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Relationships between the Content of C, N, P and their Stoichiometry in the Soils of Selected Reserves of the Białowieża Primeval Forest †

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Abstract: The aim of the study was to evaluate the correlation between the content of C, N, P and their stoichiometry in the soils of Białowieża Primeval Forest in northeastern Poland. The studies were carried out in the forest reserves of Białowieża Primeval Forest, i.e. in Władysław Szafer Landscape (WS), in Dębowy Grąd (DG), in Lipiny (LP), and in Koryciny Reserve (KOR) in northeastern Poland (Podlaskie Voivodeship). A close relationship was found between the content of TC and TN in the soils of the studied deposits, which is confirmed by positive linear relationships for the soils of the 0-5 cm layer (r = 0.965 ***, r2 = 0.931) and 5-40 cm depth (r = 0.959 * **, r2 = 0.919). The C:N ratio ranged from 17.40 to 24.50, with the highest content recorded in the soil of Lipiny reserve, which may indicate slowed decomposition processes and accumulation of organic matter in the soil due to a very acidic soil reaction (pH_(CaCl_2) 3.98). The C:P ratio in reserves of Koryciny and Dębowy Grąd ranged from 114.4-229.7, and the mean value in the litter was 547.8. The value of C:P ratio > 300 may favor biological sorption of phosphorus in the soil of Lipiny reserve. The values of stoichiometric C:N:P ratio may be an indicator of the intensity of nutrient flux entering forest soils from dead plant residues.

Keywords: carbon; nitrogen; phosphorus; forest reserves; soil; litter forest stoichiometry

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1. Introduction

Forest ecosystems contribute significantly to preventing the negative impacts of climate change by reducing CO_2 emissions to the atmosphere and increasing carbon sequestration in wood and soil [3]. Natural forest ecosystems are particularly valuable because they provide the ecosystem with plant debris, including dead wood, as a source of carbon and minerals. Forest soils in equilibrium with the natural ecosystem have high carbon density and the ratio of soil to vegetation density increases with latitude and depends on climatic factors, chemical composition of dead wood, and decomposition processes by soil microorganisms [6, 5, 16]. In assessing soil fertility and nutritional status of forest ecosystems, ecological stoichiometry can play an important role, which is an indicator of the intensity of chemical element cycling and the balance of ecological interactions [1, 4, 14]. The aim of the study was to evaluate the correlation between the content of C, N, P and their stoichiometry in the soils of the Białowieża Primeval Forest in northeastern Poland.

2. Materials and Methods

2.1. Study Area

The studies were conducted in forest reserves of Białowieża Primeval Forest in northeastern Poland (Podlaskie Voivodeship). The study areas were four nature reserves, namely Władysław Szafer Landscape Reserve (52°42'20'N; 23°42'59'E), Dębowy-Grąd Reserve (52°44'12'N; 23°41'30'E), Lipiny Reserve (52°45'12'N; 23°38'40'E), and the Koryciny Reserve in Rudka Forest Area (52°40'10'N; 22°44'30'E). Władysław Szafer Landscape Reserve- forest and coniferous forest reserve with an area of 1,343.91 ha- was established in 1969 to protect and preserve the natural state of virgin forests. The main tree species present at the area are: Picea abies L., Quercus petraea, Quercus robur, Pinus silvestris, Betula pendula and Ulmus minor Mill. The average age of the stand is 123 years. The reserve is located on brown earth (BR), rusty earth (RD). Reserve Dębowy Grąd Reserve in Białowieża Primeval Forest is a forest reserve with an area of 100.47 ha, established in 1985 to protect a fragment of Białowieża Primeval Forest with natural oak-hornbeam complexes. The predominant tree species include: Quercus petraea, Quercus robur, Fraxinus excelsior L., (Picea abies L., Acer platanoides L., Carpinus betulus L. The average stand age is 119 years. The reserve is located on typical ground vegetation (P). Lipiny Reserve in Białowieża Primeval Forest is a forest reserve with an area of 56.28 ha. It was established in 1962 to preserve the only stands of Białowieża Primeval Forest with a significant proportion of sessile oak (Quercus sessilis Ehrh). The average age of the stands is 110 years. The reserve is located on depleted brown soils (BBwy). Koryciny Reserve is a forest reserve with an area of 87.72 ha and was established in 1975 to preserve a fragment of a natural oak forest and to preserve natural and semi-natural oak-hornbeam forests ecosystems with a monumental oak stand. The main species are: Quercus petraea, Carpinus betulus L.). The age of the stand is 168 years. The reserve is located on Lessive (P), Rusty soils (RD) and Pseudogley soils (OG). The research area is located in the natural forest area: Mazowiecko-Podlaska (IV), mesoregion: Zambrowsko-Bielski (IV.7) [17]. The climate in Podlaskie Voivodeship is temperate, transitional with continental influences. The average annual temperature is 7.5°C and the average annual precipitation is 598 mm. The characteristics of the studied objects are shown in Table No. 1. according to the classification of Poland into climatic regions [15]. Soil samples for the studies from the above-mentioned reserves of the Białowieża primeval forest were collected in autumn 2021 from a 0.5 m x 0.5 m x 0.5 m open pit. The 1 kg samples were placed in appropriately labeled cloth bags. From each open pit, 6 soil samples were collected at depths up to 40 cm (0, 0-5, 5-10, 10-20, 20-40 cm).

2.2. Laboratory Analysis

After samples were collected for delivery to the IBL laboratory, they were dried in an oven at the temperature of 40°C. The samples were then sieved through a sieve with a mesh size of 2 mm [11]. Each sample was divided into two parts, i.e. (i) first part: sieved samples for the determinations: pH_{CaCl_2} (ratio 1:10), particle size distribution; and the second part of the sample (ii) was ground in an agate mortar for the determination of carbon (TC), nitrogen (TC) and phosphorus (TP).

The following parameters were determined in the prepared samples for physico-chemical analyzes, i.e. :

- Soil pH in 0.01 mol/l $CaCl_2$ by the potentiometric method, according to PN-EN ISO 10390:1997 [9] using a pH meter,
- Total nitrogen (TN), by the high temperature combustion method with TCD detection, according to PN-ISO 13878:2002 [13], using an elemental analyzer,
- Total carbon (TC), by the method of high temperature combustion with TCD detection, according to PN-ISO 10694:2002 [12], using an elemental analyzer,
- Phosphorus (TP), by the method of atomic emission spectrometry with excitation in inductively coupled plasma (ICP-OES), according to PN-EN ISO 11885:2009 [10].

All chemical analyzes were performed in the accredited Laboratory of Environmental Chemistry (AB 740) of the Forest Research Institute in Sękocin Stary in Poland.

2.3. Statistical Analyses

The obtained test results were statistically analyzed using the STATISTICA 13 Version software. Stoichiometric ratios for C:N and C:P in soils were calculated for selected occurrences of the Białowieża Primeval Forest, and overall linear Pearson correlations for pH_{CaCl_2} and the content of TC, TN, TP as well as C:N and C:P in forest litter "0" and in soils from layers up to 40 cm deep were determined for the studied objects. The significance of Pearson's linear correlation coefficients was evaluated at three significance levels p: 0.05; 0.01 and 0.001. In addition, a two-way ANOVA analysis was performed for pH_{CaCl_2} and such components as C, N, P and their stoichiometric ratios as a function of the location of the studied objects. The significance of the differences between the mean contents of the components were evaluated using Tukey's test at the significance level of $p < 0.05$.

3. Results and discussion

The soils of the Białowieża primeval forest reserves, up to 40 cm deep, were strongly acidic (pH_{CaCl_2} 4.40 at SD 0.72), and the forest litter "0" was similarly acidic. A weak positive effect of soil reaction on the content of TP in forest litter ($r = 0.554$ *** and $r^2 = 0.307$) and TC, TN and TP in soil up to 40 cm depth ($r < 0.600$ and $r^2 < 0.300$) was demonstrated.

The content of carbon (TC) in the soil of the studied objects was not very diverse and ranged from 72.63 - 110.0 g TC·kg^{-1} DM (Tab.). The lowest content of this component was in the leached brown soil in Lipiny reserve, and the highest TC in the soil in Dębowy Grąd reserve, which is partially located on typical soils lessives. It can be assumed that in such an acidic response of the habitat soil organic carbon is the most important carbon component. Using a two-way ANOVA analysis of variance, the TC content was shown to vary with soil depth. The average content of total carbon (TC) in the forest litter of all objects was 379.4 g C·kg^{-1} DM ± 82.0 and was almost nine times higher than in the 0-5 cm deep layer (42.38 g C·kg^{-1} DM ± 25.9). The TC content in the deeper soil layers was much lower, ranging from 6.25 to 25.03 g C·kg^{-1} DM.

Significant interdependence was found between the content of TC and TN and TP in soil up to 40 cm depth and for TP in litter. The relationship between TC and TN in soil up to 40 cm depth was strong ($r = 0.959$ *** with $r^2 = 0.919$) and the relationships between TC and TP were much weaker, and the highest value was found in litter ($r = 0.658$ *** and $r^2 = 0.433$). The TC content in the soil and litter affected the C:P value, and the highest value of this coefficient was found for the deeper soil layer (5-40 cm depth) ($r = 0.816$ *** and $r^2 = 0.666$).

The mean content of total nitrogen (TN), similar to TC, was lowest in the soil of Lipiny Nature Reserve (2.95 g TN DM ± 5.48). Regardless of location, litter that was on the surface of the forest floor was most abundant in TC and TN. Two-factorial ANOVA analysis of variance showed that the content of both components was significantly higher in litter than in soil to a depth of 40 cm. The TN content in the soil depended on the TC content. This is confirmed by the obtained positive linear relationships for the soil in the layer of 0-5 cm ($r = 0.965$ ***, $r^2 = 0.931$) and for the deeper layers up to 40 cm ($r = 0.959$ ***, $r^2 = 0.919$).

The quantitative ratio of C:N is considered one of the most important indicators of the quality of a forest habitat, and the smaller it is, the more fertile the soil and the faster the decomposition of organic matter occurs. The ratio of the two components is particularly important for maintaining the biodiversity of forest habitats. In the studied reserves of Białowieża Primeval Forest, the C:N ratio averaged between 17.40 and 24.5 (for Koryciny and Dębowy Grąd Reserves and for Lipina, respectively). The wider range in the

soil of Lipiny Reserve indicates slowed processes of decomposition and accumulation of organic matter.

Based on two-factorial ANOVA analysis of variance, significant differences in C: N value were found between the litter and soil to a depth of 40 cm. The C:N value of the litter had a wide C:N ratio of almost 32:1 regardless of the sampling location, which could indicate low biological activity and slowed decomposition of soil organic matter, while the ratio in the soil layers up to a depth of 40 cm was much narrower and was about 18:1.

The mean content of total phosphorus (TP) in the studied soils was similar, except for the Lipiny reserve, which had a slightly higher content (0.522 g TP·kg^{-1} DM ± 0.15). It appeared that the litter contained almost twice as much of this element as the soil to a depth of 40 cm (below 0.400 g TP·kg^{-1} DM.

Positive linear correlation coefficients were obtained between the TC and TP content in the litter ($r = 0.658$ ***; $r^2 = 0.433$), while their values in the soil were significantly weaker.

The C:P ratio in soil is an indicator of mineralization intensity and phosphorus release, and the narrower it is, the more phosphorus is released from organic compounds.

The studied soils had a wide range of C: P and ranged from 114.4 ± 143.4 – 180.0 ± 229.7 for Koryciny and Dębowy Grąd Reserves. The highest value of the C:P ratio for the mean of the studied objects was found in the litter and soil layer up to the depth of 5 cm, and with the decrease of the depth the C:P ratio decreased significantly. The C:P value in the litter presented the range of 547.8 ± 269.60, which could indicate increased biological sorption of phosphorus. Literature indicates that C:P ratio > 300:1 (C:P) favors the intensification of this process. The low solubility of phosphorus compounds in soil is strongly dependent on pH, and the sorption processes occurring in soils often make phosphorus a limiting component in forest ecosystems [2]. On acidic, very acidic, and even alkaline soils, phosphorus is recycled and converted to forms that are inaccessible to plants. The obtained test results indicate the mentioned process.

The obtained research results confirm the existing data [3, 6, 7, 8] about the important role of organic matter in carbon sequestration and nutrients cycling in the natural forest ecosystem. The values of stoichiometric C:N:P ratios can be an indicator of the intensity of nutrient fluxes entering forest soils from dead plant residues.

4. Conclusions

- A lose relationship was demonstrated between the content of TC and TN in the soil, which was confirmed by positive linear relationships for the soil from the 0-5 cm layer ($r = 0.965$ ***, $r^2 = 0.931$) and 5-40 cm depth ($r = 0.959$ ***, $r^2 = 0.919$).
- The C:N ratio in the soil of Białowieża Primeval Forest Reserves ranged from 17.40 to 24.5. The largest C:N range was found in the soil of Lipiny Reserve and may indicate slow processes of decomposition and accumulation of organic matter in the soil caused by a very acid soil reaction (pH_{CaCl_2} 3.9).
- The studied soils had a wide range of C:P and ranged from 114.4 ± 143.4 – 180.0 ± 229.7 for Koryciny and Dębowy Grąd Reserves, and the mean value for litter was 547.8 ± 269.60. asignificant value of this C: P ratio > 300 may favor the increase of biological sorption of phosphorus in Lipiny Reserve soil.
- Forest management and especially leaving dead wood in the reserves can significantly contribute to carbon sequestration and be a source of nutrients necessary for maintaining biodiversity in forest ecosystems.

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Conflicts of Interest: The authors declare no conflict of interest.

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