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**ORIGINAL PAPER** 

# COMPARISON OF NITROGEN AND PHOSPHORUS ACCUMULATION IN PLANTS ASSOCIATED WITH STREAMS AND PEATBOGS IN MID-FOREST HEADWATER ECOSYSTEMS\*

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#### ABSTRACT

The paper presents results of a 3-year-long study (2012-2014) into the nitrogen and phosphorus bioaccumulation in 22 plant species associated with mid-forest headwater streams and in 10 plant species associated with peatbogs in the valley of the Kamienna Creek, a left bank tributary of the Słupia River, northern Poland. Plants were sampled in May, July and September of each year from three locations along the streams and three locations within the peatbogs. Every month stream water and ground water were sampled in the same locations using 30 cm piezometers. The content of nitrogen and phosphorus was determined in the plant and water samples. In the plant samples, total nitrogen was assessed with the Kjedahl method and phosphorus was determined with the molybdenum method. Concentrations of NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3</sup><sup>-</sup> in ground water samples were determined using ion chromatography. Stream and ground water contained low concentrations of mineral forms of these elements, which is associated with the strongly limited influence of anthropogenic factors and almost complete afforestation of the creek's catchment. Despite this, the supply of plants with nitrogen and phosphorus was sufficient, which is confirmed by the concentrations of these elements in plants. However, peatbog plants accumulated the elements more intensively. The highest accumulation of nitrogen and phosphorus was found in the shoots of Urtica dioica. Among the stream plants, Brachythecium rivulare and *Carex remota* accumulated the least nitrogen. The average N:P ratio in plants, which was <10, suggests that nitrogen rather than phosphorus is a limiting factor for the plant growth in the analyzed ecosystem. Statistically significant Spearman correlation coefficients indicate that the concentrations of N and P in shoots of the tested plants were closely interdependent. Veronica beccabunga, Chrysosplenium alternifolium and Urtica dioica, accumulating the greatest amounts of N and P in their shoots, can be taken into account when constructing artificial wetlands intended to act as forest buffer zones.

**Keywords:** headwater ecosystems, herbaceous plants, bioaccumulation, nutrients, biological turnover.

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## INTRODUCTION

Riparian forests are of high natural and economic value as they significantly increase water retention in river valleys (MAZUREK 2008). Riparian areas with diversified spatial and age structure of tree stands are distinguished by their superior richness of both forest-marsh flora and fauna (Osadowski 2006, Pielech et al. 2015, Parzych et al. 2017). The species composition of flora of forest headwater ecosystems is closely related to the chemical composition of soils (JONCZAK 2010, JONCZAK et al. 2015) and groundwater flowing out onto the surface (MAZUREK 2006, PARZYCH et al. 2016). Water, spring and rush species are found mainly on the bottom of niches, while on peatbogs raised above the bottom of the niche there are both forest and meadow species. The plant cover of riparian forests on the banks of streams contributes to the stabilization of the coastline and formation of the chemical composition of the flowing waters (IQBAL, TACHIBANA 2007, SOUZA et al. 2013, PARZYCH et al. 2016). By catching and accumulating nitrogen and phosphorus compounds, for example, herbaceous plants greatly contribute to the protection of river waters from eutrophication (BASTVIKEN et al. 2005, HAZLETT et al. 2008). According to the research conducted by JANSSON et al. (2007) and KUGLEROVÁ et al. (2014), the availability of nutrients to riparian plants is greater than in other forest ecosystems, as the flowing water contains minerals and organic matter dissolved in bioavailable forms. The amount of nutrients taken up by plants primarily depends on the species, age and developmental phase of plants as well as the availability of individual components (VESELKIN et al. 2014). Riparian forests occupy river valley habitats located on fertile soils (PUSŁOWSKA-TYSZEWSKA et al. 2014), although the continuous flow of water and the outflow of many nutrients connected with it is reflected in the supply of plants with basic nutrients (PARZYCH et al. 2017) and in the diversity of plant communities (OSADOWSKI 2006).

The aim of the study was to compare the accumulation of nitrogen and phosphorus in the aerial shoots of herbaceous plants growing on the banks of headwater streams and on the domes of peatbogs in riparian forests. Considering the possibility of an outflow of large amounts of bioavailable nitrogen and phosphorus forms with the waters, it was assumed that the higher N and P accumulation would be shown by the species growing on the peatbogs than by ones covering the banks of headwater streams.

## MATERIAL AND METHODS

#### Study area

The study was conducted within a large, mid-forest spring niche situated in the upper part of the valley of Kamienna Creek (54°19'N; 17°10'E), a left

bank tributary of the Slupia River. It is an area with average annual precipitation of about 770 mm and average annual air temperature of 7.6°C (KIRSCHENSTEIN, BARANOWSKI 2008). The area of the catchments of Kamienna Creek is nearly completely covered by forests with spatially diverse species composition, with the dominant beech, pine and spruce trees in its plateau part and common alder (Alnus glutinosa) in the bottom of the valley. Within the studied headwater riparian forest, there was a high proportion of species commonly recognized as distinctive for the system: Galium palustre, Lycopus europaeus, Lythrum salicaria, Lysima chiavulgarisi and Solanum dulcamara. The tree layer consists of Alnus glutinosa with some addition of Betula pubescens and Sorbus aucuparia. Forest stands cover the dome peatbogs, which are cut with headwater streams and composed of alder peat with thin alternating layers of alder-sedge peat (JONCZAK et al. 2015). The soils within the riparian zone studied exhibited a number of specific characteristics resulting from their functioning in the headwater area, being a transition zone between the underground and the surface part of the water cycle in river basins (JEKATIERYNCZUK-RUDCZYK 2007). In these soils, there is a continuous, concentrated flow of groundwater towards the river, resulting in characteristic vertical gradients of concentrations of various elements (JONCZAK et al. 2015).

#### Sampling and analysis of water

In the years 2012-2014, at monthly intervals, samples of water from 3 streams (in the initial, middle and end part of each stream) and groundwater samples (from 3 piezometers installed at the central points of three dome peatbogs) from a depth of 30 cm (due to the largest concentration of roots of herbaceous plants) were collected. The samples of waters were collected into 0.5 dm<sup>3</sup> polyethylene containers. Water from the streams was collected directly into containers, whereas groundwater was sampled with a peristaltic pump, every time after the piezometers were emptied. The measurements of water reaction (pH-metr CPI 551, Elmetron, Poland) and electrolytic conductivity (CC 315, Elmetron, Poland) were taken. The content of  $\rm NH_4^+$ ,  $\rm NO_2^-$ ,  $\rm NO_3^-$  and  $\rm PO_4^{3-}$  ions was determined by ion chromatography (881 Compact IC pro, Metrom, Switzerland). Before being poured into a chromatographic column, the water samples, were filtered through 0.2 µm porosity filters and diluted with deionized water in the proportion of 1:4. All water analyses were performed on the day of sampling.

### Sampling and analysis of plants

The study covered 22 species of herbaceous plants growing on the banks of 3 headwater streams with mineral substrates and 10 species of plants growing on dome peatbogs in the zone of headwater riparian area (Figure 1). The selection of species was driven by their highest frequency and density on the surveyed sites. For chemical research, the samples of aerial shoots of the



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Fig. 1. Average and range of N concentrations (mg kg<sup>-1</sup>) in shoots of stream (a) and peatbog (b) plants in 2012-2014: Agr\_sto – Agrostis stolonifera L., Aju\_rep – Ajuga reptans L., Ath\_fem – Athyrium filix-femina (L.) Roth, Ber\_ere – Berula erecta (Huds.) Coville, Bra\_riv – Brachythecium rivulare Schimp., Cal\_pal – Caltha palustris L., Car\_ama – Cardamine amara L., Car\_ech – Carex echinata Murray, Car\_pan – Carex paniculata L., Car\_rem – Carex remota L., Chr\_alt – Chrysosplenium alternifolium L., Epi\_pal – Epilobium palustre L., Fil\_ulm – Filipendula almaria (L.) Maxim., Ger\_rob – Geranium robertianum L.,

Gle\_hed - Glechoma hederacea L., Gym\_dry - Gymnocarpium dryopteris (L.) Newman,

Men\_aqu – Mentha aquatica L., Oxa\_ace – Oxalis acetosella L.,

Pel\_end – Pellia endiviifolia (Dicks.) Dumort., Ran\_acr – Ranunculus acris L., Rum\_ace – Rumex acetosa L., Sci\_syl – Scirpus sylvaticus L., Sol\_dul – Solanum dulcamara L., Ste\_nem – Stellaria nemorum L., Urt\_dio – Urtica dioica L., Val\_off – Valeriana officinalis L., Ver\_bec – Veronica beccabunga L.

plants were collected three times in the growing season (May, July, September), in the years 2012-2014. Each single sample consisted of shoots from several specimens of a given species. 288 plant samples were collected for the study. After transport to the laboratory, the shoots of plants were washed in distilled water in order to remove mineral particles of the soil. Then, they were dried at a temperature of 65°C and homogenized in a laboratory grinder (IKA A11, Germany). In order to determine the total nitrogen and phosphorus content, 0.5 g of a plant sample was digested in a mixture consisting of 98%  $H_2SO_4$  and 30%  $H_2O_2$  in the volumetric ratio of 1: 1, until clear and colourless solution was obtained. In the solutions, nitrogen was determined using the Kjeldahl method (Büchi K-350, Switzerland) and phosphorus was analyzed with the spectrophotometric method using ammonium molybdate (UV-VIS, Hitachi U-5100, Japan). All the analyses were performed in three replicates.

### Data development

The distribution of the data obtained was examined with the Shapiro-Wilk test. Due to the absence of normal distribution, the variability of physicochemical properties of waters in streams and groundwater in the years 2012-2014 was compared using the Mann-Whitney U test. Relationships between N and P concentrations in shoots of plants growing on the stream banks and on the dome peatland were expressed as N: P ratios and the Spearman correlation coefficients between N and P concentrations in shoots of herbaceous plants. All calculations were performed using the software package Statistica 7.1.

### Quality assurance/quality control (QA/QC)

QA/QC of the analytical procedures was carried out by analyzing the standard certified reference material of water (Multielements Ion Chromoatography 89 866-50ML-F, Fluka), and of aquatic plants (CRM 060), adopting the same procedures for the analyzed samples. The results of the experimental measurements agreed with the recommended reference value material. Recoveries were calculated as a ratio of determined value to a certified one and were within the confidence intervals of the certified values. Recoveries were as follows:  $99 \pm 2\%$  (N) and  $98 \pm 3\%$  (P).

## **RESULTS AND DISCUSSION**

#### Characteristics of streams water and ground water

The stream water reaction was slightly alkaline, with an average pH value of 7.9, and the reaction of the groundwater was close to neutral, with an average pH of 7.4 (Table 1). The lowest pH values were observed at all sites in May, which was a result of the early growing season and increased demand of plants for alkaline cations (PARZYCH et al. 2016). The waters were

Table 1

Parameters	Stream waters	Groundwaters	Mann-Whitney $U$ test ( $p < 0.05$ )
pH	$7.9 \pm 0.4$	$7.4 \pm 0.4$	0.000003
EC ( $\mu$ S cm <sup>-3</sup> )	$273 \pm 66$	$237\pm52$	0.008230
$\mathrm{NH}_4^+$ (mg dm <sup>-3</sup> )	$0.1 \pm 0.2$	$0.9 \pm 1.0$	0.000099
$\mathrm{NO}_{2}^{-}$ (mg dm <sup>-3</sup> )	$0.2 \pm 0.3$	$0.3 \pm 0.4$	-
$\mathrm{NO}_{3}^{-}$ (mg dm <sup>-3</sup> )	$1.9 \pm 1.2$	$1.6 \pm 1.5$	-
$\mathrm{PO}_{4}^{3}$ (mg dm <sup>3</sup> )	$0.4 \pm 0.6$	$0.9 \pm 0.8$	-

Characteristics of stream and ground waters with the Mann-Whitney U test results

p – level of statistical significance, EC – electrolytic conductivity

characterized by relatively low mineralization, which was reflected by the electrolytic conductivity measured at an average level of 273  $\mu$ S cm<sup>-3</sup> in the stream water and 237  $\mu$ S cm<sup>-3</sup> in the groundwater (PIELECH et al. 2015). The concentration of  $NH_4^+$  ions was on average 0.1 mg dm<sup>-3</sup> in the stream waters and 0.9 mg dm<sup>3</sup> in the groundwater. The concentration levels of NO<sub>2</sub>,  $NO_3^{-1}$  and  $PO_4^{-3-1}$  ions were also low and amounted to, respectively, 0.2 mg dm<sup>-3</sup>, 1.9 mg dm<sup>-3</sup> and 0.4 mg dm<sup>-3</sup> in the waters of the streams and 0.3 mg dm<sup>-3</sup>, 1.6 mg dm<sup> $\cdot$ 3</sup> and 0.9 mg dm<sup> $\cdot$ 3</sup> in the groundwater (Table 1). Statistically significant differences in reaction, electrolytic conductivity and ammonium ion concentration  $(NH_4^+)$  in the stream waters and groundwater were found. Nitrates  $(NO_3)$  easily migrate in waters and are less likely to be subject to sorption (BERNOT, DODDS 2005, PARZYCH 2011). In a growing season, associated with the dynamic growth and development of plants, nitrate ions V are intensively absorbed by plants, leading to a decrease in their concentration in both the stream and groundwater (SICKMAN et al. 2003, PARZYCH 2011). With slightly alkaline and neutral pH of water, numerous plant species obatin more nitrate than ammonium ions (BRITTO, KRONZUCKE 2002).

#### Bioaccumulation of nitrogen and phosphorus in plants

The content of nitrogen and phosphorus in the shoots of stream bank and peatland plants showed considerable variation (Figures 1, 2). Peatbog plant species (B) were characterized by good N and P supply. The highest concentrations of nitrogen were found in Urt dio and Sol dul shoots, and phosphorus in Urt dio and Sci syl. The smallest amounts of nitrogen were demonstrated in Val\_off and Car\_ech shoots, and phosphorus in Val\_off and Men aqu. Urt dio is one of the species that efficiently accumulate nitrogen and phosphorus (PARZYCH, KANCLERSKA 2016). The plants growing directly by the streams (A) were characterized by a slightly weaker supply of nitrogen. In the case of *Bra* riv, nitrogen concentrations remained at the lowest level  $(7967 \text{ mg kg}^{-1})$  but they reached higher values in the shoots of Chr alt  $(20\ 676\ \text{mg kg}^{-1})$  and  $Rum\_ace\ (18\ 291\ \text{mg kg}^{-1})$ . The supply of phosphorus to the plants growing by the streams was good. The highest amounts of P were found in *Car\_ama* (4346 mg kg<sup>-1</sup>) and *Ver\_bec* (4661 mg kg<sup>-1</sup>) shoots. The results of the studies by SAMECKA-CYMERMAN and KEMPERS (2007) show that at higher phosphate concentrations in water, Ver\_bec can accumulate significantly higher amounts of phosphorus in its shoots (18 048 - 27 937 mg kg<sup>-1</sup>). According to OSTROWSKA (1999), the natural content of nitrogen and phosphorus in green parts of most plants is usually in the range of 13 000 to 31 000 mg kg<sup>1</sup> (N) and from 1000 to 4000 mg kg<sup>-1</sup> (P), respectively. Nitrogen is an essential nutrient that ensures proper plant growth and development, playing a key role in shaping the size and certain qualities of plants. Plants draw nitrogen from water mainly in the form of  $NH_4^+$  and  $NO_3^-$  ions, depending on the water reaction. In an acid environment, nitrogen is most commonly absorbed in the form of NH<sub>4</sub><sup>+</sup> ions, and the intensity of ammonium uptake by plants is reduced in favour of NO<sub>3</sub> in an alkaline medium (PARZYCH et al. 2017).  $NH_4^+$ 



Fig. 2. Average and range of P concentrations (mg kg<sup>-1</sup>) in shoots of stream (a) and peatbog (b) plants in 2012-2014. \* Explomations see Figure 1

ions that were not taken by plants can be transferred into the sorption complex of the soil as a result of interchangeable sorption, and  $NO_3^{-1}$  ions – due to their high mobility – are most often eluted to deeper layers of a soil profile (PARZYCH 2011). Phosphorus is one of the basic building blocks of plant material. Unlike nitrogen, it accelerates the development and stimulates the generative organs of plants. Plants take phosphorus from water in the form of  $H_2PO_4^{-1}$  and  $HPO_4^{-2}$ . The uptake of these ions is dependent on the soil pH and moisture content (CIERESZKO 2005). Both phosphorus and nitrogen are found in the forms that are easily accessible for plants at pH from 6.0-6.5 to 8.0. Continuous concentrated flow of spring waters in the riparian forest studied ensures high moisture of soil both along the streams and on the dome peatbogs (JONCZAK et al. 2015), which promotes the maximum phosphorus uptake by plants. Despite geochemical stabilization, phosphorus is subject of migration in the environment and exerts negative pressure on the biogeochemical balance.

The values of nitrogen and phosphorus content in the shoots of the riparian species surveyed were characteristic for both aquatic (PARZYCH et al. 2015) and terrestrial plants (PARZYCH 2010, 2016).

### Relationship between N and P in plant shoots

The state of mineral nutrition of plants with nitrogen and phosphorus depends on the content of the individual elements in plant shoots and on their mutual balance. The N:P ratios in the stream bank plants ranged from



4 to 6 and were lower than those in the plants growing on the dome peat bogs, where the N: P ratio ranged mostly from 6 to 7 (Figure 3). Differences in the N:P values between the analyzed plant groups (A and B) were statistically significant (p < 0.001). Numerous studies indicate that the ratio of N: P in plants on natural sites generally takes values from 12 to 13 (GÜSWELL, KORSELMAN 2002). According to Zhiguo et al. (2007), the maximum plant growth and maximum biogenic supply occur at N:P values close to 9.5. The plants of the headwater ecosystems studied herein showed significantly lower values of the N:P ratio than the majority of plants outside riparian forests (PARZYCH 2010) which is the result of continuous leaching of mineral nitrogen and phosphorus forms outside the root system of plants by the flowing headwaters. An example may be  $NO_3^-$  ions (Bernot, Dodds 2005, PARZYCH 2011). The value of the N:P ratio is characteristic for a given species (Townsed et al. 2006), but is also modified by environmental factors (PARZYCH et al. 2017). With the average ratio N:P <10 determined in this study, it was assumed that the growth and development of both stream bank (A) and peatbog (B) plants was inhibited by the limited concentration of bioavailable forms of nitrogen within the root system of the plants (Table 1).

Nitrogen and phosphorus concentrations in plant shoots are varied, which results from the variable demand of the tested plant species for nutrients in the growing season. The study showed that the levels of nitrogen and phosphorus in the growing seasons analyzed were significantly correlated, which was confirmed by the values of the Spearman correlation coefficients (r = 0.38 for group A and r = 0.45 for group B at p < 0.05), (Figure 4). Significant correlations between the N and P content in the shoots of different plant species were reported by McJANNET et al. (1995), GÜSWELL (2004) and PARZYCH (2010).



Fig. 4. Relationships between the concentrations of N and P in stream (a) and peatbog (b) plants

## CONCLUSIONS

The stream waters and groundwater were characterized by low concentrations of mineral nitrogen and phosphorus forms, resulting from the low nutrient content in spring waters supplying the niches, intensive nutrient uptake by plants during the growing season and export of significant amounts of ions beyond the reach of the root system. Significantly larger amounts of nitrogen were accumulated in the shoots of peat plants than in the stream bank plants. Among the species growing on the peatbog, the highest accumulation of nitrogen and phosphorus was found in *Urtica dioica* shoots. Among the stream bank plants, the highest amount of nitrogen was accumulated by Chrysosplenium alternifolium and Rumex acetosa, while the smallest amount of N was determined in *Brachythecium rivulare* and *Carex remota*. The average N:P < 10 ratio in the shoots of the plants studied indicates that the growth and development of plants is inhibited by the limited concentration of bioavailable forms of nitrogen within the root system of these plants. Statistically significant values of correlation coefficients show that N and P concentrations in plant shoots were closely related. Species such as Chrysosplenium alternifolium, Rumex acetosa, Urtica dioica and Solanum dulcamara accumulating in their shoots the highest amounts of N and Cardamine amara, Veronica beccabunga, Scirpus sylvaticus and Urtica dioica which accumulate the highest amounts of P may be included in the construction of artificial wetlands intended to act as buffer zones.

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