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THE EFFECT OF ABIOTIC FACTORS ON DENITRIFICATION RATES IN SEDIMENT OF SOLINA RESERVOIR, POLAND

Seasonal variations in denitrification rates (D_{tot}) were determined in bottom sediments of the Solina Reservoir using the isotope pairing technique (^{15}N IPT). D_{tot} rates ranged from about 3 to 163 μmol N_2 $m^{-2}h^{-1}$ (at temperature ranging from 6 to 24 °C). The denitrification activity at great depths was very weak because temperature there did not exceed 10 °C. As a pronounced effect of temperature was observed at all shallow places in the reservoir, temperature may be an important factor controlling denitrification rate. However, a total denitrification rate (D_{tot}) is also dependent on by NO_3^- and dissolved oxygen (DO) concentration in the overlying water, as well as on the availability of sedimentary organic matter. The use of the isotope pairing technique (^{15}N IPT) provided an opportunity to distinguish the denitrification of NO_3^- diffusing from the overlying water (D_w) from the coupled nitrification–denitrification within sediment (D_n). It was possible to observe an influence of such factors as temperature, NO_3^- and DO concentration in the overlying water on the contribution of D_w and D_n to D_{tot}.

1. INTRODUCTION

Although anthropogenic inputs of combined nitrogen cause serious eutrophication of many aquatic environments [9], [19], it can be removed from the environment. Denitrification in particular is an important process removing nitrogen from the system and transferring it to the atmosphere as gaseous dinitrogen [12], [20], [25]. Denitrifying bacteria in the sediment are able to reduce nitrate that diffuses from the overlying water to the anoxic layers of bottom sediment, as well as nitrate formed by nitrification in the oxic bottom sediment layer just below the sediment–water interface.

The mass balance of nutrients in the Solina Reservoir in south-eastern Poland reveals its high nitrogen loading, as well as high nitrogen retention and a rapid acceleration of eutrophication [24]. Since denitrification can play a major role as a nitrogen sinks to the bottom of this reservoir [22], it was important to quantify nitrification rates in the sedi-

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ment. We sought to do that as well as to draw a distinction between the denitrification supported by NO_3^- supply from the overlying water and that reflecting coupled nitrification–denitrification within sediments. More specifically, efforts were made to examine the factors affecting rates of denitrification in the reservoir sediment, notably temperature, the concentration of nitrate ($N-NO_3^-$) and dissolved oxygen (DO) in overlying water, and organic matter content (OM) in the sediment.

2. STUDY SITE

The investigations into the denitrification process were carried out in the Solina Reservoir in 2002 and 2003. This is a large and deep water body, whose selected morphometric parameters are as follows: volume – 504 million m^3, area – 22 km^2, watershed area – 1175 km^2, maximum depth – 60 m, average depth – 22 m, retention time – 215 days. The reservoir is on the San River in the Bieszczady Mountains, south-eastern Poland. It is the largest manmade lake in Poland, initially used by the power industry to generate electricity, but later also serving as a source of water supply and a recreation centre. Complementing the natural tributaries of the Solina Reservoir is the supply of water from the rotation turbines of a power plant situated within the lower-lying 10.9 million m^3 Myczkowce stilling basin. The reservoir's drainage basin is of a highland nature, and is dominated by forests, meadows and pastures. Arable land accounts for just a small percentage of the area and the area is sparsely populated and lacking in industrial plants. The limited agricultural use of the area is made, farming and animal husbandry activities being concentrated in the immediate vicinity of the reservoir, as well as in river valleys. A few small towns in the area mainly make a living from tourism.
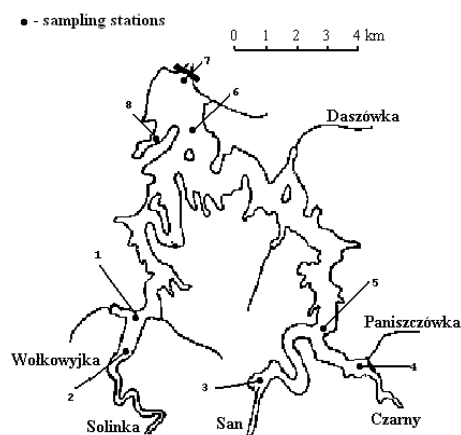

Fig. 1. Location of the sampling stations in the reservoir

In line with the specifics of the reservoir, samples were collected at eight sites, four littoral (1–4) and four profundal (5–9). The locations of sampling stations are as shown in figure 1.

3. METHODS

Undisturbed sediment cores were collected using a sampler of the author's own construction, and then transferred to a thermostatic container. During transportation, the container was filled with water overlying the sediment. The water column overlying the sediment ensured a sufficiency of oxygen and nutrients. In the laboratory, ^{15}N-labelled NO_3^- was added to the overlying water prior to the water in the tubes being replaced with reservoir water. The cores with the overlying water were then closed and incubated for 1.5 hour under in situ conditions. Water above the sediment was stirred gently with a small Teflon-coated magnet suspended 5 cm above the sediment. Momentum for the rotation of the magnets was provided by a large external magnet rotating at 50 r.p.m. to ensure homogeneous mixing of the water column. Once the incubation of cores had been completed, samples for determination of the ^{15}N$_2$ (^{29}N$_2$, ^{30}N$_2$) concentration were collected, following a careful mixing of the sediment with the overlying water. A ZnCl$_2$ solution was added to reduce a bacterial activity. N$_2$ was extracted from the water in glass gas-tight vials, by replacing 2 cm^3 of the sample with helium and shaking vigorously for 5 minutes. The gaseous phase was then analyzed for the N$_2$ concentration and isotopic distribution of ^{29}N$_2$ and ^{30}N$_2$ with a combined gas chromatograph–isotope ratio mass spectrometer (IRMS DELTA$^+$ Finnigan on line with GC/CIII).

A total denitrification in the sediment was calculated as the sum of the N$_2$ production due to nitrates from overlying water and nitrates produced as a result of nitrification within the sediment. The isotope pairing approach was used to calculate the rate of denitrification [10], [15]. The denitrification rates of added ^{15}NO$_3^-$ (D_{15}) and in situ ^{14}NO$_3^-$ (D_{14}) were calculated from the measured production of ^{14}N^{15}N (p^{29}N$_2$) and ^{15}N^{15}N (p^{30}N$_2$): D_{15} = p^{29}N$_2$ + 2p^{30}N$_2$ and D_{14} = (p^{29}N$_2$/2p^{30}N$_2$)·D_{15}. The in situ rate of denitrification of nitrates from a the water column (D_w) was calculated from D_{15} and the ^{15}N atom% of NO_3^-: D_w = (100·D_{15}/ ^{15}N atom%) − D_{15}. Coupled nitrification–denitrification rate (D_n) was calculated as the difference: $D_n = D_{14} − D_w$. The ^{15}N atom percentage of NO_3^- was estimated by mass spectrometry after biological reduction to N$_2$ [14].

The concentration of nitrogenous compounds in the overlying water was determined using colorimetric analysis (the Spectroquant technique with a WTW PhotoLab S12 photometer). The amount of dissolved oxygen and water temperature were meas-

ured using a WTW–CELLOX 325 oxygen probe with a temperature sensor. Bottom sediment was sampled each time at all stations. The sediment samples were dried, ground and analyzed for OM as loss on ignition at a temperature of 550 °C. For porosity measurements, the water content per volume of sediment was determined by drying a known volume of fresh sediment to a constant weight at 105 °C.

4. RESULTS AND DISCUSSION

The findings of the study are summarized in tables 1 and 2; the parameters of overlying water and bottom sediment being shown in table 1, and the rates of total denitrification (D_{tot}), coupled nitrification–denitrification (D_n), and denitrification of NO_3^- supplied from the water column (D_w) in table 2.

Table 1

Selected overlying-water and bottom-sediment parameters

Parameter	Reservoir	
	Shallow stations (1–4)	Deep stations (5–8)
	Overlying water	
Water temperature (°C)	6–24	8–10
Nitrate nitrogen (mg dm^{-3})	0.7–2.3	1.2–3.0
Nitrite nitrogen (mg dm^{-3})	0.011–0.093	0.010–0.025
Ammonium nitrogen (mg dm^{-3})	0.08–0.65	0.11–0.41
Total nitrogen (mg dm^{-3})	1.5–4.0	2.1–3.2
Dissolved oxygen (mg dm^{-3})	4.08–9.40	4.75–9.55
	Sediment	
Porosity (cm^3 H$_2$O cm^{-3} sed.)	0.28–0.62	0.40–0.66
OM (% of dry weight)	0.88–10.64	4.75–9.55

Table 2

Rates of total denitrification (D_{tot}), coupled nitrification–denitrification (D_n), and denitrification of NO_3^- supplied from the water column (D_w). Mean values shown in parentheses, n = numbers of measurement series

Stations	Denitrification rate (µmol N$_2$ m^{-2} h^{-1})		
	D_{tot}	D_w	D_n
Station 1, $n = 11$	9.26–163.54 (93.60)	2.35–136.26 (67.44)	6.91–49.56 (26.17)
Station 2, $n = 11$	16.29–155.15 (95.16)	5.30–112.69 (54.84)	10.99–68.12 (40.32)
Station 3, $n = 11$	8.62–82.36 (47.87)	2.63–64.79 (30.67)	5.84–36.47 (17.20)
Station 4, $n = 11$	3.12–66.22 (34.31)	0.74–52.29 (23.92)	2.34–15.06 (10.39)
Station 5, $n = 5$	16.32–30.31 (24.39)	9.62–26.90 (18.08)	3.41–20.70 (8.74)
Station 6, $n = 5$	9.20–31.44 (19.43)	5.89–27.51 (15.53)	2.31–5.45 (3.90)
Station 7, $n = 4$	6.10–19.87 (12.49)	3.87–17.42 (9.85)	2.23–3.20 (2.64)

Station 8, $n = 5$	14.10–32.37 (22.94)	10.67–28.27 (19.29)	2.76–5.07 (3.65)

Total denitrification rate over the period of the study ranged from 3.12 to 163.54 µmol N_2 m^{-2} h^{-1}, and temperature varied between 6 and 24 °C. The *t*-Student test confirmed the statistical significance of the difference between the mean denitrification rates at the sites in shallow and deep water ($t = 4.445$, $p < 0.001$).

Denitrification rates in the deep places were low (maximally 33 µmol N_2 m^{-2} h^{-1}). No significant differences between stations were found (ANOVA, $F = 2.091$, $p = 0.144$). The mean value of the denitrification rate at deep stations oscillated around 20 µmol N_2 m^{-2} h^{-1}. Such a limited activity being attributable to the low (8–10 °C) temperature of the overlying water.

Differences among stations were observed when it came to the denitrification rates for shallow sites around the reservoir (Kruskal–Wallis Test, $H = 14.137$, $p = 0.0027$). Mean values for stations 1 and 2 were almost three times as great as the maximum noted for deep places in the reservoir. In turn, the lowest value of the denitrification rate obtained for station 4 can be attributed to the decidedly different structure of the sediment there, which was compact, sandy and characterized by a low OM content.

The use of the isotope pairing technique (^{15}N IPT) allowed the quantification of both coupled nitrification–denitrification and the denitrification of NO_3^- from the overlying water. Results of the ^{15}N addition experiment conducted on the sediment cores indicate that NO_3^- in the water column was the major source of substrate for the denitrification process (rather than the NO_3^- produced in the sediment by nitrification). The rate of the latter process was sporadically higher than that of the former, as in the shallow places of the reservoir when temperature of overlying water was low. The results presented here are thus close to those of MENGIS et al. [7] or RYSGAARD et al. [18], being almost identical to those of PIND et al. [12], who found that D_w accounted for 75–90% of a total denitrification activity. In turn, those results of other studies with isotope tracers [13], [17], which have demonstrated significant contribution a coupled nitrification–denitrification makes to overall denitrification rates, contrast with our results.

Denitrification activity is controlled by population size, the availability of organic carbon as an energy source, substrate (NO_3^-) supplied from different sources, temperature, and oxygen penetration into the sediment. In consequence, with a view to the contribution of D_w and D_n to D_{tot} being determined, we examined the effects on rates of the processes ascribable to temperature, concentration of nitrate and dissolved oxygen in overlying water, and OM content in the sediment. The relationships obtained are presented in figures 2–7, in which the *X*- and *Y*-axes depict mean values from each respective station.

As was mentioned earlier, denitrification in deeps was very limited, on account of

the low temperature of the overlying sediment water throughout the year. However, a pronounced effect of temperature was observed at all the shallow sites. The positive
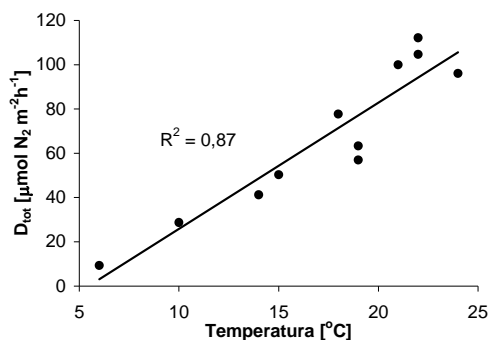
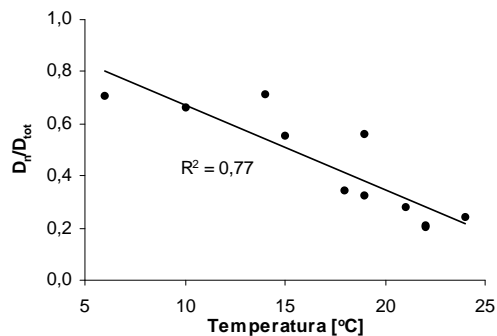

Fig. 2. Denitrification rate
as a function of temperature

Fig. 3. The contribution of D_n to D_{tot}
as a function of temperature

correlation between the total denitrification rate and temperature of overlying water ($R^2 = 0.87$) is shown in figure 2. This is in agreement with the results obtained for other freshwater ecosystems [11], [12], [20], [25]. Our investigation also reveals that a temperature increase in the overlying water ensures a greater contribution of D_w to D_{tot} ($R^2 = 0.77$) (figure 3). Thus, D_w showed the same seasonal pattern as D_{tot}, accounting in our experiments for up to 90% of the total denitrification activity at the highest temperatures. In contrast, at low temperature, D_n prevailed in D_{tot}, and accounting for up to 76% of the total process. Although the outcomes of our observations concerning the contribution of D_w and D_n to D_{tot} are similar to those reported by PIND et al. [12], they are not in accord with the statements of other investigators, e.g., CHRISTENSEN et al. [3].
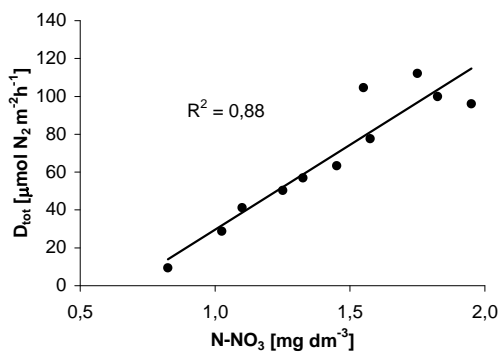

Fig. 4. Denitrification rate as a function of nitrate
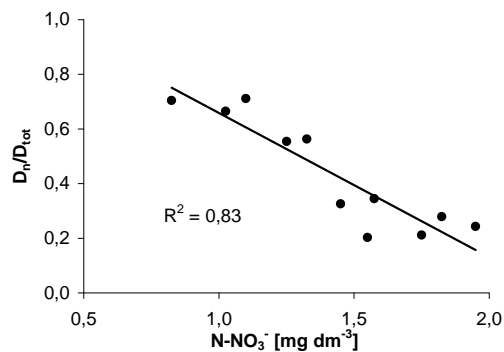concentration in overlying water

Fig. 5. The contribution of D_n to D_{tot} as a function
of nitrate concentration in overlying water

As is shown below, our observations of the influence of remaining abiotic factors on the rate of denitrification process point to the concentration of NO_3^- in the overlying water as the major factor controlling the rate of denitrification in the sediment and influencing the contribution of D_w and D_n to D_{tot}. There was a significant, positive correlation between seasonal variations in denitrification rates and NO_3^- concentration ($R^2 = 0.88$) (figure 4). Similarly, a positive linear correlation was presented by NIELSEN et al. [9], JENSEN et al. [4] and KANA et al. [6]. In turn, only one item of information disconfirming this relationship has been found [11]. In accordance with the results from other ecosystems examined by several investigators [1], [5], [16], [17], the increase in the nitrate concentration of the overlying water also points to its strong influence on the contribution of D_w and D_n to D_{tot} (resulting in a decrease in the D_n/D_{tot} ratio). This relationship is shown in figure 5. The significant correlation ($R^2 = 0.83$) indicates that D_w is regulated by the NO_3^- concentration. On the basis of the above, certain other factors were assumed to cause a reduced denitrifying activity during the period with the highest NO_3^- concentration in the overlying water. Our hypothesis became evident during analysis of the influence of OM content in the sediment and DO concentration on denitrification. As is shown in figure 6, the correlation between OM concentration and denitrification rates was rather weak ($R^2 = 0.26$), a situation that can be attributed to the differences in OM content for stations in shallow parts of the reservoir and the unstable availability of organic carbon [22]. Increased water flows in upper parts of the reservoir are a likely cause of erosion of the sediment in shallow places, leading to a loss of particulate organic material and a consequent loss of substrate for microbial metabolism [23].
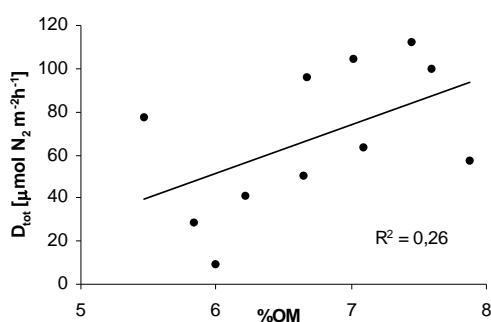

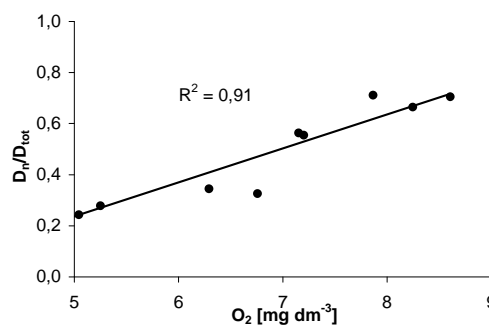Fig. 6. Denitrification rate as a function of organic matter content in bottom sediment

Fig. 7. The contribution of D_n to D_{tot} as a function of oxygen concentration in overlying water

The positive correlation ($R^2 = 0.91$) found between DO concentration in the overlying water and the D_n/D_{tot} ratio (figure 7) implies that the raising of DO concentration in overlying water led the nitrate produced in the oxic sediment layer via the nitrifica-

tion process to become a more significant substrate for denitrification (causing a greater contribution of D_n to D_{tot}). Similar findings were formulated by, e.g., CHRISTENSEN et al. [2]. A higher DO concentration in the overlying water can cause the extension of an oxic layer of the sediment, which in consequence stimulates nitrification and suppresses the process of nitrate diffusion from overlying water into anoxic-anaerobic sediment layers [8]. In our case, in which denitrification is driven by NO_3^- diffusing into the sediment from the water column, increased sediment oxygenation results in a longer diffusion path for NO_3^- to encounter the reducing conditions necessary for denitrification, thereby reducing D_{tot} rates. Our observations are close to those presented by RISGAARD-PETERSEN et al. [13]. The concentration of DO in overlying water is usually low (especially for deep reservoirs), and penetration of O_2 into sediment is not deep [20]. We therefore assume that the influence of the DO concentration in overlying water on the rates of D_{tot} is of lesser significance.

5. CONCLUSIONS

The denitrification rates in the sediment of the Solina Reservoir resemble those reported for many eutrophic reservoirs. The denitrification activity in the deeps was found to be weak, on account of a low temperature of the overlying water. A pronounced effect of temperature on nitrification was observable in all shallow places in the reservoir, indicating the importance of temperature as a factor controlling this process .

A significant correlation between seasonal variation in denitrification rates and NO_3^- concentration in the overlying water indicates that this is probably the major factor controlling the process. We have also found denitrification activity to be regulated by the availability of organic matter and DO concentration in the overlying water. As the regulation of denitrification activity is thus shown to be of a complex nature, we advocate the use of the multiple regression method of analysis for examining the combined influence of abiotic factors on the rate of denitrification.

The ^{15}N isotope pairing technique is helpful in distinguishing the denitrification of NO_3^- diffusing from the overlying water from the coupled nitrification–denitrification ongoing within sediment. This method has afforded an opportunity to quantify the contributions of both D_w and D_n to D_{tot}.

Our observations make it clear that factors such as temperature as well as the concentrations of nitrate and dissolved oxygen in the overlying water influence the contribution made by nitrification–denitrification to total denitrification rates. An increase in temperature and NO_3^- concentration reduced the contribution of D_n to D_{tot}, whereas a higher DO concentration in the overlying water led to the nitrate produced in the oxic layer of sediment through the nitrification process becoming a more significant

substrate for the denitrification process (in consequence ensuring a greater contribution of D_n to D_{tot}).

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WPŁYW CZYNNIKÓW ABIOTYCZNYCH NA SZYBKOŚĆ DENITRYFIKACJI W OSADACH DENNYCH SOLIŃSKIEGO ZBIORNIKA ZAPOROWEGO

Sezonowe zmiany szybkości denitryfikacji oznaczano w osadach dennych zbiornika zaporowego w Solinie. Do badań stosowano metodę ^{15}N IPT (z zastosowaniem stabilnego izotopu azotu). Uzyskane szybkości denitryfikacji mieściły się w zakresie od około 3 do 163 µmol N_2 $m^{-2}h^{-1}$ (w zakresie temperatur od 6 do 24 °C). W głębokich miejscach aktywność denitryfikacyjna była mała, ale temperatura wody nadosadowej nie przekroczyła tam 10 °C. Te obserwacje sugerują, że temperatura jest jednym z głównych czynników limitujących aktywność denitryfikacyjną. Znaleziono również korelacje, które sugerują, że całkowita szybkość procesu denitryfikacji (D_{tot}) jest kontrolowana przez stężenie azotanów i tlenu rozpuszczonego w wodzie nadosadowej oraz zawartość materii organicznej w osadach. Zastosowanie do badań metody ^{15}N IPT pozwoliło wyznaczyć nie tylko całkowitą szybkość denitryfikacji (D_{tot}) w osadach, ale również szybkość denitryfikacji azotanów produkowanych w tlenowej warstwie osadów w procesie nitryfikacji (D_n) oraz azotanów dyfundujących do osadów z wody nadosadowej (D_w). Zaobserwowano, że na wielkość udziału D_n i D_w w D_{tot} mają wpływ takie czynniki jak temperatura oraz stężenie azotanów i tlenu rozpuszczonego w wodzie.