

The Fate of ^{15}N -Nitrate in Healthy and Declining *Phragmites australis* Stands

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Received: 24 July 1996; Accepted: 5 December 1996

ABSTRACT

The dissimilatory nitrate-reducing processes, denitrification, and dissimilatory nitrate-reduction to ammonium were studied in freshwater lake sediments within healthy and degrading *Phragmites australis* (reed) stands. The samples from the healthy vegetation site contained roots and rhizomes. Cores were supplied with $1.9\text{--}5.2\ \mu\text{g}\ ^{15}\text{N-NO}_3^- \text{g}^{-1}$ dry sediment in the laboratory and subsequently incubated for 8 h at 20°C , in the dark. The ^{15}N compounds were determined before (natural percentage of ^{15}N) and after 1 and 8 h of incubation.

The uptake of ^{15}N by the roots and rhizomes in the healthy vegetation was 61%. Nitrogen losses, interpreted as denitrification, accounted for 25 and 84% of the added $^{15}\text{N-NO}_3^-$ in sediment from the healthy and degrading vegetation sites, respectively. The percentages of nitrate reduced to ammonium were 4 and 9% in sediment from the healthy vegetation and degrading vegetation sites, respectively. The percentage of ^{15}N -total N in the sediment of the healthy vegetation site was 10%, whereas for the degrading vegetation site this percentage was 7%. The percentage of nitrate reduced to ammonium could be potentially underestimated by the percentage of ^{15}N measured in the sediment. In this case, in healthy and degenerating *P. australis* stands, the percentage of produced ammonium accounted for 14–16%.

The nitrate reduction rates were calculated based on an incubation period of one hour. The denitrification rate in sediment from the degrading vegetation site was higher than from the healthy vegetation site. The rate of dissimilatory nitrate reduction to ammonium was almost tenfold higher in sediment from the degrading vegetation site compared to sediment from the healthy vegetation site. The significantly lower percentages of dissimilatory nitrate reduction to ammonium and denitrification in the healthy stand compared to the degrading stand was probably due to the presence of roots and rhizomes. In the sediments of healthy and degrading *P. australis* stands, denitrification was the main nitrate-reducing process.

Introduction

Two dissimilatory pathways for nitrate reduction can be distinguished: denitrification and dissimilatory nitrate reduction to ammonium (DNRA). The reduction of nitrate (NO_3^-) via denitrification produces gaseous products such as nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2). The process of DNRA leads to the production of ammonium (NH_4^+) via nitrate (NO_2^-). The two processes are performed by different groups of microorganisms. Denitrifying bacteria are obligatory oxidative organisms [27], whereas bacteria that dissimilate nitrate to ammonium have a fermentative metabolism [26]. The conversion of significant amounts of nitrate to ammonium is restricted to highly reduced conditions, therefore being of little significance in oxic soils [8, 30]. In waterlogged sediments, the percentage of nitrate reduced to ammonium has been reported to vary widely [11, 13]. Under anoxic conditions, with high pH values and large quantities of readily oxidizable organic matter, the reduction of nitrate to ammonium was considerable [12, 24, 26]. However, some studies showed that DNRA could be overestimated in waterlogged or estuarine sediments, due to incomplete blockage of nitrous oxide reductase by acetylene or nitrate uptake by microphytes [4, 21]. The main factors regulating which of the two nitrate-reducing processes will be favored are the concentrations and ratios of available electron donors and acceptors [20, 27]. Aerenchymatous plants create an oxic-anoxic interface in the root zone by releasing oxygen into this zone [2, 22]. The release of oxygen by the roots may stimulate nitrification [5, 9, 19] and, subsequently, denitrification after diffusion of nitrate into the reduced zone of the sediment.

The decline of the aerenchymatous plant *Phragmites australis* (Cav.) Trin. ex. Steudel (reed) in Central Europe has been proceeding very rapidly, especially during the last 25 years. The die-back of *P. australis* can be caused by abiotic circumstances, hydrological and climatological factors, or by biotic parameters [18]. Eutrophication is considered to be the main factor for the rapid decline of healthy *P. australis* stands. The effect of eutrophication on *P. australis* appears to be indirect, via accumulation of organic matter. Eutrophication could lead to anoxia in the below ground growing parts of *P. australis* because the increased litter production may result in an increase of decomposition activity in the sediment compartment. Also, it has been shown that at the die-back sites, aeration by plants was affected negatively due to blockages in the aerenchymatous tissue [2].

In the present paper, we hypothesize that the accumula-

tion of organic matter leads to an increase in electron donor availability, and, more importantly, due to the blockage in aerenchymatous tissue, to a diminished oxygen release into the root zone. This, in turn, leads to a shift in the composition of the dissimilatory nitrate-reducing bacterial community in favor of fermentative bacteria dissimilating nitrate to ammonium. Hence, the percentage of nitrate reduced to ammonium will be higher at sites with a diminished oxygen release, which are the declining *P. australis* stands. The aim of the study is, therefore, to quantify denitrification and DNRA in healthy and declining *P. australis* stands, using $^{15}\text{N}\text{-NO}_3^-$.

Materials and Methods

Sampling Site

Lake Grosser Kelpinsee (53°03'N, 13°44'E), a natural eutrophic lake in eastern Germany, was chosen as the field location. Sediment cores (tubes 25 cm in depth, 10 cm in diameter) were taken in early October 1994 from a healthy *P. australis* stand (a nonaccumulation zone of organic matter), a declining *P. australis* stand (an accumulation zone of organic matter), and a control site without *P. australis* vegetation. From every type of sediment, 15 cores were taken from the 15-cm sediment layer. Only sediment samples from the healthy vegetation site contained roots and rhizomes. The tubes with the cores were filled with lake water to diminish oxygen penetration into the sediments, closed, and stored at approximately 4°C for transport to the laboratory. The stems of *P. australis* from the healthy vegetation site had to be cut to allow closure of the tubes. Sediment temperature during sampling was 12°C. The dissolved NO_3^- concentration in the water column was lower than the detection level of $10\ \mu\text{mol l}^{-1}$, the NH_4^+ concentration was $2.5\ \mu\text{mol l}^{-1}$. The dissolved phosphate concentration was $17.5\ \mu\text{mol l}^{-1}$. From every sediment type, three cores were used to determine initial characteristics of the sediments and of the rhizomes and roots, i.e., inorganic and total nitrogen concentrations, natural percentage of ^{15}N , pH, percentage of organic matter, and organic carbon content. The remaining 36 cores were stored for 15–29 days after sampling, at 4°C, before use.

Incubation Conditions

Labeled $^{15}\text{N}\text{-NO}_3^-$ was used to study dissimilatory nitrate reduction in the three sediment types. The experiment was performed in four sets of nine cores, each containing three cores of each sediment type. The cores were placed at 20°C the night before the incubation. The cores were injected from a reservoir (800 ml) containing $^{15}\text{N}\text{-KNO}_3$ (99.5 atom % ^{15}N), chloramphenicol, and potassium acetate. This reservoir was dispersed simultaneously to the cores by the use of four syringes, to ensure homogeneous distribution of the K^{15}NO_3 solution. The final concentrations were $20\ \mu\text{g K}^{15}\text{NO}_3\ \text{[1]}$ ($2.8\ \mu\text{g }^{15}\text{N}\text{-NO}_3^-$), $0.27\ \text{mg chloramphenicol [23]}$, and $0.13\text{--}0.39\ \text{mg potassium acetate g}^{-1}\ \text{dry sediment}$. The remaining stock so-

lution was used to determine the exact initial concentrations of $^{15}\text{N-KNO}_3$. The cores were incubated at 20°C , in the dark, and one core of each sediment type was harvested after 1, 8, and 24 h. Nonenriched potassium nitrate was added in one of the four series to determine the natural abundance ratios of the different nitrogen components: NO_3^- , NH_4^+ , and total nitrogen in sediments and in roots and rhizomes. To determine the dissolved $^{15}\text{N-N}_2\text{O}$ and $^{15}\text{N-N}_2$ concentrations, 1-ml water samples were taken and evacuated to 3-ml venoject tubes before the cores were harvested.

Determinations of Percentage of ^{15}N

A diffusion procedure was used to prepare samples for percent $^{15}\text{N-NH}_4^+$ and percent $^{15}\text{N-NO}_3^-$ analysis [7, 25]. Sediment:KCl solution ratios (w/v) used for the analysis were 1:4.53 for samples from the healthy vegetation site, 1:31.6 for samples from the degrading vegetation site, and 1:3.61 for samples from the control site. These ratios were based upon measurements performed on cores used for determinations of initial sediment characteristics. The samples were made alkaline by adding 0.3 g MgO ($\text{pH} \approx 10.0$), and the evolving NH_3 was trapped by glass microfiber filters (Whatman GF/C) (Springfield Mill, Maidstone, Kent, England) acidified with $10\ \mu\text{l}$ 2.5 M KHSO_4 . The filters were packed in teflon and floated on the KCl extract. The flasks were gently shaken for 6 days at 20°C . The filters with the NH_4^+ fraction were collected and replaced by new ones; 0.5 g Devarda's alloy was added to the flasks to reduce NO_3^- and NO_2^- to NH_4^+ . The flasks were shaken for another 6 days. The two sets of glass fiber filters containing the percentage of ^{15}N from NH_4^+ and $\text{NO}_3^- + \text{NO}_2^-$ were dried in an NH_3 -free atmosphere for 12 h at room temperature. The filters were analyzed to determine the percent ^{15}N fraction of the N-NH_4^+ , N-NO_3^- , and N-NO_2^- . Natural percent ^{15}N values were determined on a Hereaus-Finnigan MAT 251 mass spectrometer, and the enriched percent ^{15}N values were determined on a Carlo Erba-SIRA combination.

Calculation Methods

During the diffusion procedure, random samples from the KCl extract were taken to determine if NH_4^+ was totally removed before Devarda's alloy was added. The results showed that, especially for samples from the degrading vegetation site, NH_4^+ was still present in the KCl extract. Consequently, the percent ^{15}N on the filters derived from the NO_3^- fraction might be underestimated due to NH_4^+ dilution. To determine the exact concentrations of $^{15}\text{N-NH}_4^+$ and $^{15}\text{N-NO}_3^-$, the inorganic nitrogen concentrations were determined in extracts that were also used for pH measurements. Together with the percent ^{15}N from the NH_4^+ filters, the concentration of $^{15}\text{N-NH}_4^+$ could be calculated. Since there was no nitrate present before incubation, and the injected $^{15}\text{N-NO}_3^-$ concentration was known, the $^{15}\text{N-NO}_3^-$ could be calculated without using the $^{15}\text{N-NO}_3^- + \text{NO}_2^-$ filters.

Sediment Measurements

Before harvest, the redox potentials in the sediments were measured with platinum electrodes and a calomel reference cell and corrected for differences in pH.

For the determination of inorganic nitrogen concentrations and pH (H_2O and KCl) of the healthy and control sediment sites, sediment was shaken at 20°C , with deionized water 1:5 (w/v), for 2 h. After measurement of the $\text{pH}(\text{H}_2\text{O})$, samples were taken for inorganic nitrogen determinations. The $\text{pH}(\text{KCl})$ was measured after addition of 25 ml of 2 M KCl and another incubation period of 2 h. For samples of the degrading vegetation site, the sediment: water ratio (w/v) was 1:37.5 for the first two incubation series, and 1:50 for the last two. The samples were centrifuged at 15,000 g in a Biofuge A (Heraeus Christ) (Dijkstra Verenigde, Almere, The Netherlands) bench centrifuge for 10 min. The supernatants were analyzed for NO_3^- , NO_2^- , and NH_4^+ , using a Technicon Traacs 800 autoanalyzer (Technicon Instr. Corp., Tarrytown, N.Y., USA) with a detection limit of $10\ \mu\text{mol l}^{-1}$, for all three compounds. For the total nitrogen concentrations and percent ^{15}N determinations, the sediment was dried (70°C) and ground. The percent ^{15}N was also determined using the Carlo-Erba SIRA combination. The total nitrogen concentrations were measured spectrophotometrically in digests obtained by treating a sediment sample with a mixture of H_2SO_4 -Se and salicylic acid [17].

The organic matter was determined as loss on ignition (4 h at 550°C). Total organic carbon of the sediment was determined according to the Mebius procedure [15].

Rhizome and Root Measurements

The roots and rhizomes present in the cores of the healthy site were dried (70°C) and ground (0.5 mm), and inorganic and total nitrogen, and total percentage of ^{15}N were determined. Inorganic and total nitrogen concentrations of plant roots and rhizomes were determined as in sediment samples [17]. The percentages of ^{15}N were determined using the Carlo-Erba SIRA combination.

Data Analysis

Sediment characteristics were analyzed by one-way ANOVA. Differences between the sediment means were tested for significance using Tukey's test. If necessary, data were \ln transformed to obtain homogeneity of variances. Since the reduction of NO_3^- to N_2 or NH_4^+ is a linear process (as long as no substrate is limited) and no pool dilution of the enriched potassium nitrate occurred under anoxic conditions, it was not necessary to use a simulation model [28, 29]. Differences in the percentages of ^{15}N pools were arcsin(sqrt) transformed. The percentages of denitrification were inhomogeneous of variances. This data was compared using the one-way, nonparametric Krushal-Wallis test. The denitrification rates and the dissimilatory rates of nitrate reduction to ammonium were calculated based on 1 h of incubation. The values of the dissimilatory rates of nitrate reduction to ammonium were analyzed by one-way ANOVA. Differences between the means were also tested for significance using Tukey's test. The denitrification rate data were not sufficient to allow statistical analysis.

Results

Sediment Characteristics

Differences in sediment parameters of the three sites are given in Table 1. The pH (both H_2O and KCl) was signifi-

Table 1. Original characteristics of sediments from the healthy *Phragmites australis* vegetation site, the degrading *P. australis* vegetation site, and the control site without vegetation^a

Vegetation type	pH		Inorganic N		Total N	Organic matter (%)	Organic carbon (%)	C/N ratio
	H ₂ O	KCl	NO ₃ [−]	NH ₄ ⁺ (μg N g ^{−1})				
Healthy	7.8 ^c	8.0 ^c	0.0	1.4 ^b	769 ^c	2.1 ^c	1.1 ^b	14.3 ^a
Declining	7.6 ^b	7.9 ^b	0.0	145.5 ^c	22896 ^d	48.0 ^d	25.7 ^c	11.3 ^a
Control	8.0 ^d	8.3 ^d	0.0	1.0 ^b	392 ^b	0.9 ^b	0.5 ^b	12.8 ^a

The means were statistically analyzed with one way ANOVA, followed by Tukey's test. Total N was analyzed after ln-transformation; the data was presented untransformed. Significant differences ($P < 0.05$) within columns are indicated by different letters

cantly different for the three sediments; NO₃[−] and NO₂[−] (data not shown) were below the detection level. The NH₄⁺ concentrations were low, except in sediment from the declining *P. australis* vegetation that had a value of 145.5 μg N-NH₄⁺ g^{−1} dry sediment. Another marked difference between the sediments was the organic matter content. The sediment of the declining site contained 48% organic matter, whereas sediment from the healthy and control sites contained 2.1 and 0.9%, respectively. The organic carbon content was significantly higher in the sediment from the degrading site (25.7%) than in the sediments from the healthy (1.1%) and control sites (0.5%). The total nitrogen concentrations were significantly different for the three sediment types. The C/N ratios (w/w) of the three sediments ranged from 11.3 to 14.3.

The inorganic and total nitrogen concentrations of the roots and rhizomes of the healthy vegetation were not significantly different before or after the incubations with enriched KNO₃. NO₃[−] could not be detected in the roots and rhizomes, and the NH₄⁺ concentrations varied between 23 and 42 μg N-NH₄⁺ g^{−1} dry roots and rhizomes (data not shown).

¹⁵N-Nitrate Incubation Experiments

After determining all nitrogen compounds and percentage of ¹⁵N, the results showed that NO₃[−] could not be detected after 24 h of incubation. For a valid determination of the nitrate-reducing rates, NO₃[−] must be available. The presented results concerning the nitrate-reducing processes are, therefore, based on 8-h incubations.

¹⁵N Conversions During the Incubation Periods

In Table 2, the concentrations of ¹⁵N-NO₃[−], ¹⁵N-NH₄⁺, ¹⁵N-total N, and ¹⁵N for roots and rhizomes during 8-h incuba-

tions for all sites are given. These have been corrected for the ¹⁵N percentage at the beginning of the incubation. Due to different amounts of dry sediment per core, the actual ¹⁵N-NO₃[−] concentration at the beginning of the incubation was different in every core. Therefore, the result of every single core was included. During the incubation period, some cores from all sites leaked interstitial water. These cores were excluded from the results.

Healthy Vegetation Site

None of the sediment parameters (pH, organic matter, organic carbon, and C/N ratio) changed significantly during the incubation with enriched nitrate. The Pt-electrode redox potentials ranged from −70 to +226 mV, which is between nitrate reduction and the reduction of ferric to ferrous iron [14]. Natural percentages of ¹⁵N were NO₃[−]: 0.36259 (δ¹⁵N −10.13), NH₄⁺: 0.36537 (δ¹⁵N −2.42), total N: 0.36873 (δ¹⁵N 6.64), and roots and rhizomes: 0.36666 (δ¹⁵N 1.26). The actual ¹⁵N-NO₃[−] concentration ranged from 1.86 to 3.36 μg ¹⁵N g^{−1} dry sediment. The ¹⁵N concentration in the roots and rhizomes varied largely between cores. The percent ¹⁵N recovery was 94 ± 19% (data not shown).

Degrading Vegetation Site

Comparing the sediment parameters after incubation to their original values, the pH(H₂O) was significantly lower after 1 and 8 h of incubation (7.3 and 7.0, respectively). Also, the pH(KCl) was lower than before incubation (7.5), although no differences were detected during the incubation period. The organic matter, organic carbon, and C/N ratios were not significantly affected by the incubation. Redox potentials ranged from −32 to +90 mV, i.e., between the re-

Table 2. Concentrations of the different ¹⁵N-pools after 1 and 8 h of incubation for the healthy vegetation and degrading vegetation sites and the control site^a

Vegetation site	Actual ¹⁵ N-NO ₃ ⁻ (t = 0)	After 1 h of incubation				Actual ¹⁵ N-NO ₃ ⁻ t = 0	After 8 h of incubation			
		¹⁵ N-NO ₃ ⁻	¹⁵ N-NH ₄ ⁺	¹⁵ N-Total N	¹⁵ N-Roots and rhizomes		¹⁵ N-NO ₃ ⁻	¹⁵ N-NH ₄ ⁺	¹⁵ N-Total N	¹⁵ N-Roots and rhizomes
Healthy	2.11	0.85	0.01	0.12	0.61	2.32	0.0	0.11	0.18	0.85
	(0.01)	(0.02)	(0.00)			(0.16)	(0.0)	(0.01)		
	1.86	0.76	0.03	0.11	0.92	2.49	0.89	0.09	0.15	1.23
	(0.02)	(0.01)	(0.01)			(0.01)	(0.01)	(0.00)		
Degrading	N.D. ^b	N.D.	N.D.	N.D.	N.D.	3.36	0.40	0.19	0.37	1.75
						(0.04)	(0.02)	(0.01)		
	5.23	1.39	0.17	0.31	—	2.45	0.00	0.28	0.15	—
	(0.03)	(0.03)	(0.00)			(0.44)	(0.00)	(0.00)		
Control	2.93	0.00	0.20	0.19	—	3.97	0.00	0.30	0.20	—
	(0.04)	(0.00)	(0.00)			(0.03)	(0.00)	(0.01)		
	N.D.	N.D.	N.D.	N.D.	—	3.71	0.00	0.53	0.29	—
						(0.05)	(0.00)	(0.02)		
Healthy	2.94	0.81	0.01	0.09	—	3.11	0.91	0.06	0.11	—
	(0.39)	(0.03)				(0.04)	(0.03)	(0.00)		
	2.56	0.82	0.00	0.09	—	2.71	0.78	0.05	0.14	—
	(0.06)	(0.04)				(0.07)	(0.04)	(0.00)		

^a The ¹⁵N concentrations are corrected for the ¹⁵N percentage at the beginning of the incubation. The concentrations are given in μg ¹⁵N g⁻¹ dry sediment or roots. The data in parenthesis are the standard deviations of the measurements

^b N.D., Not determined

duction of ferric iron and the reduction of sulfate [14]. The natural percent ¹⁵N in NO₃⁻, NH₄⁺, and total N was 0.36521 (δ¹⁵N -3.0), 0.36827 (δ¹⁵N 4.47), and 0.36737 (δ¹⁵N 3.06), respectively.

The enriched nitrate concentration, directly after injection, varied from 2.45 to 5.23 μg ¹⁵N g⁻¹ dry sediment (Table 2). After 8 h, NO₃⁻ could no longer be detected, which may indicate high rates of nitrate reduction. The ¹⁵N-NH₄⁺ concentrations increased to a maximum of 0.53 μg ¹⁵N-NH₄⁺ g⁻¹ dry sediment after 8 h of incubation. The percent ¹⁵N recovery was very low in all cores, between 13 and 36%.

Control Site

Redox potential from the control site ranged from -64 to +70 mV, indicating that the sediment was slightly reduced [14]. The pH(KCl) (8.5) was significantly higher after incubation than before. The other sediment parameters, i.e., pH(H₂O), organic matter, organic carbon, and C/N ratio (w/w), were not significantly affected by the incubation, compared to the results presented in Table 1. The natural percent ¹⁵N for NO₃⁻, NH₄⁺, and total N in the sediment was 0.36362 (δ¹⁵N -7.34), 0.36667 (δ¹⁵N -0.66), and 0.36896 (δ¹⁵N 7.28), respectively.

The actual ¹⁵N-NO₃⁻ concentration varied from 2.56 to 3.11 μg ¹⁵N g⁻¹ dry sediment. The ¹⁵N-total N concentration in the sediment remained around 0.10–0.14 μg ¹⁵N g⁻¹ dry sediment during incubation. The ¹⁵N-NH₄⁺ concentration increased, but remained low. The variation between the cores was low, regardless of the actual ¹⁵N-NO₃⁻ concentration. The ¹⁵N recovery was low, and varied between 14 and 35%.

Percentage ¹⁵N Produced and the Nitrate-Reduction Rates

Potential nitrogen conversion processes occurring in the cores are illustrated in Fig. 1. The injected ¹⁵NO₃⁻ could have been reduced via denitrification to ¹⁵N₂ (1) or via dissimilatory ¹⁵NO₃⁻ reduction to ¹⁵NH₄⁺ (2). Nitrate could have been produced by nitrification (3). ¹⁵NO₃⁻ uptake by plants could occur in the cores of the healthy vegetation site (7), and nitrate could have been assimilated by the microbiota (8). NH₄⁺, either originally present or formed during the incubation, could have been immobilized by the microbiota (5) or taken up by the plant (4). Throughout the incubation, ammonium could have resulted from mineralization (6).

The percentage of ¹⁵N recovered in N₂, NH₄⁺, sediment,

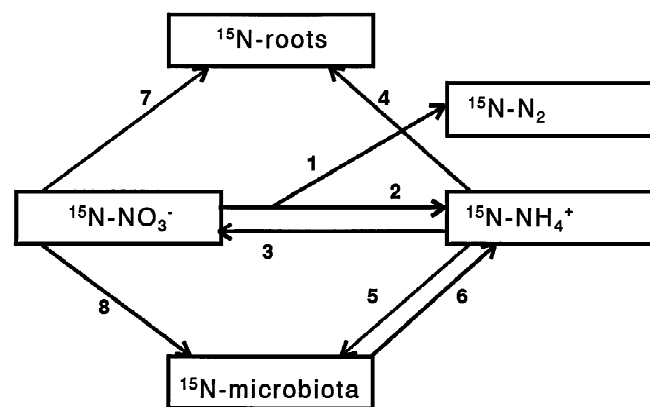


Fig. 1. The nitrogen conversion processes contributing to the $^{15}\text{N-NO}_3^-$ distribution in freshwater sediment in the rhizosphere of *Phragmites australis*. (1, Denitrification; 2, dissimilatory nitrate reduction to ammonium (DNRA); 3, nitrification; 4, ammonium uptake by the plant; 5, immobilization; 6, mineralization; 7, uptake of nitrate by the plant; 8, assimilatory nitrate reduction.)

and roots and rhizomes was independent of the $^{15}\text{N-NO}_3^-$ concentration and whether NO_3^- was still present after 8 h of incubation. The assumption was made that there was no pool dilution of the produced ^{15}N pools during the 8-h incubation. The percentage $^{15}\text{N-NO}_3^-$ reduced to either $^{15}\text{N-N}_2$ or $^{15}\text{N-NH}_4^+$ should be the same in all cores of the same sediment. It was not possible to determine the $^{15}\text{N-N}_2$ concentration, since the total N_2 concentration in the venoject tubes could not be accurately measured over atmospheric background. Therefore, the percent $^{15}\text{N-N}_2$ was calculated from the unaccounted ^{15}N . The percentages of ^{15}N in N_2 , NH_4^+ , sediment, and roots and rhizomes are given in Table 3.

The percentage of missing nitrogen and, thus, the putative denitrification were significantly lower in sediment from the healthy vegetation site compared to sediment from the degrading vegetation site (25 and 84%, respectively). The estimated percentage of denitrification in the sediment from the degrading site did not significantly differ from that of the control site (93%). The percentages of $^{15}\text{N-NH}_4^+$ formed from $^{15}\text{N-NO}_3^-$ were lower. In the healthy vegetation, the $^{15}\text{N-NH}_4^+$ produced was 4%, which was not significantly different from that of the degrading vegetation site (9%) or the control site (2%). Although the percent $^{15}\text{N-NH}_4^+$ was low in sediment from the degrading *P. australis* stand, it was significantly higher in comparison with the control site (2%). The percentages of ^{15}N in the sediment were nearly the same at the degrading and control sites (7 and 5%, respectively). For sediment from the healthy vegetation site,

the percentage was significantly higher (10%). The percent ^{15}N in roots and rhizomes was 61%.

Regarding the dissimilatory nitrate-reducing rates, it was necessary that nitrate be available, otherwise the rates were dependent on nitrate availability and not on the nitrate-reducing bacterial community present. After 8 h of incubation, NO_3^- could no longer be detected (Table 2). Therefore, presumed denitrification rates and the rate of dissimilatory nitrate reduction to ammonium were calculated on the basis of the different ^{15}N concentrations after 1 h of incubation. Almost all cores showed detectable nitrate after 1 h of incubation (Table 2). The assumption was made that, in cores with the same sediment, the composition of the nitrate-reducing community was the same. Since there were too few measurements for the putative denitrification rates, only an estimate could be obtained from the results. The putative denitrification rate in sediment from the healthy vegetation site was $0.280 \mu\text{g N-N}_2 \text{ g}^{-1} \text{ dry sediment h}^{-1}$. In sediment from the degrading vegetation site and the control site, the estimated denitrification rates were 3.361 and $1.820 \mu\text{g N-N}_2 \text{ g}^{-1} \text{ dry sediment h}^{-1}$, respectively (Table 3). The dissimilatory rates of nitrate reduced to ammonium for sediments from the healthy vegetation site and control site were 0.020 and $0.005 \mu\text{g N-NH}_4^+ \text{ g}^{-1} \text{ dry sediment h}^{-1}$, respectively. The rate of dissimilatory nitrate reduction to ammonium in sediment from the degrading vegetation site, $0.184 \mu\text{g N-NH}_4^+ \text{ g}^{-1} \text{ dry sediment h}^{-1}$, was 9.2 to 36.8 times greater in comparison with the healthy vegetation and the control sites.

Discussion

In the degrading *P. australis* stand, the concentrations of ammonium, total nitrogen, organic matter, and organic carbon were very high (Table 1). Regarding the condition of *P. australis*, there is a positive correlation between the concentrations of nitrogen and phosphorus present in the sediment and the softening of *P. australis* stems [6]. The indirect effects of the high nitrogen concentrations at the accumulation site could contribute to the degradation of *P. australis*.

The following assumptions were made, with respect to nitrogen conversion processes (Fig. 1), during the interpretation of the $^{15}\text{N-NO}_3^-$ results: (1) $^{15}\text{N-NH}_4^+$ was homogeneously distributed on the glass microfiber filters; (2) there was no isotopic discrimination between ^{14}N and ^{15}N in the formation of N_2 and NH_4^+ from NO_3^- by the bacteria; (3) ^{15}N not accounted for represented ^{15}N gasses; (4) total ^{15}N in the roots and rhizomes was a measure of the ^{15}N uptake;

Table 3. The percentages of ^{15}N after the incubation with enriched $^{15}\text{N-NO}_3^-$ in the different N-pools of sediments from the healthy *Phragmites australis* stand, the degrading *P. australis* stand, and the control site without vegetation. Also, the nitrate reduction rates are given, based on the ^{15}N values after 1 h of incubation

Vegetation type	% ^{15}N				Nitrate reduction rates	
	N_2	NH_4^+	Sediment	Roots and rhizomes	Denitrification	Dissimilatory nitrate reduction to ammonium (DNRA) ($\mu\text{g } ^{15}\text{N g}^{-1} \text{ dry sediment h}^{-1}$)
Healthy	25	4 ^{ab}	10 ^b	61	0.280	0.020 ^a
Degrading	84	9 ^b	7 ^a	—	3.361	0.184 ^b
Control	93	1 ^a	5 ^a	—	1.820	0.005 ^a

The data was presented untransformed. The percentages of ^{15}N pools were arcsin(sqrt) transformed. The means were statistically analyzed with one way ANOVA, followed by Tukey's test. Significant differences ($P < 0.05$) within columns are indicated by different letters. The percentage denitrification from the healthy vegetation site was significantly different from the degrading vegetation site, according to the one-way nonparametric test. No statistic was performed on the few nitrate reducing data regarding the denitrification rate.

(5) the incubation period was too short for the produced $^{15}\text{N-NH}_4^+$ to become immobilized, assimilated, or diluted by mineralization; (6) nitrate assimilation by the microbiota was negligible; (7) nitrification was of little importance in water-saturated samples. Several of these assumptions merit further scrutiny. Concerning assumption 3, the percentage of presumed denitrification might be overestimated due to NH_3 evolving from the three sediments with relatively high pH. Regarding assumption 5, only the microbiota present at the time of sampling were involved in ammonium uptake, as the addition of chloramphenicol arrested further cell growth. Since ammonium was present and nitrate assimilation was inhibited in the presence of ammonium [11], all samples fulfill the criteria for assumption 6. Even for samples from the healthy vegetation site, assumption 7 is probably fulfilled, as stems were cut and all samples were inundated, sealed, and kept in the dark.

Since little was known concerning the quality of the organic material present in the sediments, acetate was added during the nitrate-reducing incubations to ensure the availability of an electron donor. It is not possible to measure the actual nitrate-reducing rates, because the addition of $^{15}\text{NO}_3^-$ will always influence these rates. The rates of nitrate reduction must be regarded as potential activities, depending on the composition of the bacterial community present at sampling time. For the sediments from the degrading vegetation site and the control site, there was a great loss of ^{15}N (84 and 93%, respectively) (Table 3). For sediment from the healthy vegetation site, where 61% of the ^{15}N was recovered from roots and rhizomes, the loss of enriched nitrogen was much lower (25%). Since plants may be better competitors for nitrate than nitrate-reducing bacteria [10], the assumption was made that the percent ^{15}N in the roots and rhizomes originated from uptake of $^{15}\text{NO}_3^-$, and not from $^{15}\text{NH}_4^+$.

The percentages of $^{15}\text{N-NH}_4^+$ recovered from $^{15}\text{NO}_3^-$ were rather low for all three sites, ranging from 2 to 9%.

The nitrogen turnover rates in a riparian fen, determined by ^{15}N dilution, showed that DNRA accounted for only 3–6% of the nitrate reduced [1]; whereas at least 80% of the nitrate reduction in soil was due to denitrification, with the remainder being reduced by nitrate assimilation and, to a lesser extent, by DNRA. The DNRA was slightly higher than reported [1], but within the same order of magnitude.

The percentages of ^{15}N -total N in sediment of the healthy and degrading sites were 10 and 7%, respectively. Assuming that all ^{15}N -total N was first reduced to ammonium, the percentages of DNRA were potentially underestimated in accordance with the percentages found in the total nitrogen fraction. Since the sums of $^{15}\text{N-NH}_4^+$ and ^{15}N -total N recovered were 14–16% for the healthy and the degrading sites, respectively, there was no difference in the percentage of ammonium formed in these sediments.

Regarding the percentages of putative denitrification and DNRA of the total nitrate reduction, the ratio between inferred denitrification and DNRA calculated with and without the percent ^{15}N -total N recovered via $^{15}\text{N-NH}_4^+$ from the healthy vegetation, degrading vegetation, and control sites were 1.8–6.3, 5.3–9.3, and 13.3–46.5, respectively. This indicates that the percentage of nitrate reduced to ammonium was higher in the sediment from the healthy vegetation site compared to the other sites. Regarding the nitrate-reducing rates, one has to keep in mind that these rates are based on 1 h of incubation. Nothing is known about the possible lag time in activity. Therefore, the rates should be considered as potential dissimilatory nitrate reducing rates occurring in the field, when nitrate becomes available. The presumed denitrification rate in sediment from the degrading vegetation was higher than in sediment from the healthy

vegetation site. The rates of dissimilatory nitrate reduction to ammonium were much higher in sediment from the degrading stand than in sediment from the healthy vegetation stand. These results, to a large extent, agree with the original hypothesis that accumulation of organic matter leads to a shift in the composition of the dissimilatory nitrate-reducing bacterial community in favor of fermentative nitrate-reducing bacteria. In sediment from the degrading *P. australis* stand, there were no roots or rhizomes present, with *P. australis* floating on top of the water. Oxygen transport through the aerenchymatous tissue and the release of oxygen into the root zone was, therefore, not possible. Also, the organic carbon content was extremely high, as were, most likely, the oxygen-consuming processes. The high rate of dissimilatory nitrate reduction to ammonium can be explained by the continuous anoxic conditions and the limited amount of nitrate (Table 1). Fermentative nitrate-reducing bacteria are able to grow under these conditions, in contrast to denitrifying bacteria. This is supported by other studies in which the composition of the nitrate-reducing community in waterlogged sediments was investigated. Under nitrate limitation, fermentative bacteria were dominant, but, when nitrate was added, denitrifying bacteria became dominant [16].

The percentage of nitrate reduced to ammonium will be high when nitrate becomes available after an oxic period. However, denitrifying bacteria have a higher growth rate and are also better competitors for nitrate than fermentative bacteria [16]. Therefore, with longer incubation times, the percentage of nitrate reduced to dinitrogen will increase. After 8 h of incubation, there is no difference in the percentage of ^{15}N recovered in $^{15}\text{N-NH}_4^+$ between healthy and degrading vegetation stands. The oxygen release into the root zone by *P. australis* at the healthy vegetation stand may explain the lower rate of dissimilatory nitrate reduction to ammonium at this site. Likewise, the composition of the nitrate-reducing community in sediment from the healthy vegetation site is probably different from that at the degrading vegetation site. In sediment of the healthy *P. australis* stand, the uptake of nitrate by the plant is an important process, despite the cutting of the stems. This is probably the reason that the presumed percentage of denitrification is lower in sediment from the site with healthy vegetation than in sediment from the site with degrading vegetation. Regardless of the sediment type, denitrification is the main nitrate-reducing process. The presence of the aerenchymatous plant is important for the uptake of nitrate and the release of oxygen into the root zone. Furthermore, the concentrations and the ratio of

the available electron donor and acceptor were found to be less important than previously reported [12, 20, 25, 26, 27], at least in these freshwater saturated sediments in the presence of an aerenchymatous plant.

Acknowledgments

This study was supported by the EC-EUREED project: Effects of interactions between eutrophication and major environmental factors on the ecosystem stability of reed vegetation in European land-water ecotones. The author acknowledges Ab Wijnhuizen and Willy Keultjes for their help concerning the equipment necessary for field sampling; Wiecher Smant for the total nitrogen and organic carbon measurements; and the Netherlands Energy Research Foundation (ECN) in Petten, the Netherlands, for the ^{15}N determinations. Valuable comments on the manuscript were provided by Sep Troelstra, Jack Middelburg, Jan Woldendorp, and George Kowalchuk. Special thanks are due to Wim Wessel for his useful and positive ideas concerning the percent ^{15}N results.

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