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COMPETITIVENESS OF DISSIMILATORY NITRATE REDUCTION PROCESSES IN BOTTOM SEDIMENT OF RZESZÓW RESERVOIR

Seasonal variations in the rates of dissimilatory nitrate reduction processes were analysed in the bottom sediment of Rzeszów Reservoir using the isotope pairing technique (^{15}N IPT). This method is based on enriching the overlying water with ^{15}N -labelled NO_3^- and observing how it has been transformed. Another aim was to determine the influence of chosen abiotic factors on the competitiveness of the processes examined. It was found that temperature, organic matter contents and the C/N ratio in the bottom sediment are statistically significant factors controlling the competitiveness of the transformations mentioned above.

1. INTRODUCTION

In anoxic conditions, in the top layer of the bottom sediment, nitrates may be reduced. The nitrate reduction is dominated by two processes: denitrification, in which nitrates are converted into gaseous oxides (NO and N_2O) and nitrogen, and dissimilatory nitrate reduction to ammonium (DNRA), (known also as nitrate ammonification), in which the final products are ammonium ions [11]. Due to denitrification, nitrogen is lost (released into the atmosphere), which leads to its depletion in aquatic environment. On the other hand, DNRA allows ammonia nitrogen to be available in the food-chain.

Until recently, large majority of researchers focused on denitrification. However, a growing number of research has been done lately on the nitrate ammonification process as well. According to some scientists, it may be as significant as denitrification itself [2], [16], whereas others claim that it achieves only minor values [1], [12]. The results of the research often differ a lot even in very similar environments.

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Therefore, the recognition of nitrate reduction processes together with the factors controlling them seems of importance, especially in the aquatic ecosystems threatened with eutrophication.

2. STUDY SITE

Investigations of dissimilatory nitrate reduction processes were conducted in the bottom sediment of a small, shallow and eutrophic Rzeszów Reservoir in the years 2002 and 2003.

Rzeszów Reservoir on the Wisłok River in south-eastern Poland was constructed in 1973. After 20 years of exploitation, its water depth decreased considerably, and fast growth of aquatic plants encroached on the previously open surface water. Consequently, large areas of the reservoir have silted up and gradually have been transformed into land [18]. Its volume decreased from 1.18 to 0.5 mln m³ and its mean depth from 1.5 to 0.5 m. The Wisłok River and the Strug River, main tributaries of the reservoir, are highly polluted with nutrients [10], which flow directly into the reservoir. The drainage basin of the reservoir has an agricultural character with a few industrial centers. Sediment and overlying water samples were collected at 4 stations, located on the shallow part of the reservoir (figure 1).

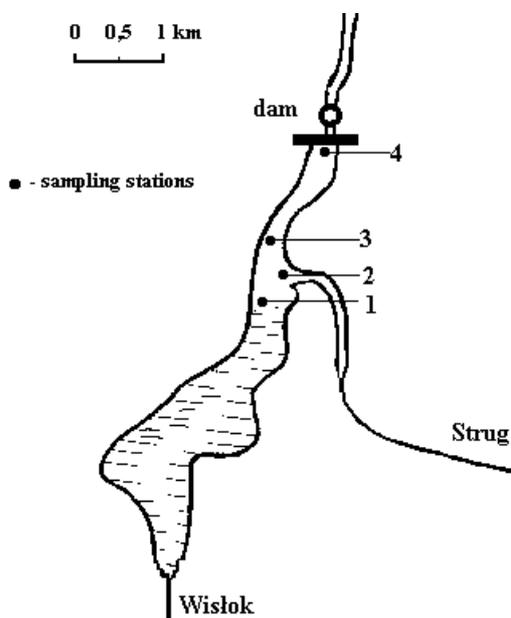


Fig. 1. Location of sampling stations on Rzeszów reservoir

3. METHODS

18 series of measurements were carried out during the period of investigation. The overlying water temperature ranged from 6 to 24 °C, and the water depth at the sampling stations was about 0.5 m. Each sampling series involved taking three sediment cores from every station. Undisturbed sediment cores were collected by means of plexiglass tubes, driven directly into the sediment. After closing the tubes with a rubber stopper at the bottom, they were placed in a thermostatic container, which enabled us to preserve natural conditions, and transported from the sampling site to the laboratory. During transportation, the container was filled with a 20-centimeter layer of overlying water from the sediment, which was a source of oxygen and nutrients. In the laboratory, the water in the tubes was replaced with some fresh water from the reservoir, which was allowed for about 15 minutes to equilibrate with the sediment pore water. The overlying water in each core was enriched with ^{15}N (0.1 cm³ of 15 mM K¹⁵NO₃). The amount of $^{15}\text{NO}_3^-$ added caused an increase of the in situ nitrate concentration in the overlying water of less than 10%. The sediment cores with the overlying water were then closed and incubated for 1.5 hours. In situ conditions (temperature) were maintained during that time. The water above the sediment was stirred gently by small Teflon-coated magnets suspended 5 cm above the sediment. A large external magnet rotated small magnets at 50 r.p.m. to ensure homogeneous mixing of the water columns, without affecting the sediment layer. When the incubation was stopped, the samples for ^{29}N and ^{30}N analyses were collected after carefully mixing the sediment (pore water) with the overlying water. Bacterial activity was inhibited by 250 µm³ of 7 M ZnCl₂. The same sample was collected for $^{15}\text{NH}_4^+$ analysis, and a few drops of chloroform inhibited bacterial activity. Denitrification and DNRA rates were calculated as a mean from the in vitro incubation results obtained for 3 cores sampled from each station.

N₂ was extracted from the water in glass gastight vials by replacing 2 cm³ of the sample with helium and shaking it vigorously for 5 minutes. The gaseous phase was then analyzed for the concentrations and isotopic distribution of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ with a gas chromatograph combined with isotope ratio mass spectrometer (IRMS DELTA⁺ Finnigan on line with GC/CIII). The concentration of $^{15}\text{NH}_4^+$ was determined by the use of the microdiffusion technique for KCl extracts of the sediment–water suspensions as described by RISGAARD-PETERSEN and RYSGAARD [14]. The $^{15}\text{NH}_4^+$ analysis was conducted using C and N Flash Elemental Analyzer, model EA 1112 (ThermoQuest), combined with DELTA^{PLUS} mass spectrometer (Finnigan).

The denitrification rates of added $^{15}\text{NO}_3^-$ (D₁₅) and *in situ* $^{14}\text{NO}_3^-$ (D₁₄) were calculated from the measured production of $^{14}\text{N}^{15}\text{N}$ (p²⁹N₂) and $^{15}\text{N}^{15}\text{N}$ (p³⁰N₂) [13]: D₁₅ = p²⁹N₂ + 2p³⁰N₂; D₁₄ = (p²⁹N₂/2p³⁰N₂)·D₁₅. The total *in situ* rate of dissimilatory $^{14}\text{NO}_3^-$ reduction to NH₄⁺ (DNRA) was estimated from the production rate of $^{15}\text{NH}_4^+$ (p¹⁵NH₄⁺) in anoxic–anaerobic NO₃⁻ reduction zone. Assuming that DNRA takes place

in the same sediment stratum as denitrification, the ^{15}N atom% of NO_3^- reduced to NH_4^+ is the same as the ^{15}N atom% of NO_3^- reduced to N_2 . The in situ rate of DNRA can therefore be calculated as: $\text{DNRA} = p^{15}\text{NH}_4^+ \cdot (D_{14}/D_{15})$. Further details are given by RISGAARD–PETERSEN and RYSGAARD [14].

In the overlying water its enrichment with ^{15}N was determined. The sediment samples were dried, ground and analyzed for organic matter (OM) as a loss in ignition at the temperature of $550\text{ }^\circ\text{C}$ [6], and the content of C_{tot} and N_{tot} was estimated by means of a CN Flash EA 1112, ThermoQuest Analyzer. For porosity measurement, the water content per volume of sediment was determined in each sediment by drying a known volume of the fresh sediment to a constant weight at $105\text{ }^\circ\text{C}$.

The Statistica PL program was used for statistical calculations. Multiple regression analysis method was used to determine simultaneous influence of a few independent variables on a dependent variable.

4. RESULTS AND DISCUSSION

The results obtained are shown in table 1. Denitrification rates in the bottom sediment of Rzeszów Reservoir over the two-year investigations ranged from 25.89 to $610.0\text{ }\mu\text{mol m}^{-2}\text{h}^{-1}$ (at the temperature between 7 and $23\text{ }^\circ\text{C}$). Fewer nitrates were reduced in the dissimilatory nitrate reduction to ammonium nitrogen than in the process of denitrification. During the study, DNRA rates were found to range from 0.2 to $41.09\text{ }\mu\text{mol m}^{-2}\text{h}^{-1}$.

Table 1

Denitrification and DNRA rates ($\mu\text{mol m}^{-2}\text{h}^{-1}$), mean values are shown in parentheses, $n = 18$

	Station 1	Station 2	Station 3	Station 4
Denitrification	35.2 – 610.0 (259.9)	27.6 – 441.7 (221.2)	25.9 – 478.8 (196.0)	26.3 – 414.2 (206.1)
DNRA	0.20 – 41.1 (14.5)	0.40 – 23.4 (9.1)	0.35 – 24.6 (10.9)	0.40 – 24.9 (10.7)

Neither denitrification rate (ANOVA, $F = 0.704$, $p = 0.553$), nor DNRA rate (Kruskal–Wallis Test, $H = 0.776$, $p = 0.855$) prove any significant statistical differences between particular stations. The details about the process rates as well as the description of abiotic factors controlling these rates were discussed in the former papers [4], [5], [19].

The main objective of this paper was to establish the influence of some chosen abiotic factors on the competitiveness of denitrification and DNRA. These factors can be itemized as follows: temperature, organic matter content and C/N ratio in the bottom sediment. Figures 2–4 show the relationships between mean values of the parameters examined.

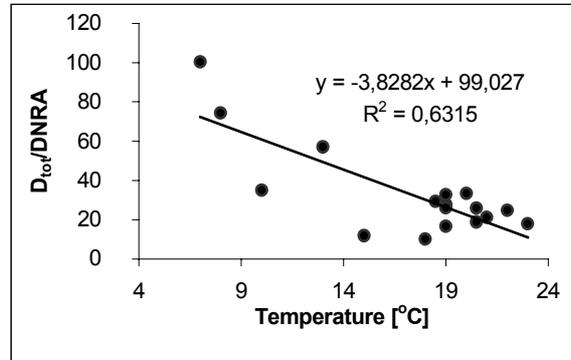


Fig. 2. $D_{tot}/DNRA$ ratio as a function of temperature

One of the factors influencing the competitiveness of the dissimilatory nitrate reduction processes in the bottom sediment is temperature. Negative correlation between temperature and $D_{tot}/DNRA$ ratio was found to reach a highly significant level of $p < 0.05$. An increase in temperature ensures greater contribution of DNRA to the whole process of dissimilatory nitrate reduction (figure 2). A similar correlation was presented by King and Nedwell, as can be seen in [7]. They show that lower temperature favours denitrification, while higher temperature favours DNRA, which implies that denitrification bacteria are psychrophiles, and bacteria ammonifying nitrates are mesophiles. KELLY-GERREYN et al. [7] also conclude that temperature is a significant factor, which influences the partitioning of nitrate reduction in the denitrification and DNRA processes. However, in their experiment, denitrification was a favoured pathway only in a narrow range of temperatures, i.e. 14 °C–17 °C, while above and below these temperatures DNRA occurred.

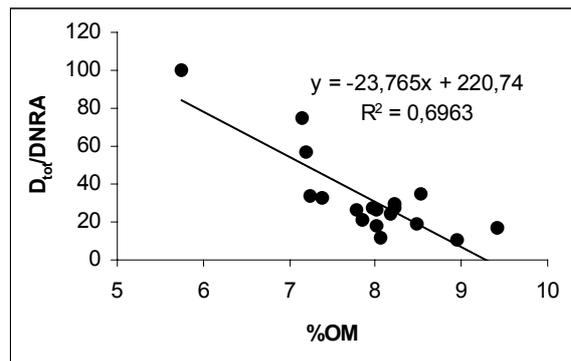


Fig. 3. $D_{tot}/DNRA$ ratio as a function of organic matter content in bottom sediment

Organic matter is connected with both DNRA and denitrification; directly by providing the electron donors and indirectly by taking up oxygen, thus creating anoxic conditions [9]. Our research showed that the content of organic matter in the sediment influences the competitiveness of the denitrification and DNRA processes. Negative, linear correlations were found between the $D_{tot}/DNRA$ ratio and the organic matter content (figure 3) ($p < 0.05$), which implies that a higher content of organic matter favours DNRA process.

Other researchers also generally agree that DNRA is favoured at the expense of denitrification in the environments rich in organic matter [1], [9], [20]. DNRA is enhanced in the sediments of a very high organic matter content, although usually its function in the bottom sediments does not seem essential [1], [3], [15].

The influence of C/N ratio on the competitiveness of the denitrification and DNRA processes was also investigated. It was observed that higher values of C/N ratio increased the DNRA contribution as compared to denitrification (figure 4).

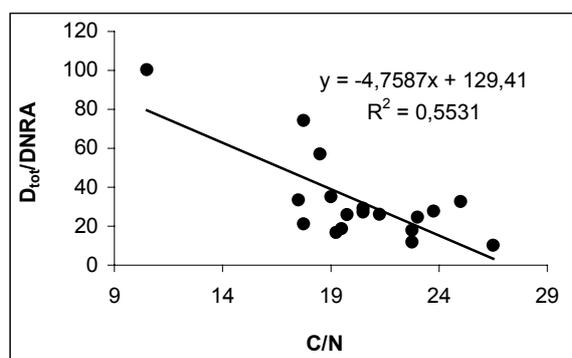


Fig. 4. $D_{tot}/DNRA$ ratio as a function of C/N ratio in bottom sediment

The relations obtained were statistically significant ($p < 0.05$). The ratio of the number of the available electron donors (e.g. carbon) to electron acceptors (e.g. NO_3^-) is an important factor influencing not only the type of transformation, but also its final products. DNRA is the favoured pathway when a limiting factor is NO_3^- , whilst denitrification is more significant when a limiting factor is carbon [1], [8], [17].

The multiple regression analysis (for the results see table 2) was used to determine the simultaneous influence of the parameters under examination on the competitiveness of the denitrification and DNRA processes in the bottom sediment. The model obtained describes about 43% ($R^2 = 0.4303$) of the total variation of a dependent variable. Such independent variables as temperature, C/N ratio and organic matter content in the bottom sediment were statistically significant ($p < 0.05$), despite a relatively low value of R^2 coefficient.

Table 2

Results of multiple regression analysis of influence of selected parameters on competitiveness of denitrification and DNRA processes for Rzeszów Reservoir

$R = 0.6560$; $R^2 = 0.4303$. Standard error of estimation: 23.154; $F(3.67) = 16.872$; $p < 0.001$				
	Coefficient B	Standard error of B	Significance level p	Semi-partial correlation
Intercept	132.628	17.044	< 0.001	
Temperature (T)	-3.260	0.654	< 0.001	-0.459
C/N ratio	-1.007	0.495	0.046	-0.188
Organic matter (OM)	-2.866	2.041	< 0.001	-0.129

The following model was obtained:

$$D_{\text{tot}}/\text{DNRA} = -3.260 T - 1.007 \text{ C/N} - 2.866\% \text{ OM} + 132.628 \pm 23.154.$$

Coefficients B in the model have negative values, which means that an increase in a given independent variable results in an increase of the contribution of DNRA process to the whole process of dissimilatory nitrate reduction. The analysis of the semi-partial correlation coefficients helped in ascertaining that (other variables excluded) temperature accounts for over 20% of the variance of the dependent variable. Therefore, temperature is the most significant factor influencing the competitiveness of the nitrate reduction processes. Its rise results in a higher contribution of DNRA process to the reduction in comparison with denitrification. For other variables, semi-partial correlation coefficients assume significantly lower values, the variables C/N and %OM are responsible for 3.5% and 1.7% of the variation in the dependent variable, respectively.

5. CONCLUSIONS

1. Denitrification rates in the bottom sediment of Rzeszów Reservoir over the two-year period of the study ranged from 25.9 to 610 $\mu\text{mol m}^{-2}\text{h}^{-1}$. The values are similar to the ones reported for eutrophic reservoirs.

2. Large majority of nitrates was reduced to N_2 in the denitrification process. The rate of dissimilatory nitrate reduction to ammonium was insignificant in comparison with that of denitrification, and the scanty values ranged between 0.2 do 42 $\mu\text{mol m}^{-2}\text{h}^{-1}$.

3. The temperature increase ensures a greater contribution of dissimilatory nitrate reduction to ammonium in the whole process of dissimilatory nitrate reduction.

4. Higher organic matter content in the bottom sediment improved the rate of both processes, which resulted in a bigger contribution of DNRA to the nitrate reduction.

5. The C/N ratio in the bottom sediment was significant in terms of the competitiveness of the dissimilatory nitrate reduction processes in bottom sediment. A higher value of C/N ratio increased the DNRA contribution as compared to denitrification.

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KONKURENCYJNOŚĆ PROCESÓW DYSYMLACYJNEJ REDUKCJI AZOTANÓW W OSADACH DENNYCH ZBIORNIKA ZAPOROWEGO W RZESZOWIE

Szybkość dysymilacyjnych procesów redukcji azotanów badano w osadach dennych zbiornika zaporowego w Rzeszowie. Do badań wykorzystano metodę ^{15}N IPT opartą na wprowadzeniu do układu stabilnego izotopu badanego pierwiastka (^{15}N) i śledzeniu ścieżek jego przekształceń. Podjęto również próbę określenia wpływu wybranych czynników abiotycznych na konkurencyjność badanych procesów. Stwierdzono, że temperatura, zawartość materii organicznej oraz stosunek C/N w osadach dennych są parametrami wpływającymi w sposób statystycznie istotny na konkurencyjność tych przekształceń.