

## INVESTIGATING THE SOURCE OF NITRATE IN POLAND'S SAN RIVER WATER BY ANALYSING THE NITROGEN AND OXYGEN ISOTOPIC RATIO

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**Abstract:** Nitrate concentrations and loads, and their nitrogen ( $\delta^{15}\text{N}$ ) and oxygen ( $\delta^{18}\text{O}$ ) stable isotope ratios were analysed in samples collected in SE Poland, from the River San and two of its tributaries. The river waters studied were in fact nitrate-poor (with ca.  $2 \text{ mgN L}^{-1}$ ), with loads calculated indicating an agricultural impact (range ca.  $2\text{-}40 \text{ kg N ha}^{-1}\text{year}^{-1}$ ). Nevertheless, the isotopic signature ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  within  $4.5\text{-}7\text{‰}$  and  $9.5\text{-}17\text{‰}$  respectively) for nitrate implies the influence of wastewaters and manure. This is confirmed by modeling using *IsoSource 1.3* software, which reveals shares of nitrate from the nitrification of wastewaters and manure in the range  $48\text{-}80\%$ . Multiple regression methodology was then used with results obtained in the present study and cited *in litt.*, in order to estimate the impact of the basin's agricultural area and the relationship between nitrate isotopic composition and nitrate content in surface waters. Therefore, the results suggest that stable isotope analysis can supply key information as regards the origins of nitrate, beyond that available using classical models based solely on catchment management and concentration data.

**Keywords:** Nitrate, stable isotopes, land use, superficial water, framework water directive

### 1. INTRODUCTION

Nitrate is one of more characteristic pollutants of waters, and derives from a variety of sources (Hill 1996). Its over-enrichment from anthropogenic sources is one of the major pressure on water ecosystems. Mostly, excess nitrate increase biological production and the availability of organic carbon within an ecosystem or eutrophication (Smolders et al., 2009; Koszelnik & Bartoszek 2011; Dunalska et al., 2013; Gołdyn et al., 2013). In general, two pathways of nitrate are distinguished: the direct discharge from industry or sewage management, (point sources), and scattered inputs, which are emitted over large areas what makes them difficult to locate and to attribute to a certain source (diffuse sources). Point sources are regulated relatively easily via optimization procedures applied to environmental technologies, whose steady development reduce emissions of pollutants into the aquatic environment (Kaleta et al., 2007; Tomaszek & Grabas 2007; Wiatkowski 2009; Josimov-Dunderski et al., 2012;). Diffuse sources can in turn

include agricultural run-off that may contain nitrate-containing fertilizers and manure (Fetter et al. 2012; Ristić et al., 2013). Moreover, atmospheric deposition of nitrate is connected with the natural oxidizing of atmospheric dinitrogen, as well as with industrial emissions of the oxides of nitrogen (Koszelnik 2007; Tobari et al., 2010; Fang et al., 2011; Tabayashi & Koba 2011). Despite atmospheric loads of nitrate make a negligible contribution to total nitrogen mass balances, very often due to the large supply of nitrate from surface sources (Koszelnik & Tomaszek 2000; Gaj & Bellaloui 2012) rainfall can deliver sizeable amounts of digestible nitrate to oligotrophic waters, especially in the circumstances of a polluted watershed and/or more intensive precipitation (Koszelnik 2007; Lo & Chu 2006).

The European Union's Nitrate Directive (ND; 1991) aims to safeguard the quality of water across Europe against eutrophication by preventing nitrate from agricultural sources from polluting surface waters, not least by promoting good farming practices. The designation of "vulnerable zones", i.e.

areas draining into polluted or threatened waters and contributing to nitrate pollution is one of the main topics of the ND. In Poland, levels of fertilizer use with artificial nitrogen sources reach  $150 \text{ kgN ha}^{-1}$ , the average for the country as a whole being  $50 \text{ kgN ha}^{-1}$ . The use of manure is on a similar level (Król & Sokół 2006; Gaj & Bellaloui 2012). Fertilizer and sewage causing harm, not only to inland waters, but also to the Baltic Sea, which receives a higher loadings of pollutants from the territory of Poland than any other state around the Sea's coast (Giełczewski et al., 2013).

Nitrate may be applied in large amounts to the arable land. Many of the fertilizer is then taken up by crop plants (as intended), while a further 20% becomes bound to the ground and then surface waters. However,  $\text{NO}_3^-$  is not simply externally loaded to waters, but also continually turned over within the catchment by internal biogeochemical processes such as assimilation, remineralization, nitrification and denitrification. The only natural process that may control the  $\text{NO}_3^-$  load of a bodies of water is bacterial denitrification (Sebilo et al., 2003; Sutyla et al., 2009; Gruca-Rokosz et al., 2009).

This complexity as regards sources makes interpretation of the economy of nitrate in the environment problematical. However, in recent years, the stable isotope signatures of nitrogen ( $\delta^{15}\text{N}$ ) and oxygen ( $\delta^{18}\text{O}$ ) have been used successfully in attributing the nitrate present in both the ground and surface waters (Mayer et al., 2002; Voss et al., 2006; Tobari et al., 2009; Tabayashi & Koba 2011; Koszelnik 2009; Lee et al., 2009; Li et al., 2010; Itoh et al., 2011). The methodology is based on the observation of isotopic fractionation, resulting in an isotopic signature present at various stages of the nitrate flux. The nitrate in fertilizers produced from atmospheric air has an N isotope ratio close to air dinitrogen (0‰) and oxygen (23.5‰) (Amberger & Schmidt 1987). The isotopic composition of nitrate resulting from mineralization of organic nitrogen and then nitrification in soils is depleted of the heavier  $^{15}\text{N}$  when compared with the substrate ( $\text{N}_{\text{org}}$  or ammonia). Otherwise, nitrate from manure and wastewaters arising from the same biochemical fluxes loses  $^{14}\text{N}$  due to volatilization (Mayer et al., 2002, Kendall et al., 2007, Snider et al., 2010).  $\delta^{18}\text{O}$  of nitrate from nitrification originates mainly from  $\text{H}_2\text{O}$  and is thus dependent on the water source (Lee et al., 2009; Snider et al., 2010). Even during denitrification, when systematic depletion of the lighter isotopes of both N and O is observed,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of the residual nitrate are increased (Knöller et al., 2011).

For these reasons, analysis of both  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values for nitrate provides a tool by which to distinguish between the sources of nitrate mentioned, as is shown on figure 1.

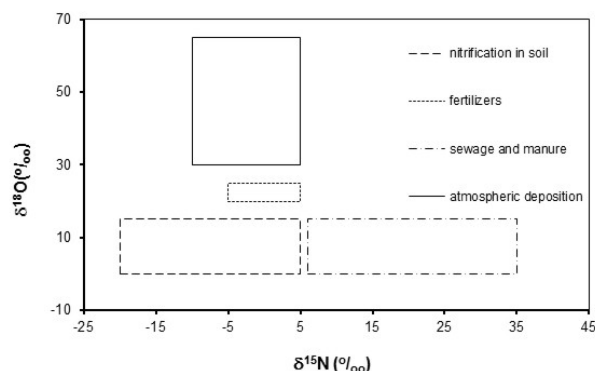


Figure 1. Isotopic signatures for different nitrate sources.

This paper reports the analysis of nitrogen content and loads, as well as the contribution of nitrate sources supplied, in the case of the San river system (SE Poland), where the catchment is of a kind that results in eutrophication being noted in the river waters (Lipińska et al., 2009). In our opinion the paper brings the new concept of interpretation of nitrate sources in water with low nitrate concentrations. The results obtained are to improve management of nitrate sources in water management in line with Water Framework Directive, Nitrate Directive and Helsinki Convention. It was thus deemed likely that results obtained would be of more general application to nitrate management in various of the Central-Eastern Europe countries, and most especially their Carpathian regions. A final objective was to test for whether nitrate source expressed in terms of the N and O nitrate isotopic composition would show a relationship with nitrate content in river waters on sustainable forestry and agricultural catchments.

## 2. MATERIALS AND METHODS

### 2.1. Studied area

The River San flows through SE Poland, (Fig. 2). The river arises at an elevation of 900 m a.s.l. (at  $49^{\circ}00'10''\text{N}$   $22^{\circ}52'30''\text{E}$ ). It is a right-bank tributary of the Vistula, with a length of 443 km making this Poland's 6th-longest river. It's the point of its confluence is at 200 m a.s.l. (and at  $50^{\circ}44'14''\text{N}$   $21^{\circ}50'36''\text{E}$ ).

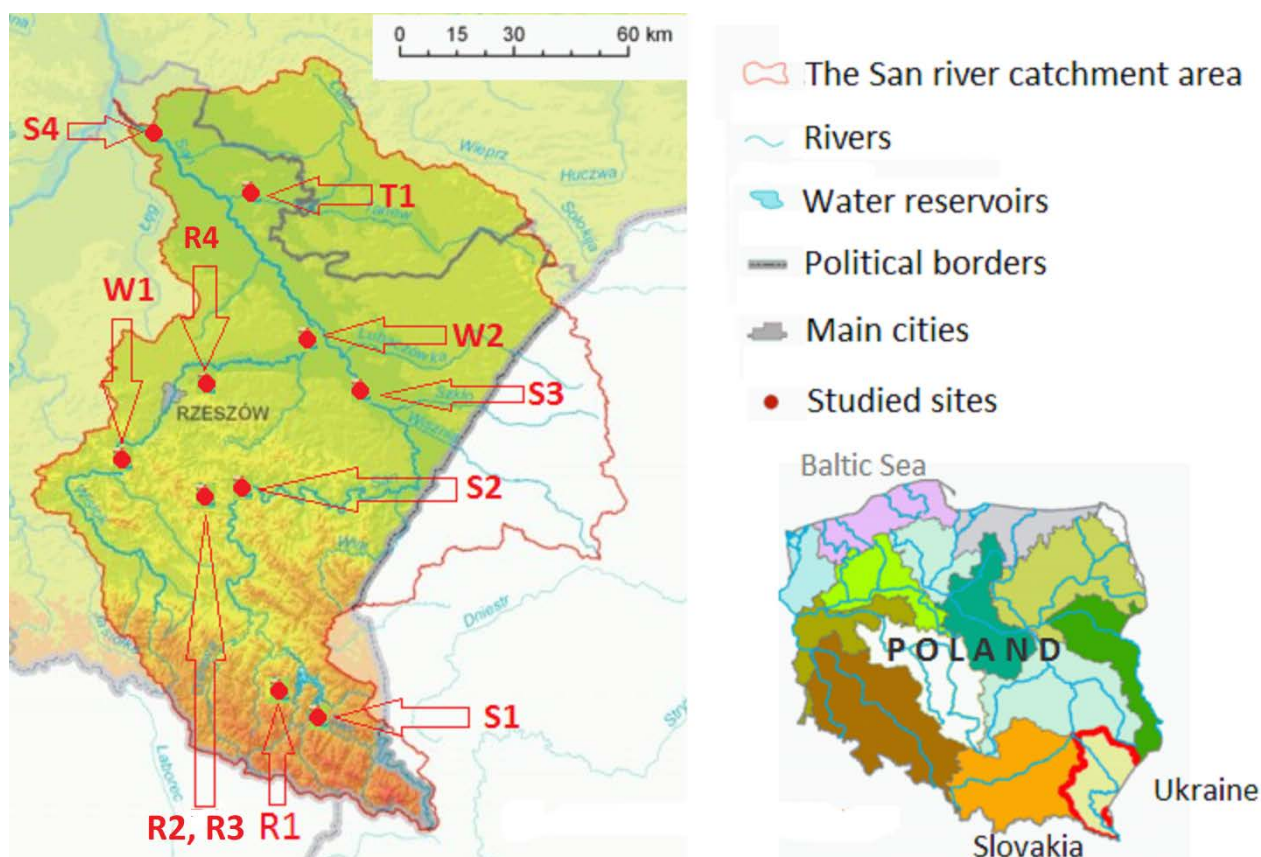


Figure 2. Location of study sites.

The San and its tributaries represent a major source of drinking water for 60% of the population living within its catchment area, and are also important elements where local management, planning and the landscape are concerned. The upper part of the river catchment comprises mountainous areas dominated by forest cover. Farmed agricultural land and settlements are mainly located at the mouths of tributary valleys, and it is in such areas that the San passes through the complex of bodies of water known as the Solina-Myczkowce Reservoirs. Together, the latter account for some 15% of overall water storage in Poland, having a volume of c. 500 Mm<sup>3</sup>, and a surface area of 22 km<sup>2</sup>. The mean hydraulic retention time (HRT) of the reservoirs is 270 days, and mean depth is 22 m (max 60 m) Koszelnik et al., 2007).

As the San passes down through its catchment area it assumes features more and more typical of a lowland river, following through an increasingly agricultural landscape. Nevertheless, farms are small here (*ca.* 5 ha) and characterised by “outdated” extensive farming methods. Organic fertilizers are used mainly, besides nitrate are most common artificial fertilizers (85%) (Król & Sokół 2006, Koszelnik 2009). Overwhelmingly, the farm households in question produce for their own needs only, while about half of the households concerned

are not connected to either sewer or septic systems. After the changes forced by the economic crisis in the 80's, both farming and land use as well as the population density did not change significantly. The catchment characteristics are as shown in table 1.

Table 1. Parameters of the analysed catchment areas.

Site	Location on river course (km)	Catchment area (km <sup>2</sup> )	Mean long-term flow (m <sup>3</sup> s <sup>-1</sup> )	Agricultural (%)	Meadows and pastures (%)	Forests (%)	Other (%)	Population (Inh. km <sup>-2</sup> )
S1	352.2	603	20.1	3.1	15.3	80.0	1.6	6.1
S2	234.9	2922	47.2	13.9	18.0	63.1	5	33.5
S3	124.8	7014	73	25.4	12.7	55.5	6.4	52.0
S4	2.0	16861	124	37.0	15.1	42.2	5.7	108.3
T1	1.0	2340	13	47.0	9.9	32.6	10.5	61.5
W1	91.7	1420	14.2	43.5	16.5	34.9	5.1	124.1
W2	5.8	3540	26	44.7	18.1	30.4	6.8	195.1

River water was sampled monthly between March 2007 and February 2008, at four sites along the San (Fig. 1: S1-S4); two sites along its largest left-bank tributary the Wisłok (Fig. 2: W1-W2); as well as from its largest right-bank tributary, the

Tanew (Fig. 2: T1). Site S1 is located next to the point of discharge of the river into the Solina Reservoir. Sites S4, W2 and T1 are located at rivers mouths. All the studied sites fall within the hydrological network of the Polish Institute of Meteorology and Water Management, at which daily rates of water flow (Q) are monitored continuously.

Furthermore, to provide for a reference study, tests for the N and O stable isotope ratio in the nitrate, and mineral nitrogen content in water from different sources were carried out. The selected study sites were characterised by a unilateral kind of management, therefore the analysed nitrate was not derived from a number of sources but only from one. Three areas under different forms of management were selected for this purpose. Firstly, waters from shallow wells and a drainage ditch, where natural nitrification is the most likely source of nitrate ( $N_{\text{soil}}$ ), were sampled and analysed (site R1). Secondly, the same analysis was conducted for an agricultural area, where there is intensive use of synthetic fertilisers ( $N_{\text{fer}}$ ). Within the studied area the most common  $KNO_3$  fertilisers were used (site R2). Water from a drainage ditch was studied in this case. Thirdly, determinations were made for water from a drainage ditch running through a village that lacked sewerage, in which wastewater is consequently discharged into leaky septic tanks (site R3). It was assumed that nitrate in these samples of water originated in the sewage or manure ( $N_s$ ). Moreover, atmospheric water was also analysed (as  $N_{\text{at}}$ ) within the R1 site area, and at site R4, located near the city of Rzeszów, with a view to identifying the influence of industry. The results of reference isotopic analyses for nitrate from the different sources are shown in Table 2. ANOVA showed that the end-member values are differ from one another and may be used. Details of the reference study was described in former paper (Koszelnik & Gruca-Rokosz, 2013).

## 2.2. Sampling and analytical methods

For the nitrate origin study, each site provided ca. 7 L of water (about 10 mg of nitrate-nitrogen in each sample) collected for the elemental and isotopic analyses. Water was sampled using a telescopic sampler - into rinsed plastic bottles, subsequently being cooled and processed within 4 hours. Conductivity, total oxygen content, pH and temperature were measured in situ with a MultiLine P5 meter (WTW, Germany). About 0.5 L of glass-fibre-membrane-filtered water was made subject to spectrophotometric determinations for concentrations of nitrate-nitrogen ( $N-NO_3^-$ , salicylate method, coefficient of variation for the

procedure – CVP:  $\pm 1.5\%$ ), nitrite-nitrogen ( $N-NO_2^-$ , Griess reaction, CVP:  $\pm 1.7\%$ ), ammonium-nitrogen ( $N-NH_4^+$ , Berthelot's reaction, CVP:  $\pm 1.4\%$ ) and phosphate-phosphorus ( $P-PO_4^{3-}$ , Molybdate method, CVP:  $\pm 1.8\%$ ) using a PhotoLab S12 spectrophotometer (WTW, Germany). Moreover, Kjeldahl nitrogen ( $N_{\text{Kjeld}}$ ), as well as total phosphorus (TP, oxidation to phosphate), were analysed in non-filtered samples. Total nitrogen (TN) was calculated as the sum of nitrate- and nitrite-nitrogen and  $N_{\text{Kjeld}}$ . All methods with the exception of those for ammonium- (Rand et al., 1976) and nitrate-nitrogen (Monteiro et al., 2003) were after Hermanowicz et al., (1999). Nutrient loads from the catchment were calculated for each gauging station using known catchment area, Q and concentrations, while concentrations between sampling days were approximated using the least squares method.

Table 2. Mean values  $\pm$  standard deviations for nitrate ( $N-NO_3^-$ ), ammonium ( $N-NH_4^+$ ) concentration and the oxygen ( $\delta^{18}O$ ) and nitrogen ( $\delta^{15}N$ ) isotopic signature in nitrates from reference waters (upper box) used in modeling calculations. p-values of ANOVA for nitrogen (*italic*) and oxygen (**bold**) isotopic signatures in nitrates from reference waters (lower box). Abbreviations as in text. Data from Koszelnik & Gruca-Rokosz (2013).

Index	Unit	$N_{\text{soil}}$	$N_{\text{fer}}$	$N_s$	$N_{\text{at}}$
	Site	R1	R2	R3	R1,R4
$N-NO_3^-$	mg L <sup>-1</sup>	2.96 $\pm 2.11$	7.62 $\pm 3.24$	5.84 $\pm 3.21$	0.53 $\pm 0.33$
$N-NH_4^+$	mg L <sup>-1</sup>	0.21 $\pm 0.15$	2.31 $\pm 2.05$	3.79 $\pm 2$ .98	0.31 $\pm 0.11$
$\delta^{15}N$	‰	2.9 $\pm 1.5$	0.4 $\pm 4.1$	8.9 $\pm 1.8$	48.0 $\pm 8.3$
$\delta^{18}O$	‰	2.8 $\pm 0.7$	17.9 $\pm 5.2$	7.7 $\pm 8.7$	-1.4 $\pm 2.7$
ANOVA $\alpha < 0.05$	$N_{\text{soil}}$	-	0.1593	0.0000	0.0026
	$N_{\text{fer}}$	<b>0.0008</b>	-	0.0007	0.3415
	$N_s$	<b>0.0875</b>	<b>0.0359</b>	-	0.0000
	$N_{\text{at}}$	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	-

Preparation for isotopic analyses of nitrate followed Silva et al. (2000). 4-6 L of sampled water was filtered through a glass membrane and transferred to the anion-exchange resin (Bio-Rad, AG1-X8, 200-400 mesh, Cl<sup>-</sup>) to immobilization of nitrate. The anion was then eluted with 20 cm<sup>3</sup> of 3M HCl, the obtained solution being neutralized to pH 5-6 using Ag<sub>2</sub>O. Precipitated AgCl was drained off, and the solution containing AgNO<sub>3</sub> freeze-dried. 30% of the solid AgNO<sub>3</sub> was placed on a silver capsule for nitrogen isotope analysis in the system:

Elemental Analyzer (Thermo Quest, Flash 1112) coupled with an IRMS spectrometer (DELTA<sup>Plus</sup>, Finnigan Mat). The rest of the sample was weighed into a quartz tube with 2 mg of pure graphite (Merck). The evacuation tube was then sealed, and combusted in a furnace at 850°C to produce CO<sub>2</sub>. The resulting gas was purified cryogenically on a vacuum line and transported to a Gas Chromatograph Combustion III system coupled with IRMS spectrometer for oxygen-isotope analysis. The data for isotope <sup>15</sup>N:<sup>14</sup>N and <sup>18</sup>O:<sup>16</sup>O ratios showed as δ<sup>15</sup>N and δ<sup>18</sup>O were expressed in permil (‰) versus Air and VSMOW standards respectively, as follows:

$$\delta^{15}\text{N} \text{ or } \delta^{18}\text{O} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \quad (1)$$

where *R* denotes <sup>15</sup>N:<sup>14</sup>N and <sup>18</sup>O:<sup>16</sup>O. The precision of the measurement was verified by analysis of laboratory standard IAEA-NO3. The standard deviation for the isotopic analysis was of less than 0.2‰.

To simulate the shares of different nitrate sources, the *IsoSource 1.3* software package was applied, this calculating ranges of source proportional contributions to a mixture on the basis of stable isotope analyses when the number of sources is too large to permit a unique solution (Phillips et al., 2005). All obtained results were processed using *MS Excel* tools. Descriptive statistics, correlation analysis, multiple regression analysis and ANOVA have also been applied.

### 3. RESULTS

#### 3.1. Chemistry of analysed waters

The analysed river waters were slightly alkaline (max. noted pH 8.21; Table 3) and well oxygenated (Table 3). Moreover, these waters were nitrate-poor (Table 3). Higher concentrations of nitrate were noted at the San mouth, mean 2.23 mg N L<sup>-1</sup>. Much nitrate was present at upper site S1, at which the mean concentration amounted to 1.66 mg N L<sup>-1</sup>. The waters of the River Tanew yielded a mean concentration of just 0.88 mg N L<sup>-1</sup> (Table 3). The variability of nitrate concentrations was negligible, a maximum of 4.1 mg N L<sup>-1</sup> was observed at S4, and the standard deviations for values at the different sites ranged between 0.22 and 0.83 mg N L<sup>-1</sup>. Total nitrogen mean concentrations ranged between the 1.84 mgN L<sup>-1</sup> noted in the River Tanew and the 2.82 mg N L<sup>-1</sup> reported for the water at the San mouth (Table 3). Nitrite-nitrogen concentrations ranged from 0.01 to 0.06 mgN L<sup>-1</sup>. In the case of ammonium-nitrogen, differences

associated with the locations of stations were not noted, other than at station W2. Mean concentrations varied from 0.09 to 0.21 mgN L<sup>-1</sup>, but at W2 the value was as high as 0.61 mgN L<sup>-1</sup>.

Mean concentrations of both total P and phosphate-phosphorus increased steadily down the San, from 0.12 mgP L<sup>-1</sup> and 0.05 mgP L<sup>-1</sup> at S1 to 0.21 mgP L<sup>-1</sup> and 0.11 mgP L<sup>-1</sup> at S4 respectively. Water from the Tanew was phosphorus-poor (mean 0.11 mgP L<sup>-1</sup> of TP and 0.04 mgP L<sup>-1</sup> of phosphate). TP values for the River Wisłok were on average 0.18 mgP L<sup>-1</sup> and 0.19 mgP L<sup>-1</sup>, while phosphate values were highest (of 0.12 and 0.14 mgP L<sup>-1</sup> on average, at stations W1 and W2 respectively. Molar ratios for total forms of N:P were usually higher than stoichiometric 16:1. The mean values for the ratio decreased down the San river, from 41:1 to 21:1 at S1 and S3 respectively, though an increase to 32:1 was noted at S4. At the mouths of tributaries of the San similar values of 37:1 (W2) and 38:1 (T1) were observed. The routine study carried out by the Environmental Protection Inspectorate points to eutrophication of the waters of the San. *Chl. a* concentration downriver reaches 20 µg L<sup>-1</sup> (Table 3).

#### 3.2. Land use and nitrogen mass-balance

Under the FAO classification, the soils in the catchment are defined mainly as podzols. The river valleys are filled with alluvial soils, while the upper catchment (in the Bieszczady Mountains) features Carpathian flysch. Watershed parameters are as shown in Table 1. Data corresponding to site S4 are identical to the characteristics for the San watershed as a whole. The river system under analysis is located upstream of the Vistula river watershed, and forests constitute more than 40% of its area. The arable area increased downstream from 3% upstream of site S1 to 38% of the area. The agricultural impact is more noticeable within the catchment of the Wisłok, where arable land accounts for between 43 and 47% of the area, as well as in the area of the Tanew (47%). The value for grassland cover is 15% overall, this varying rather little in different parts, between 9.9 and 18.1%. Urban and industrial areas are insignificant, owing to the low population density (Table 1), these therefore being included in the “others” category (accounting for ca. 5% overall), in relation to their limited importance.

Descriptive statistics (median, mean, maximum and minimum values) for TN and N-NO<sub>3</sub><sup>-</sup> runoff from monitoring sites are as summarized in figure 3. Mean total nitrogen runoff from the whole San catchment area (S4) was of 9.82 kg ha<sup>-1</sup>year<sup>-1</sup>, and ranged from 39.32 to 1.93 kg ha<sup>-1</sup>year<sup>-1</sup>.

Table 3. Mean values  $\pm$  standard deviations for analysed indexes in the studied river waters. Range of results are presented in the case of *Chl a*, temperature and pH. *Chl a* from Lipińska et al. 2009

Index	Site	S1	S2	S3	S4	W1	W2	T1
	Unit							
N-NO <sub>3</sub> <sup>-</sup>	mg L <sup>-1</sup>	1.66 $\pm$ 0.34	1.26 $\pm$ 0.45	1.25 $\pm$ 0.24	2.23 $\pm$ 0.83	1.33 $\pm$ 0.43	1.46 $\pm$ 0.52	0.88 $\pm$ 0.22
N-NO <sub>2</sub> <sup>-</sup>	mg L <sup>-1</sup>	0.01 $\pm$ 0.00	0.02 $\pm$ 0.20	0.01 $\pm$ 0.01	0.01 $\pm$ 0.00	0.06 $\pm$ 0.05	0.07 $\pm$ 0.06	0.01 $\pm$ 0.00
N-NH <sub>4</sub> <sup>+</sup>	mg L <sup>-1</sup>	0.16 $\pm$ 0.07	0.21 $\pm$ 0.15	0.09 $\pm$ 0.05	0.10 $\pm$ 0.09	0.08 $\pm$ 0.08	0.61 $\pm$ 0.38	0.12 $\pm$ 0.08
N <sub>tot</sub>	mg L <sup>-1</sup>	2.47 $\pm$ 0.27	2.46 $\pm$ 0.54	2.39 $\pm$ 0.49	3.82 $\pm$ 0.88	2.49 $\pm$ 0.35	3.79 $\pm$ 1.27	1.84 $\pm$ 0.51
P-PO <sub>4</sub> <sup>3-</sup>	mg L <sup>-1</sup>	0.05 $\pm$ 0.05	0.05 $\pm$ 0.07	0.07 $\pm$ 0.08	0.11 $\pm$ 0.13	0.12 $\pm$ 0.11	0.14 $\pm$ 0.11	0.04 $\pm$ 0.02
P <sub>tot</sub>	mg L <sup>-1</sup>	0.12 $\pm$ 0.09	0.18 $\pm$ 0.11	0.19 $\pm$ 0.11	0.21 $\pm$ 0.19	0.18 $\pm$ 0.17	0.19 $\pm$ 0.14	0.11 $\pm$ 0.08
N:P	Mol:1	41 $\pm$ 24	27 $\pm$ 21	21 $\pm$ 14	32 $\pm$ 23	26 $\pm$ 20	38 $\pm$ 27	37 $\pm$ 21
<i>Chl a</i>	μg L <sup>-1</sup>	1 $\div$ 2	4 $\div$ 6	7 $\div$ 13	15 $\div$ 20	-	-	-
pH	-	7.84 $\div$ 8.21	7.32 $\div$ 7.81	7.27 $\div$ 7.76	7.22 $\div$ 7.60	7.34 $\div$ 7.49	7.12 $\div$ 7.27	7.11 $\div$ 7.27
O <sub>2</sub>	mgO <sub>2</sub> L <sup>-1</sup>	8.67 $\pm$ 1.91	7.91 $\pm$ 1.49	7.79 $\pm$ 1.24	7.45 $\pm$ 1.25	7.51 $\pm$ 1.94	7.44 $\pm$ 2.17	8.55 $\pm$ 1.66
Temperature	°C	1 $\div$ 21	1 $\div$ 24	1 $\div$ 24	1 $\div$ 25	1 $\div$ 23	1 $\div$ 24	1 $\div$ 22

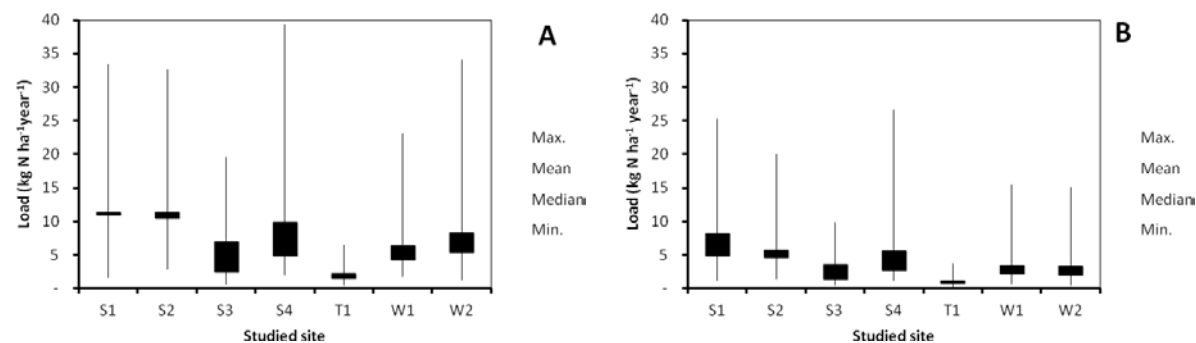


Figure 3. Boxplots of total nitrogen (A) and nitrate-nitrogen (B) discharge from the studied basins. Sequentially from top: maximum, median, mean and minimum values. Abbreviations as in text.

Considerably higher loads of nitrogen of about  $11.3 \text{ kg ha}^{-1}\text{year}^{-1}$  were exported from the upper parts of the river watershed (S1 and S2) with a range from *ca.* 33 to  $2 \text{ kg ha}^{-1}\text{year}^{-1}$ . An increase in mean TN loads from  $6.38$  to  $8.34 \text{ kg ha}^{-1}\text{year}^{-1}$  with a wide range from  $1.71$  to  $23.1 \text{ kg ha}^{-1}\text{year}^{-1}$  and  $1.20$  to  $34.13 \text{ kg ha}^{-1}\text{year}^{-1}$  was observed along the Wisłok, at stations W1 and W2 respectively. The TN export from the Tanew river catchment area (T1) was inconsiderable: mean  $2.23 \text{ kg ha}^{-1}\text{year}^{-1}$ , with a range of  $0.42$ - $6.53 \text{ kg ha}^{-1}\text{year}^{-1}$ . Nitrate-nitrogen loads accounted for the greatest share of total nitrogen (50-70%), while variations in loads were similar to those noted for TN. Shares above 50% were reported as characteristic of the agricultural area, with a predominance of non-point sources of nitrogen (Billen et al. 1991, Voss et al. 2006). Other than at station T1, mean TN loads ranged between  $6.38$  and  $11.36 \text{ kg ha}^{-1}\text{year}^{-1}$ .

### 3.3. Isotopic composition of the studied waters

Statistical analysis of the isotopic compositions for nitrate sampled from the analysed gauging stations indicates three statistically distinguishable kinds of station (Fig. 4). Mean values (of  $9.3\text{‰}$  and  $4.6\text{‰}$  respectively) for the  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  in nitrate differed significantly between site S1 and the others. Except at S1, depletion of heavier isotopes  $\text{N}^{15}$  and  $\text{O}^{18}$  was noted along the San (mean from  $7.1$  and  $17.7\text{‰}$  respectively at S2 to  $5.9$  and  $16.3$  respectively at S4), these values being indistinguishable. The inverse phenomenon was observed in the waters of the Wisłok, in which mean  $\delta^{15}\text{N}$  decreased from  $5.8\text{‰}$  (W1) to  $6.4\text{‰}$  (W2) and  $\delta^{18}\text{O}$  from  $12.9\text{‰}$  to  $13.4\text{‰}$ . The stable-isotope composition for nitrate sampled from the T1 site was close to that for sites W1 and W2 (mean  $\delta^{15}\text{N}$ :  $6.1\text{‰}$  and  $\delta^{18}\text{O}$ :  $13.3\text{‰}$ ).  $\delta^{15}\text{N}$  varied seasonally across a wide range of values (Std. Dev.:  $1.6$ - $3\text{‰}$ ), as compared with  $\delta^{18}\text{O}$  (Std. Dev.:  $2.2$ - $4.1\text{‰}$ ).

### 3.4. Nitrate source

To identify the source of mixed material on the base of its isotopic signature, end-member mixing models are often used. These models are limited in scope to two components using a single tracer or three components using a pair of tracers. In the case of the *IsoSource 1.3* model used in the study, each of the individual solutions represents a combination of four source proportions that satisfies the isotopic mass balance in the mixing model. Descriptive statistics (number of solutions, mean,

standard deviation, minimum, maximum) for each source are provided as a way to characterize this entire distribution of feasible solutions.

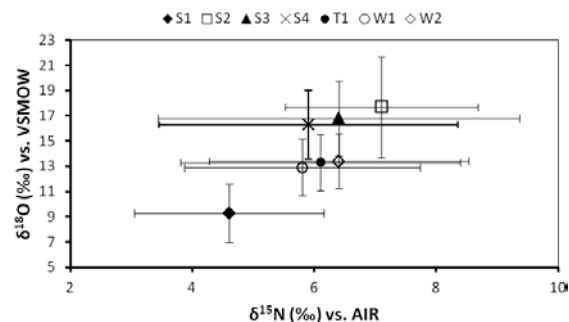


Figure 4. Mean values and standard deviations for nitrate isotopic signatures at the studied sites. Abbreviations as in text.

Table 4. Results of modeling calculation using *IsoSource 1.3*. Mean values  $\pm$  standard deviations and ranges for shares of nitrate from different sources presented. Abbreviations as in text.

Source (%)	S1	S2	S4	S3	W1	W2	T1
$\text{N}_{\text{soil}}$	$31.4 \pm 7.4$	$1.5 \pm 2.1$	$0.4 \pm 0.1$	$0.6 \pm 0.0$	$5.8 \pm 5.9$	$1.9 \pm 3.2$	$3.6 \pm 4.4$
	19÷43	0÷6	0÷1	0÷1	0÷19	0÷7	0÷12
$\text{N}_{\text{fer}}$	$15.3 \pm 8.7$	$2.0 \pm 8.9$	$0.4 \pm 0.0$	$0.6 \pm 0.0$	$6.9 \pm 5.9$	$2.4 \pm 3.3$	$4.3 \pm 4.5$
	2÷30	0÷8	0÷1	0÷1	0÷23	0÷8	0÷15
$\text{N}_{\text{s}}$	$48 \pm 3.8$	$71.7 \pm 2.5$	$75 \pm 3.2$	$74.6 \pm 3.4$	$73.7 \pm 4.9$	$80.6 \pm 3.5$	$77.2 \pm 4.4$
	45÷51	69÷76	74÷76	74÷76	65÷84	77÷86	71÷86
$\text{N}_{\text{at}}$	$5.3 \pm 3.1$	$24.8 \pm 2.5$	$24.2 \pm 1.9$	$24.2 \pm 1.8$	$13.6 \pm 1.7$	$15.1 \pm 1.2$	$14.9 \pm 2.2$

To avoid the misrepresentation of the results, the distribution of feasible solutions as well as the mean and standard deviation are reported in Table 4. Modeling using *IsoSource 1.3* most probably show the origin of nitrate in waters of the San as organic sources usually summarized as sewage/manure/ septic systems. A range in values of between 69 and 76 % was estimated for stations S2, S3 and S4. Simultaneously, nitrate from the atmosphere has a 24-25%, share of  $\text{NO}_3^-$ , with other sources irrelevant ( $<1\%$ ). The upper station S1 reported a more even distribution. Sewage was the main source of nitrate, ranging widely between 45 and 51%, but the influence of forest soil nitrification is also noticeable (19-45%). Moreover, 2-30% of nitrate originates from mineral fertilizer, and up to 10% from the atmosphere. In the case of both tributaries of the San under study, some 75% of identified nitrate originated from sewage, while more than 12% is from the atmosphere and only a small proportion from other sources.

## 4. DISCUSSIONS

### 4.1. Source of the nitrate

The range of calculated nitrogen loads is characteristic for a watershed in which sustainable agriculture is being practiced (Billen et al., 1991, Koszelnik et al., 2002, Mayer et al., 2002, Vink et al., 2007). In fact, this interpretation is not fully compatible with the real-life management of the catchment, in line with high forest cover (Table 1), most especially in the cases of stations S1 and S2, where mean total N-loads are of about 11 kgN ha<sup>-1</sup>year<sup>-1</sup>. The catchment management suggests a slightly more limited nitrogen supply. On the basis of land-cover models, N-loads were estimated during earlier studies at 5 kgN ha<sup>-1</sup>year<sup>-1</sup> at S1 (Koszelnik 2009). Moreover forest land generates smaller amounts of nitrate from nitrification alone in soils (Tobari et al. 2005). Generally, forest covers the upper parts of the San watershed, in contrast to the agricultural and urban areas located near the river banks elsewhere.

The important role of riparian processes in reducing nutrient- (especially nitrate-) loadings to waters is well known (Cey et al., 1999, Sebilo et al., 2003). The absence of a riparian zone may result in a shift of natural nitrogen retention and denitrification within the soil to river/lake systems. Therefore, eutrophication of inland and marine waters is not only a result of an increase in inputs of nitrogen (and phosphorus) to surface water, but also a reflection of a decrease in the retention of both elements in land-water systems. Such trends are to be observed in Europe and North America (Billen et al., 1991, Mayer et al., 2002, Zalewski 2006, Koszelnik 2009). However, deforestation is one of the most important environmental problems to affect the shaping of global climate, as well as local environmental conditions, due to the increases noted in rates of soil erosion, as well as the decline in biodiversity. Nitrogen export from the catchment is directed by interactions between the hydrological cycle and the biochemical and geochemical process occurring in waters and soils (Vink et al. 2007, Burt et al. 2010). Long-term studies suggest an increase in N-NO<sub>3</sub><sup>-</sup> concentrations (and thus also loads) in inland waters (Burt et al. 2010). Apart from the phosphorus, nitrogen loadings are a significant concern when it comes to surface-water eutrophication, ecosystem health and the quality of drinking water (Domagalski et al., 2007, Bartoszek & Tomaszek 2008, Burns et al., 2009).

Analysis of nitrate isotope signatures confirms conclusions based on calculated N-loads rather than

catchment management. Moreover, it suggests new arguments to facilitate the nitrate-origin interpretation in case S1, in which N-loads are higher than expected. The lower values of both the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  parameters noted at that site (means 4.6 and 9.3‰ respectively) suggest natural nitrification. Similar values are reported for well-forested areas (Spoelstra et al., 2001, Voss et al., 2006, Tobari et al., 2010). Nevertheless, calculations made using *IsoSource 1.3*, in relation to reference studies, showed that less than a half (c. 31.8%) of the nitrate is of soil origin, while the major source is wastewater and manure (ca. 48%). These findings may reflect an extremely low wastewater management standard. Indeed, most households are not connected to wastewater treatment plants, while mass tourism tends to generate additional pollutants discharged into the ground or escaping there via leaking septic tanks (Lipińska et al., 2009). Likewise, the slope of the mountain watershed favors the loss of fertilizers (ca. 15.3%) and manure. These observations are supported by the significant correlation obtained between N-NO<sub>3</sub><sup>-</sup> and Q at the gauging station ( $\text{N-NO}_3^- = 1.353 + 0.032Q$ ;  $R^2 = 0.637$ ;  $p = 0.002$ ). Similar findings were not obtained in relation to other sites. Anthropogenic processes within the River San shift the stable isotope distribution to more enriched levels of  $^{15}\text{N}$  and  $^{18}\text{O}$ , (Domagalski et al., 2007, Li et al., 2010) as can be observed at sites S2, S3 and S4, where N and O isotopic signatures vary across narrow ranges (see Fig. 4). These signatures, as well as those noted at the W1, W2 and T1 sites are broadly within the range typically noted for agricultural catchments (Kendall et al., 2007), although in many cases lower values of  $\delta^{18}\text{O}$  and higher values of  $\delta^{15}\text{N}$  were noted (Chang et al. 2002, Lee et al. 2009, Pellerin et al. 2009). The identified isotopic signatures may reflect the input of NO<sub>3</sub><sup>-</sup> from a variety of sources, and may be a result of nitrate fluxes on the way from S1 to S2 and further sites (Miyajima et al. 2009). For stations S2, S3 and S4, it was possible to establish a c. 70-80% share of nitrate from wastewaters/manure, with some 15-25% deriving from the atmosphere. The model used revealed only a negligible (up to 6%) influence of the other directions (Table 4). The contribution due to wastewater/manure nitrate is larger than expected, and in our opinion results from sewage rather than manure, in relation to the decline in the area of land supporting agriculture that has been noted during the last 20 years (Król & Sokół 2006, Koszelnik 2009). Modifications of wastewater management may thus be helpful in reducing nitrate loads and concentrations in river waters. Additionally, these results suggest that, within the



studied catchment, conditions conducive to the rapid nitrification of ammonia derived from sewage prevail.

Differences between sites located on the San and its tributaries result from different oxygen delta values. Variations in  $\delta^{18}\text{O}$  confirm the influence of atmospheric deposition, as is reflected in the calculated shares for nitrate loads. This is consistent with former studies (Mayer et al., 2002, Kendall et al., 2007, Lee et al., 2009, Tobari et al., 2010) in which oxygen isotope ratios are rising above +10‰, suggesting an increased contribution of atmospheric nitrate to surface runoff. In our study, the concept involving these phenomena gains strong support from the significant correlation between the mean  $\delta^{18}\text{O}$  value and the mean share of atmospheric nitrate ( $\delta^{18}\text{O} = 7.357 \text{ N}_{\text{at}} + 0.395$ ;  $R^2 = 0.985$ ;  $p=0.00$ ). The dynamics of  $\text{NO}_3^-$  in the river may be controlled by instream production, by nitrification and by removal (by way of denitrification), or affected by anthropogenic nitrate loading and internal assimilation and remineralisation, as suggested by Miyajima *et al.*, (2009) Via the first way, increases in both  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  are noted (Miyajima et al., 2009, Knöller et al., 2011), but during assimilation, the depletion of heavier isotopes is observed, especially in the case of the oxygen isotopic signature of nitrate (Miyajima et al. 2009).

The Solina-Myczkowce complex of reservoirs (of volume  $5 \cdot 10^8 \text{ m}^3$ ) is located between sites S1 and S2. Reservoir storages about 20% of supplied nitrogen and about 5% of supply is eliminated by way of denitrification (Koszelnik et al., 2007). This may be an additional cause of the differences between the nitrate isotopic signature obtained at site S1, as opposed to other sites. However, no significant correlation between nitrate content and  $\delta^{15}\text{N}$  (suggesting an impact of denitrification) was identified (see above). Sites located on tributaries of the San have similar mean  $\delta^{15}\text{N}$  value to the S2, S3 and S4 sites, though  $\delta^{18}\text{O}$  is lower by about 2 per mill (Fig. 4).

#### 4.2. Influence of nitrate source on nitrate content in water

From an applied point of view, the most important impact is that relating nitrate source to the concentration of the ion in waters. It was noted above how difficult predicting origin by reference to catchment management and loads might be, even if the stable isotope signature is a very useful tool. The influence of nitrate on eutrophication is obvious, so we looked for any influence nitrate origin might have on concentrations, and hence also on the

eutrophication of waters. Considering the model to have wider applicability, we compared data obtained with those from other watersheds in Europe and North America (Fig. 5), in which  $\text{N-NO}_3^-$  concentrations are similar (at  $<5 \text{ mgN L}^{-1}$ ). Multiple regression was used to evaluate two equivalent models:

$$\text{N-NO}_3^- = 1.030(\pm 0.823) - 0.125(\pm 0.057)\delta^{18}\text{O} + 0.080(\pm 0.009)\%Agr \quad (2)$$

and

$$\text{N-NO}_3^- = 0.414(\pm 0.772) - 0.159(\pm 0.110)\delta^{15}\text{N} + 0.087(\pm 0.009)\%Agr \quad (3)$$

where: %Agr denotes the share of a given basin that is agricultural (and other terms are as described above).

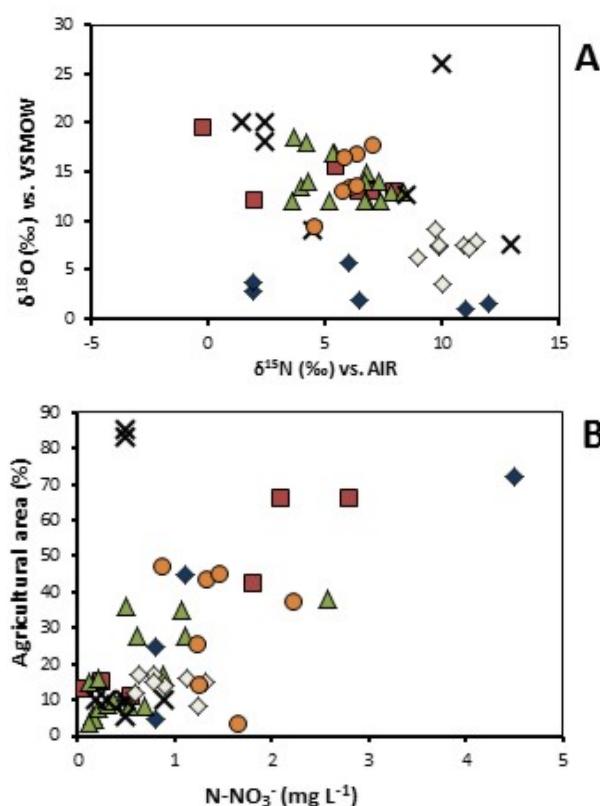


Figure 5. Catchment parameters used in multiple regression analysis. Circle symbols: this study; triangles: from Mayer et al., (2002); squares: from Voss et al., (2006); crosses: from Chang et al., (2002); dark diamonds: from Burns et al., (2009); light diamonds: from Li et al., (2010)

On the basis of the summary dependent variable regression given in Table 5 for  $\text{N-NO}_3^-$ , 63% (eq. 2) and 62% (eq. 3) of the variability to the dependent variable is seen to be described by the models. The inclusion of additional dependent variables does not result in a substantial improvement of parameters to either model. The

models show the greatest impact of the stable isotopes of  $\delta^{18}\text{O}$  (eq. 2) and  $\delta^{15}\text{N}$  (eq. 3) on the content of nitrate in the water. Depletion of the heavier isotopes of both O and N in nitrate reflect increasing nitrate concentrations with increasing agricultural area in the basin.

There is a widespread opinion that a reduction in loads of anthropogenic nitrogen to waters can be achieved if diffuse agricultural inputs are reduced (Grimvall & Stålnacke 2001), however the results of these studies point to a range of biogeochemical nitrogen fluxes. The multipath of nitrogen transformation is accompanied by isotope effects that could be an essential component of strategies for the management and protection of aquatic ecosystems.

Table 5. Parameters of multiple regression analyses.

Parameter	Eq. 1	Eq. 2
Multiple R	0.6399	0.7896
Multiple R <sup>2</sup>	0.6270	0.6234
F(2)	49.75	46.36
p	0.0000	0.0000
Standard error	2.25	2.31

## 5. CONCLUSIONS

Stable isotope study is a helpful tool when it comes to verifying the origin of the nitrate in water. A mixing model based on reference isotope values can estimate the shares accounted for by different nitrate sources, and may be useful in differentiating between nitrates from wastewaters/manure on the one hand, and the use of artificial fertilizers on the other. Knowledge about nitrate isotopic composition is useful in interpreting its origin, but also in predicting the effects of excessive nitrogen runoff. This is furthermore complementary to the traditional interpretation of the causes of eutrophication based around basin development and nitrogen loads. Finally, the method gained successful application in tracing anthropogenic N on its way to coastal waters. Studies undertaken around the world bring new information when it comes to the creation of accurate models of nitrate sources and losses, useful in water management.

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