original paper

Effects of the presence of deadwood in old natural forests and on the stoichiometric ratios of C, N and P in the soil

Irena Burzyńska

Laboratory of Environmental Chemistry, Forest Research Institute, Sękocin Stary, Braci Leśnej 3, 05−090 Raszyn, Poland

ABSTRACT

The aim of the study was to evaluate the effects of the presence of deadwood on the content of C, N and P and their stoichiometric ratios in the soils of the Białowieża Forest. The study was conducted in Hajnówka Forest District in the following reserves: Władysław Szafer Landscape Reserve, Dębowy−Grąd, Lipiny and outside the Białowieża Forest – in the Rudka Forest District, the reserve of Koryciny. Soil samples were collected in the fall (October−November 2021). Soil samples were taken from litter (O) and mineral soils up to a depth of 40 cm at several layers (0−5, 5−10, 10−20, 20−40 cm). Samples were collected under deadwood (object: 1) and at control sites without deadwood (object: 0). The pH of the samples were measured in 0.01 M $CaCl₂$ solution using the potentiometric method for; TC (total carbon) and TN (total nitrogen) with high temperature combustion with TDC detection. TP (total phosphorus) was determined by atomic emission spectrometry with inductively coupled plasma excitation (ICP−OES). Carbon content (TC) ranged from 300 to 400 g·kg⁻¹ in litter samples and from 1.43 to 100 g·kg⁻¹ in soil. Total nitrogen content (TN) was 9.31-15.59 g·kg⁻¹ in litter and 0.56-3.36 g·kg⁻¹ in soil. The presence of deadwood on the soil surface favored the accumulation of C mainly in the litter and increased the value of C:N compared to the control samples. The C:N ratio was in the range of 30−40:1 in litter and 10−20:1 in soil. C:P ratios in the samples ranged from 329:718:1 in leaf litter and 41−91:1 in soil with long−term presence of deadwood increasing C:N and C:P ratios in forest litter. A high C:N ratio may indicate slow decomposition of materials rich in compounds that are difficult to degrade (*e.g*., cellulose and lignin).

KEY WORDS

carbon, forest soil, natural forest, nitrogen, phosphorus

Introduction

Generally, natural forests are ecosystems with an high level of plant debris which include dead wood that serves as a source of carbon and other elements. It is estimated that 90−99% of wood is cellulose, hemicellulose and lignin, as well as 50% C, 44% O and 6% H and smaller amounts of N, P, K, Ca, Mg (Pettersen, 1984; Meerts, 2002; Lasota, *et al*., 2018).The stoichiometric C:N:P ratios reflect soil fertility and influence the nutritional status of plants (Batjes, 2014; Fan, 2015). The study of stoichiometry in forest ecosystems is important for understanding the mechanisms

Received: 5 April 2023; Revised: 17 July 2023; Accepted: 18 July 2023; Available online: 29 August 2023

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e−mail: I.Burzynska@ibles.waw.pl

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of natural community functioning, biodiversity conservation, and natural ecosystem functioning (Trentini *et al*., 2017). According to Buchkowski (2015) and Khalid (2019), changes in soil min− eral content affect the biomass composition of soil microorganisms and the availability of these nutrients to vegetation.

Forest soils with high C:P ratios may favour low microbial diversity, while low resource quality (*i.e*., low C:N, C:P and N:P) may favour bacterial diversity (Delgado−Baquerizo *et al*., 2017). As energy flows from plants to their consumers, the C:N:P ratio decreases and the stoichiometric ranges become smaller. The study of stoichiometric C:N:P ratios in forest soils was also conducted by Pang (2021), Torres−Duque (2022) and Sterner (2003).

Leaving dead wood in the forest environment favors the accumulation of C, N and P in the soil and affects their stoichiometric ratios, thereby increasing soil carbon sequestration, forest habitat fertility and biodiversity. For this purpose, the content of the mineral components in forest soils was compared for objects with deadwood in the sampling area and in control areas without deadwood.

The aim of the study was to investigate the effects of deadwood in forests on the content and stoichiometric ratios of C, N and P in the soil.

Materials and Methods

RESEARCH AREA. The study was conducted in northeastern Poland (Podlaskie Voivodeship) in the Białowieża Forest and Koryciny reserve. The Białowieża Forest is one of the most important and best preserved fragments of a forest woodland in the lowlands of Central and Eastern Europe (Jaroszewicz *et al*., 2019). The study was conducted on Hajnówka Forest District in the following reserves: Władysław Szafer Landscape Reserve (52.691732, 23.8357632; 52.698617, 23.815757; 52.700349, 23.868408), Dębowy−Grąd (52.721489, 23.726108; 52.725693, 23.744072; 52.745772, 23.723280; 52.721489, 32.735982), Lipiny (52.753853, 23.618982; 52.757660, 23.617617; 52.756790, 23.626887), and outside the Białowieża Forest – in the Koryciny reserve, Rudka Forest District (52.692337, 22.710309; 52.696536, 22.871981; 52.703524, 22.703380). The basis for locating the

Fig. 1.

Location of research facilities in the Białowieża Forest and Koryciny reserve

Table 1.

research plots in the nature reserves was a 1985 natural inventory study on the effects of the hurricane in the winter of 1982−1983 on the state of the forest habitat. The Białowieża Forest is located in the continental biogeographical region zone of semi−boreal forests and mixed forests (EEA, 2019). This is reflected in the species composition of the stands with a significant proportion of *Quercus robur* L., *Acer platanoides* L., *Carpinus betulus* L., *Fraxinus excelsior* L., *Tilia cordata* Mill. and a high proportion of *Picea abies* (L.) Karst. The climate is a temperate transitional climate with continental influences. The average annual temperature is 7.5°C and the average annual precip− itation is 598 mm. The characteristics of the studied objects are listed in Table 1.

Soil samples were collected from an open pit (0.5×0.5×0.5 m) in the fall (October− November 2021). Samples were collected from a depth 40 cm including litter (O) and from min− eral soil (0−5, 5−10, 10−20 and 20−40 cm). The deadwood consisted of deciduous and coniferous species (*A. platanoides*, *Q. robur*, *F. excelsior*, *T. cordata* and *P. abies*, and was in the final stages of decomposition (phase IV and V) (Maser *et al*., 1979).

LABORATORY ANALYSIS. Soil samples were stored in an oven at 40°C and sieved with a 2 mm sieve with removal of stones and roots by hand. Then the samples were divided into two groups: (i) sieved samples for determination of $pH_{CaCl₂}$ (1:10 soil to 0.01 molar CaCl₂ solution ratio), and (ii) the samples were finely ground with an agate mortar grinder for determination of carbon (TC), nitrogen (TC) and phosphorus (TP).

The following components were measured in the samples:

- $-$ Soil (pH) in 0.01 M CaCl₂ by the potentiometric method using a pH meter,
- Total nitrogen (TN) and total carbon (TC) by the high temperature combustion method with TCD detection using an elemental analyzer,
- Phosphorus (TP) samples after mineralization with aqua regia ($HNO₃ \times 3HCl$) followed by measurements using atomic emission spectrometry with inductively coupled plasma excitation (ICP−OES).

STATISTICAL ANALYSIS. The normality distribution of the variables was tested with the Shapiro− −Wilk test. A two-way ANOVA was performed for pH_{CaCl2} and total soil content of TC, TN and TP in the forest samples. Stoichiometric ratios were calculated for C:N, C:P and N:P. The sig−

Characteristic of the Białowieża Primeval Forest and Koryciny reserve research area in Podlaskie Voivodeship

nificant differences between the average contents of the components was evaluated using Tukey's HSD (honest significant difference) test with *p*<0.05.

Linear Pearson correlation coefficients were then calculated for pH_{CaCl} for the following components tested: TC, TN, TP, C:N and C:P. The significance of the linear Pearson correla− tion coefficients was tested at three significance levels with $p < 0.05(*)$, $0.01(**)$ and $0.001(**)$. All analyses were performed in Statistica 13 software (StatSoft, 2013; Dell Inc., 2016).

Results

The content of TC in samples was 284-462 g·kg⁻¹ in the litter and 9.33-44.57 g·kg⁻¹in the soil. The highest TC level in a sample was the soil of the reserve 'DG' (22.33–462.23 g·kg⁻¹) and the least was in the soil samples from the reserve 'LP' (18.10−329.84 g·kg–1) and 'WS'. (16.06− -435.50 g·kg⁻¹). The content of carbon was highest in the litter and decreased with depth (Fig. 2). Analysis of variance showed that leaving deadwood affected the carbon content, especially in the litter of the 'WS' and 'DG' reserves, and in the soil of the 'DG' reserve (Table 2, Fig. 2).

The content of TN in the forest soil reserves depended on the location of the samples taken and the depth of soil. The soil of the reserves 'DG' (1.60−15.59 g·kg–1) and 'KOR' (0.56− −13.33 g·kg–1) were the richest in TN, while it was lower in the soil of the reserves 'LP, (0.88− -9.31 g·kg⁻¹) and WS (0.84-12.07 g·kg⁻¹). Regardless of the sampling site, the highest TN level was found in the forest floor, especially under dead wood. (Table 2, Fig. 2).

The content of TP in the forest reserve soil of the Białowieża Forest was low ≤ 1.00 TP g·kg⁻¹ regardless of the location and depth of the soil sample (Table 2, Fig. 2). On the basis of two−factor method ANOVA there was no effect of deadwood on the phosphorus content in the studied forest soils.

The stoichiometric C:N ratios in the soils of the Białowieża Forest Reserves varied depending on the site and depth, and were in the range of 14-40 g·kg⁻¹ for litter and 12.94-21.16 g·kg⁻¹ for soil (Table 2). The largest C:N ratio range was found in the soil of the reserve 'LP' (20.67−40.29

Fig. 2.

Content of TC, TN, TP in soil up to 40 cm deep from reserves of the Białowieża Forest and Koryciny reserve

Soil pH_{GeO2} and total content of TC, TN and TP (g·kg⁻¹) and their stoichiometric ratios on samples with deadwood and control samples in the reserves of the Soil pH_{GG2} and total content of TC, TN and TP (g·kg⁻¹⁾ and their stoichiometric ratios on samples with deadwood and control samples in the reserves of the

Table 2.

 0 – samples with deadwood; 1 – samples without deadwood; a, A, b, B – statistically significant differences between means ($p \le 0.05$) 0 – samples with deadwood; 1 – samples without deadwood; a, A, b, B – statistically significant differences between means (*p*<0.05)

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g·kg–1), and much smaller in the soils of 'DG' (12.94−22.04 g·kg–1) and 'KOR' (15.09−25.14 g·kg–1) (Tab. 2). Regardless of the location of the study points, the largest ranges of stoichiometric ratios for both C and N were found in the litter. Based on two−factor ANOVA, a positive effect of deadwood with an increase of C:N ratios in the sampled forest litter was found at most sites, however this was not observed for the soil samples (Table 2).

The average C:P values for the forest reserves sampled ranged from 329-718 g·kg⁻¹ for litter and 41.73−91.48 g·kg–1 for soil (Fig. 3). The highest C:P ranges were found in the 'DG' (58.94− −718.32 g·kg–1) and 'WS' (45.45−708.27 g·kg–1) reserves, and the lowest in the soil of the 'KOR' reserve (41.39−406.2 g·kg–1). At most sites, with the exception of the 'KOR' reserve, leaving deadwood on the forest floor resulted in a significant increase in the C:P ratio in the litter, but had no significant effect on the value in the soil.

The N:P ratio had the highest values in litter (13.36-22.92 g⋅kg⁻¹), similar to the aforementioned cases, but with its soil value much lower (2.0−6.76 g·kg⁻¹). The largest range of N:P ratios was found in the soil of the 'DG' reserve, and the smallest in the 'WS' and 'LP' reserves (Table 2). It was found that the long−term presence of deadwood did not significantly affect the value of N:P ratios in the soil or litter.

The study evaluated the linear Pearson's correlations between the content of the total forms of TC, TN and TP in the soils of forest reserves for control and deadwood containing samples. Positive and in most cases highly significant linear correlation coefficients were obtained (Table 3). The highest values were obtained for TC and TN and ranged from $r=0.903***$ for samples with deadwood (1), $r=0.957***$ for control samples (0), and for all objects overall $(r=0.916***)$. In addition, slight linear relationships were found between the content of TC x TP (object 1: $r=0.600***$ and object 0: $r=0.792***$) and $TN\times TP$ (object 1: $r=0.600***$ and object 0: $r=0.792$).

Discussion

The content of TC and TN in the forest soils studied depended on the location of the measure− ment points, soil depth, and the presence of deadwood. Total carbon content (TC) from samples ranged from 284.24−462.23 g·kg–1 in litter to 9.33−44.57 g·kg–1 in soil. The total nitrogen content (TN) in the soil varied from 2.95 g·kg⁻¹ to 4.85 g·kg⁻¹. The presence of litter on the soil surface and its decomposition contribute to changes in the physicochemical properties of the substrate and to an increase in the contents of some nutrients, mainly C and N (Annunzio *et al.,* 2008; Łabęda and Kondras, 2020). Vesterdal (2002) and Post and Know (2008) found that a greater amount

Fig. 3.

Stoichiometric ratios of C:N and C:P in soils of forest reserves of the Białowieża Forest and Koryciny reserve

Table 3.

Pearson correlations of the pH_{CaCl_2} and C, N and P in soils of the Białowieża Forest and Koryciny reserve

0 – without deadwood; 1 – with deadwood

*, **, and *** significant correlation at *p*=0.05, 0.01 and 0.001 level

of nutrients are present in the upper surface layer of forest soils. Wang (2021) showed that the contents of TC and TN and the ratio of TC:TN, TC:TP and TN:TP decreased with soil depth and type of forest. According to Gill and Burke (1999) and Vergas (2006), the quality and quantity of litter and the cycling of its nutrients depend on the predominant forest tree species.

Soil pH, C and N content and their ratios in the soil depend on the type of forest (Quan *et al*., 2014). Wang (2021) showed that the carbon content in forest leaves and soils changes with the age of the forest stand, and that the TC, TN and TP content is higher in mature forest soils than in young and middle−aged forests. According to Han (2005), trees in deciduous forests had higher N and P concentrations than those in coniferous forests. The forest reserves of the Biało− wieża Forest, where the study was conducted, had mature stands over 110 years old, and their composition was dominated by the following tree species: *Quercus petraea* (Matt.) Liebl.*, Q. robur, Carpinus betulus* and *Picea abies*.

The C:N ratio is an important indicator of soil organic matter quality because it affects the potential of microorganisms involved in mineralization of nutrients (Hodge *et al*,. 2000). The nutri− ent content (TC, TN, TP) and their stoichiometry depend on many site variables including geographic location, geology, soil texture and type, forest type, tree species present in the stand, and their age (Koerner *et al*., 1997; Verheyen *et al*., 1999). Cools *et al*., 2014, Quan *et al*., 2014). It was found that leaving deadwood in forested areas for a longer period of time resulted in an increase in the C:N ratio in litter compared to the control samples. The C:N value in the soils ranged from 30−40:1 in the case of forest litter (except in the 'KOR' reserve, where the C:N value was 23:1) to 10−20:1 in the soil. The high C:N value in litter may indicate low biological activity and slow decomposition of organic matter. This could be due to the slowing of miner− alization of organic matter due to a high content of cellulose, lignin and hemicellulose in wood, which has been confirmed by other studies (Vargas *et al*., 2006). A high C:N ratio may indicate low C and N mass losses in the litter and soil (Kraus *et al*., 2004).

According to Qi *et al*. (2020), differences in topsoil C:N and C:P ratios amongst different tree species of deciduous and coniferous trees were significantly related to tree litter. Average C:P ratios in the forest reserves studied ranged from 329.12−718.32:1 in litter to 41.73−91.48:1 in soil. The presence of deadwood significantly increased the stoichiometric C:P ratio in the litter of the forest reserves but had a lower effect on the soil nutrient content. Degado−Baquerizo (2017) showed that forest soils with high C:P ratios can support low microbial diversity, while low resource quality (*i.e*., C:N and C:P:N) can support bacterial diversity. A wide range of stoi−

chiometric C:P ratios in litter from forest reserves may indicate increased biological absorption of phosphorus. Literature suggests that C:P>300:1 favors increased activity of this process. According to Bueis (2019), the low solubility of phosphorus compounds in soil is strongly dependent on pH, and the absorption processes occurring in the soil often make phosphorus a limiting component in forest ecosystems. In acidic, very acidic and alkaline soils, phosphorus cycling is reversed and converted to forms that are inaccessible to plants. According to Pakuła and Kalembasa (2008), forest soils contain nearly 80% organic phosphorus (in the form of phytin, phospholipids and nucleic acids). It can be assumed that the dynamics of nutrient cycling in old forests are much slower and caused by the weakening of mineralization processes of deadwood which are rich in compounds that are difficult to degrade, such as cellulose.

Conclusions

- The presence of long term deadwood favored the accumulation of TC, especially on the for− est floor, and significantly increased the C:N value compared to the control samples. The C:N value in soil varied between 30−40:1 for litter and 10−20:1 in soil. A high C:N value may indicate slow decomposition of material containing dead woody debris rich in compounds that are difficult to degrade (*e.g*., cellulose and lignins).
- The C:P ratio in the investigated forest soils varied from 329−718:1 in litter to 10−200:1 in soil. The presence of deadwood significantly increased these ratios. A high C:P ratio in forest litter >300:1 under acidic soil conditions favored reverse cycling of phosphorus to forms inaccessible to vegetation.
- The study showed that in reserves with forests that are more than 100 years old the dynam− ics of nutrient cycling are different and the relationships between nutrients have a wider range than in young and middle−aged forests. Old forests are dominated by deadwood, which decomposes slowly and gradually releases nutrients into the soil.

Conflicts of interest

The author declare that they have no known competing financial interests or personal relation− ships that could have appeared to influence the work reported in this paper.

Funding

The study was elaborated within the framework of the research project entitled 'The influence of deadwood on soil and humus accumulation processes' (no 900801) financed by subsidy funds received by the Forest Research Institute from Polish Ministry of Education and Science.

Acknowledgements

Special thanks to EngD. Karol Sokołowski for soil sampling and M.Sc. Eng. Halina Dróżdż, EngD. Krzysztof Sztabkowski, M.Sc. Marlena Romanowska, M.Sc. Grażyna Misiewicz, M.Sc. Anna Paluch for performing chemical analyses.

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Streszczenie

Pozostawianie martwego drewna w starych lasach naturalnych a zawartość i stosunki stechiometryczne C, N i P w glebie

Badania prowadzono w województwie podlaskim na terenie 3 rezerwatów leśnych Puszczy Biało− wieskiej – Rezerwatu Krajobrazowego Władysława Szafera "WS" (52°42'20"N; 23°42'59"E), Dębowego Grądu "DG" (52°44'2"N; 23°41'30"E) i Lipin "LP" (52°45'12"N; 23°38'40"E) – oraz poza jej obszarem: w rezerwacie Koryciny "KOR" (52°40'10"N; 22°44'30"E) (tab. 1; ryc. 1). Drzewostany rezerwatów leśnych były w wieku 110−160 lat, a w ich składzie dominowały *Quercus robur* L., *Carpinus betulus* L. i *Tilia cordata* Mill., ze znacznym udziałem *Picea abies* (L.) Karst. Celem badań była ocena wpływu pozostawiania martwego drewna w starych lasach naturalnych na zawartość oraz stosunki stechiometryczne C, N i P w glebie.

Próbki pobrano jesienią 2021 r. z warstw gleby 0−5, 5−10, 10−20 i 20−40 cm oraz ze ściółki (O) – bezpośrednio spod martwego drewna (obiekt 1) oraz w jego sąsiedztwie do 1 m (obiekt 0). W próbkach zmierzono pH w 0,01 M CaCl₂ metodą potencjometryczną. Po zmineralizowaniu próbek w roztworze wody królewskiej (kwas azotowy i kwas nadchlorowy 1:1) oznaczono zawar− tość TC i TN metodą wysokotemperaturowego spalania z detekcją TDC, natomiast zawartość TP metodą emisyjnej spektrometrii atomowej ze wzbudzeniem w indukcyjnie sprzężonej plazmie (ICP−OES).

Zawartość węgla całkowitego (TC) w glebie rezerwatów leśnych mieściła się w zakresach 300−400 g·kg–1 w ściółce i 1,43−100 g·kg–1 w glebie, a azotu całkowitego (TN) 9,31−15,59 g·kg–1 w ściółce i 0,56−3,36 g·kg–1 w glebie (tab. 2). Najzasobniejsza w oba składniki była gleba rezer− watu "DG", a najmniej zasobna gleba rezerwatu "LP" (tab. 2; ryc. 2). Średnia zawartość TP w ściółce leśnej mieściła się w przedziale 0,65−1,00 g·kg–1 i była wyższa niż w glebie (0,20−0,48 g·kg–1). Pozostawianie martwego drewna sprzyjało gromadzeniu TC, głównie w ściółce leśnej, i zwiększało wartość stosunku C:N w porównaniu do obiektów kontrolnych. Stosunek C:N mieścił się w zakresach 30−40:1 w ściółce oraz 10−20:1 w glebie (ryc. 3a), natomiast stosunek C:P wahał się w przedziałach 400−600:1 w ściółce i 10−200:1 w glebie (ryc. 3b). Na podstawie korelacji liniowych Pearsona odnotowano dodatnie i w większości wysoce istotne współczynniki (tab. 3). Najwyższe ich wartości otrzymano dla obiektów kontrolnych (r=0,957***), a nieco mniejsze dla obiektów z martwym drewnem (r=0,903***). Ponadto stwierdzono współzależności liniowe dla zawartości w glebie TC×TP w obiektach z martwym drewnem (r=0,600***) i w obiekcie kon− trolnym $(r=0.792***)$.

Pozostawianie martwego drewna w rezerwatach leśnych zwiększało zakres stosunków C:N i C:P w ściółce. Szeroki zakres C:N może świadczyć o powolnym rozkładzie materii organicznej

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zasobnej w związki trudno podlegające rozkładowi (celuloza, ligniny). Znaczna wartość C:P (300:1) w ściółce rezerwatów leśnych w warunkach kwaśnego odczynu gleby sprzyjała uwstecznianiu fosforu do form niedostępnych dla roślin.