations were made of *Sphagnum* biomass and *Alnus glutinosa* density. Nitrogenase activity was generalized over the upper 20 cm of peat during the period May through October. Nitrogenase activity outside this period was assumed negligible because of prevailing low soil temperatures ($< 10°C$) and a reported Q_{10} value for nitrogenase activity in peat of about 6 (Waughman 1970). Nitrogenase activity above the water table was calculated from experiments with drained peat muck, whereas activity below the water table was calculated from experiments with waterlogged samples. Water tables relative to the soil surface were determined on a weekly basis, using methods described in Koerselman (1989).

Denitrification

Denitrification rates were measured by the acetylene inhibition technique

(Yoshinara et al. 1977; Ryden & Rolston 1983) with peat muck collected 5 cm below the soil surface in September 1987. Samples of 20 ml peat muck were incubated in 40-ml serum vials. Fen water was added to completely waterlog the samples and create anaerobic conditions favourable for denitrification. The following treatments were applied with 4 replicates per treatment.

1. Acetylene was added by syringe to the vials to create a concentration of 10% (v/v) in the headspace of the vials.
2. 2 ml of a 1000 ppm nitrogen solution was added to the vials.
3. Both acetylene and a 1000 ppm nitrate solution were added to the vials.
4. No additions.

The vials were incubated in the dark at a temperature of 20°C. A 2-ml gas volume was withdrawn from the headspace of the vials (at 0, 2, 4 and 24 hours for treatment 2 and 3; at 0 and 24 hours for treatment 1 and 4). The gases were injected into 10-ml serum vials that were initially flushed with Helium to purge the system of atmospheric nitrogen compounds. The vials were stored at −15°C until they were analyzed as N_2O on a Packard 438 A gas chromatograph with electron capture detection (310°C). Gases were separated with Porapak Q at 35°C with dinitrogen as carrier gas (flow 10 ml/min). Corrections were made for the solubility of N_2O in water.

Results

Dinitrogen fixation

Acetylene reducing activity was low in peat muck and *Sphagnum* from the recharge fen throughout the season (Fig. 1A). In the discharge fen, activity was initially low but a peak was observed in both the water amended and the drained treatment in August. Acetylene reducing activity in drained samples was significantly higher ($p < 0.001$; sign test) than in waterlogged samples.

Acetylene reducing activity was associated with *Alnus glutinosa* saplings throughout the summer with low rates in October signifying the end of the active growing season (Fig. 1B). There were, however, no differences between months due to high variability of replicates. Activity during the growing season (May–September) was positively correlated with leaf area ($r = 0.68$, $p < 0.001$), leaf dry weight ($r = 0.63$, $p < 0.001$), total root nodule dry weight ($r = 0.41$, $p < 0.05$), total above-ground biomass

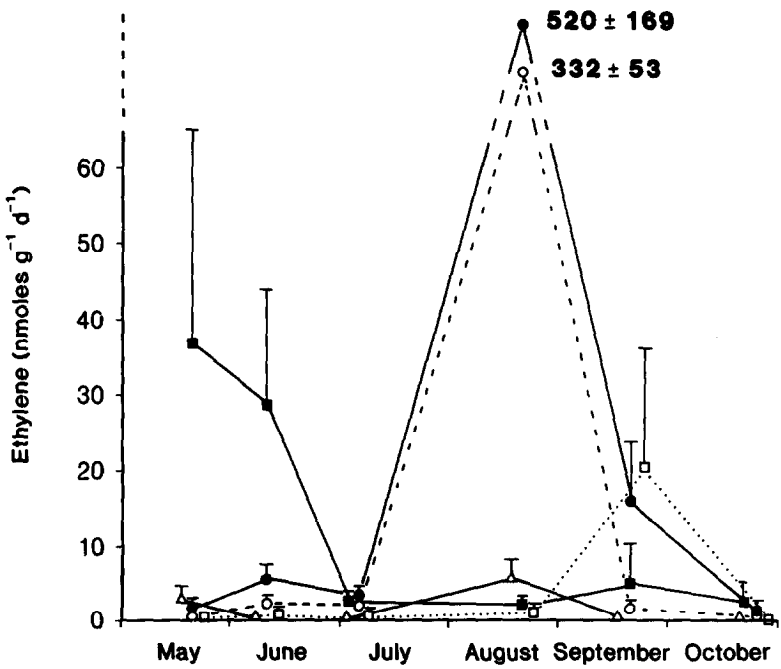

Fig. 1A. Ethylene production (nmoles/g/day) from acetylene during the period May–October 1987. Error lines represent 1 s.e.
● = Discharge fen, peat muck drained
○ = Discharge fen, peak muck waterlogged
■ = Recharge fen, peat muck drained
□ = Recharge fen, peat muck waterlogged
△ = Recharge fen, *Sphagnum*

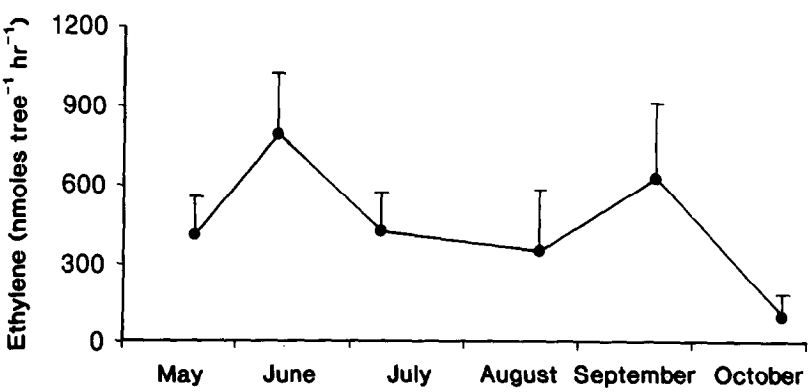

Fig. 1B. Ethylene production (nmoles/tree/hr) from acetylene by excised roots of *Alnus glutinosa* saplings in the recharge fen during the priod May–October 1987. Error lines represent 1 s.e.

Table 2. Estimated dinitrogen fixation rates on an areal basis (kg N/ha/y) for peat muck, *Sphagnum* and *Alnus glutinosa*.

Material	Peat muck	*Sphagnum*	*Alnus*
Discharge fen	12.7	–	–
Recharge fen	1.1	< 0.1	1.0

($r = 0.40$, $p < 0.05$), and living above-ground biomass ($r = 0.49$, $p < 0.05$).

Table 2 presents results of the acetylene reduction experiment converted to the amount of dinitrogen fixed on an areal basis. The amount of dinitrogen fixed in the discharge fen (12.7 kg/ha/y) was strongly influenced by the August measurement that contributed 92 percent. This clearly reduces the reliability of the estimate. The amount of dinitrogen fixed in the recharge fen (2.1 kg/ha/y) was equally split over the *Alnus* saplings and the peak muck. Dinitrogen fixation associated with *Sphagnum* species was negligible.

Denitrification

When no nitrate was added to the samples, N_2O evolution was negligible (discharge fen) or very low (recharge fen; Fig. 2B), while high rates of N_2O evolution were observed in nitrate-amended samples (Fig. 2A). N_2O evolution was significantly higher after 24 hours in the presence of nitrate (Fig. 2A). The addition of acetylene stimulated N_2O evolution and the effect increased during the course of the experiment. From Fig. 2A, it is apparent that N_2O evolution in nitrate-amended soil samples increases steadily with time, especially in samples incubated in the presence of acetylene. N_2O evolution rates in peat muck from the discharge fen were significantly higher than those in peat muck from the recharge fen for nitrate-amended samples ($p < 0.005$; sign test).

Discussion

Results of our study provide evidence that dinitrogen fixation occurred in the peat muck in both fens. Dinitrogen fixation was low during most of the season (< 0.3 μg N/g/d), but high activity was observed in the discharge fen in August, both in water-amended samples and in drained samples (2.7 and 4.2 μg N/g/d, respectively). Although we cannot explain this sudden peak in activity, this may well be a more general phenomenon, as a similar event was described for Minnesota (USA) mires by Urban & Eisenreich (1988) and Eckardt & Biesboer (1988).

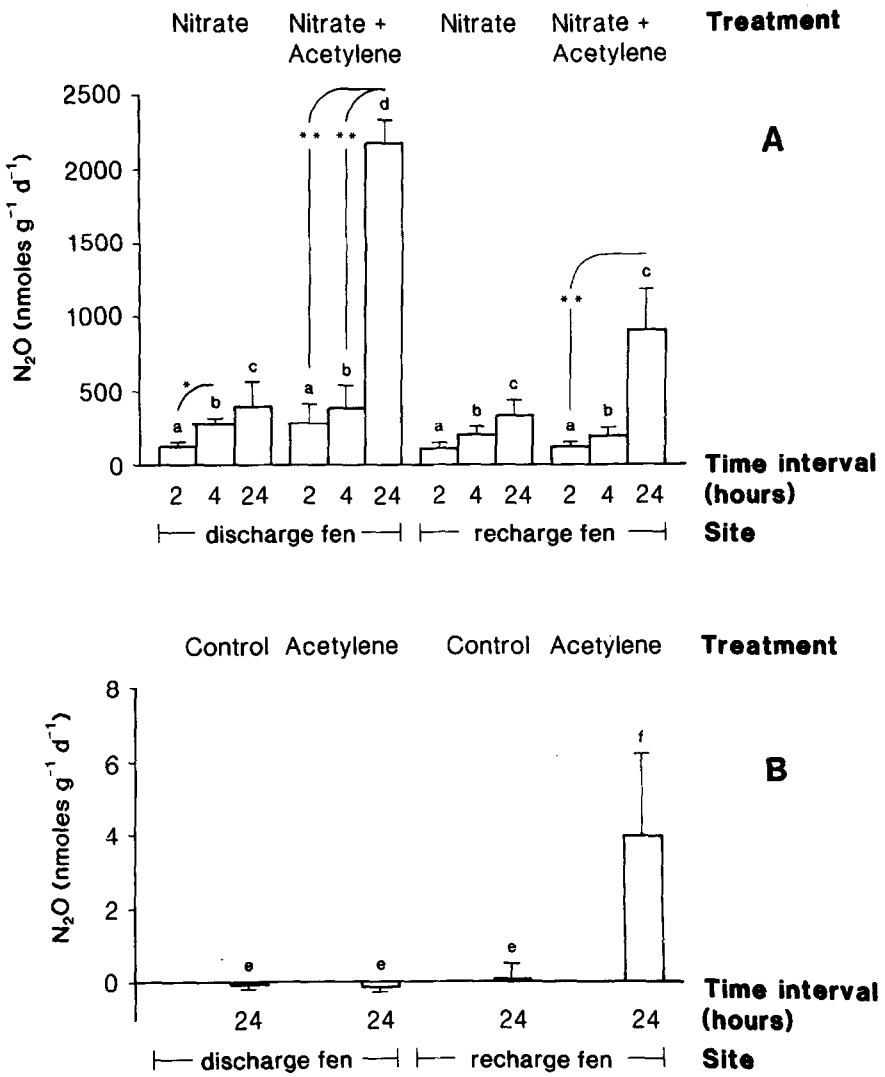

Fig. 2. N_2O evolution rates (nmoles/g/day) from peat muck. Error bars represent 1 s.e. Differences between treatments and between sites at the same sampling time (2, 4, 24 hours) are significant ($p < 0.05$; Wilcoxon test) when bars at that sampling time are headed with different letters. (A) Potential N_2O evolution rates in nitrate-amended samples. Differences over time within one time course were tested with Wilcoxon test. Lines connecting bars that are labelled with a single asterisk (*) are significantly different at the $p < 0.05$ level. A double asterisk (**) indicates a significant difference at the $p < 0.025$ level. (B) N_2O evolution rates at ambient nitrate levels (0.1 ppm).

The amounts of dinitrogen fixed by peat muck in the two fens compare well with rates reported for a Swedish subarctic bog (0.3 μg N/g/d; Granhall & Selander 1973) and data for a Minnesota mire (0 to 5.4 μg N/g/d; Urban & Eisenreich 1988). A comparison with other studies is hampered by the fact that most investigators did not present their data on a dry weight basis.

Dinitrogen fixation associated with *Sphagnum* species was negligible in our study (0 to 0.05 μg N/g/d), although it has been reported that *Sphagnum* species may fix up to 600 μg N/g/d when they are epiphytically or intracellularly associated with cyanobacteria (Granhall & Selander 1973).

More important was dinitrogen fixation by small *Alnus* saplings at rates similar to those reported by Akkermans & Van Dijk (1976) for Alder swamps in the NW part of the Netherlands (Weerribben area).

One of the aims of our study was to estimate the amount of dinitrogen that is fixed on a total ecosystem basis, in order to establish its quantitative importance in the nitrogen mass balance of the fens. Clearly, the calculation of the total amount of dinitrogen fixed in the discharge fen was greatly influenced by the August value for peat muck which contributed 92 percent. Our assay frequency was too low to enable an accurate calculation of the amount of dinitrogen fixed in this fen. Further, no study was made of spatial heterogeneity within the fens, and there were no data available on the distribution of acetylene-reduction activity over the soil profile, or during the winter. Therefore, our estimates should be regarded with caution. However, the estimated total amounts of dinitrogen fixed for the discharge fen (12.7 g N/ha/y) and the recharge fen (2.1 kg N/ha/y) compare with well estimates for rich fens (21.0 kg N/ha/y) and poor fens (5.3 kg N/ha/y) in Germany (Waughman & Bellamy 1980).

Our results must be evaluated, however, as a controversy has developed concerning the interpretation of results from the acetylene reduction assay (e.g., Giller 1987). Witty (1979) demonstrated that ethylene production is partly an endogenous process, and ethylene produced *in situ* is rapidly decomposed, but in the presence of acetylene the oxidation of ethylene is inhibited. As control measurements without acetylene do not measure endogenous ethylene production, we may have overestimated acetylene-reduction in the peat muck. However, the internal ethylene cycle described by Witty (1979) will probably have been insignificant in our waterlogged samples, that were most likely anaerobic. Moreover, when small amounts of ethylene were added to soil samples, ethylene oxidation occurred at a very low rate, if at all. Finally, no ethylene evolution occurred in acetylene- and ethylene-free controls.

Even more controversial is the interpretation of denitrification rates measured with the acetylene inhibition technique. Our results indicate that

denitrification at ambient nitrate concentrations (0.1 ppm) is negligible. In the samples from the discharge fen, no activity could be detected at all, whereas in the recharge fen denitrification approximates 0.1 μg N/g/d, or 0.3 kg N/ha/y (assuming an active soil layer of 20 cm and a Q_{10} of 2; Focht 1974; Stanford et al. 1975; Gordon et al. 1986). Although this estimate is very low, it should be realized that the N_2O evolution rate observed accounts for the loss of the total nitrate pool in the soil sample within 24 hours. Yoshinara et al. (1977) showed that at low nitrate levels, the total nitrate pool can be denitrified within 4 hours. Thus, in our experiment, the denitrification processes will most likely have ceased during the time course of the experiment due to depletion of the nitrate pool, causing our estimates of *in situ* denitrification rates in the recharge fen to be too low.

Denitrification rates in nitrate-amended soil samples were 2 to 3 orders of magnitude higher than under ambient nitrate levels, indicating potential denitrification rates of 59 and 200 kg N/ha/y for the recharge fen and the discharge fen, respectively. These findings agree well with other studies (Hemond 1983; Westermann & Ahring 1987). The increased denitrification rate during the time course of the experiment (Fig. 2A) points to proliferation of the denitrifyer populations, and/or the concurrent development of conditions favouring denitrification. The increasingly stimulatory effect of acetylene on N_2O evolution rates during the time course of the experiment, as indicated by differences between treatment 2 and 3 (Fig. 2A), indicates that during the time course of the experiment there occurred a shift from N_2O towards N_2 production. This shift can be attributed to a decrease of the redox potential due to nitrate reduction (Focht 1974). A decrease in redox potential could also explain the observed increase in denitrification rates during the time course of the experiment. The higher denitrification rates in peat muck from the discharge fen compared to the recharge fen may be due to differences in soil pH. Denitrification rates are known to decrease when the soil pH drops below 6 (Focht 1974; Firestone 1982).

Our results indicate that facultative denitrifyers do occur in peat, but that denitrification rates are low due to a low nitrate availability. Thus, *in situ* denitrification rates may well be determined by nitrate supply due to nitrification. As the acetylene inhibits nitrification (Ryden & Rolston 1983), the acetylene inhibition technique is not very suitable in soils low in nitrate; the nitrate pool will be depleted during the time course of the experiment, and supply by the nitrification process is inhibited. Thus, the quantitative importance of the process *in situ* remains questionable.

However, there is general agreement that nitrifying bacteria are scarce in most mires because of prevailing anaerobic soil conditions (Given & Dickinson 1975; Dickinson 1983; Williams & Wheatley 1988). In a blanket bog at

Moore House (UK) nitrification was estimated to be at a rate of 0.03 kg N/ha/y (Martin & Holding 1978), and Rosswall & Granhall (1980) could not detect any nitrification at all in a fen in Stordalen (Sweden). Thus, supply of nitrate by nitrification will be quantitatively unimportant, and uptake by the mire vegetation that is nitrogen limited in these fens (Verhoeven 1988) will probably account for the nitrate thus produced (see, Urban et al. 1988). Inflow of groundwater rich in nitrate does not occur in these fens (Koerselman et al. 1989a), but relatively high nitrate levels are associated with precipitation. The total nitrate-N deposition by bulk precipitation at the sites is 5.2 kg N/ha/y. If we assume that microbial and plant activity only occur in the summer, 3.0 kg N/ha/y is available for denitrification. Based on tracer experiments by Hemond (1983) it can be assumed that about 25 percent of this amount is converted to ammonium by dissimilatory nitrate reducers. We further assume that the balance is equally split over the plants and denitrifyers, as it has been shown that there is competition between denitrifyers and assimilative nitrate uptake by autotrophics (Urban et al. 1988). Based on this line of argument, denitrification rates associated with precipitation will approximate 1.1 kg N/ha/y. Potential denitrification rates that were observed in our study are well in excess of these estimated rates.

It is interesting to note that the denitrification process will likely become more important when nitrate availability in the fens increases as a result of the inflow of nitrate-rich water. Most fens in the Vechtplassen area are seriously threatened by nitrogen eutrophication (Koerselman et al. 1989a) and nitrate levels are expected to increase in the near future (e.g., Verhoeven et al. 1988). Our experiments indicate that denitrification potentially can remove excess nitrate from water entering the fens, and can do so very efficiently. This has important implications for the nutrient cycling in fens in relation to nitrogen eutrophication and deserves further study.

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