original paper

Eco−enzymatic and microbial stoichiometry of forest and agricultural soil use from a temperate climate: A case study from Southern Poland

Jarosław Lasota, Stanisław Łyszczarz[⊠], Ewa Błońska

Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Krakow, 29 Listopada 46, 31−425 Kraków, Poland

ABSTRACT

Intensification and fertilization of agricultural soils reduce the number and diversity of soil microorganisms responsible for the cycling of organic carbon (C), total nitrogen (N) and total phosphorus (P). The aim of this research was to determine the C:N:P stoichiometry of forest and agriculture land use types on soil properties. In this study, the relationship between enzyme stoichiometry, microbial biomass stoichiometry and potential regulators was explored. The study was conducted in the Rybnik Forest District of southern Poland (50°05'55"N, 18°32'42"E). The research covered soils in forestry, ecotone and agricultural areas. Transect and soil samples were collected in forest and agricultural areas and on the border between forest and agricultural areas. Six transects of 200 m length running through a forest use area, an ecotone and an agricultural use area were established. Soil samples were collected every 50 m where the midpoint of each transect was located at the edge of the ecotone. The content of C, N and P, eco−enzymatic activity and microbial biomass of C, N and P were determined in soil samples. The research confirmed the possibility of using stoichiometry to assess the impact of land use on soils. Molar stoichiometry and eco−enzymatic stoichiometry significantly distinguished forest soils from agricultural soils. The stoichiometry of microbial biomass turned out to be less sensitive in the assessment of changes in soils caused by the type of land use. The amount and quality of the supplied organic matter influenced the stoichiometry of the studied soils. The stoichiometry of the studied soils con− firmed the higher availability of nutrients in forest soils.

KEY WORDS

agriculture soils, Arenosols, Cambisols, eco−enzyme activity, forest soils, land use, microbial biomass

Introduction

In recent years, interest in soil conditions has been stimulated by the growing awareness of the fact that the soil is an important component of the biosphere. It functions not only to produce food, wood and other resources, but it is also crucial for maintaining the local, regional and global quality of the environment. Rational management of the soil is one of the elements of shaping the environment and can be achieved by taking into account all the soil's functions, identifying threats, and designating sensitive areas that are most exposed to soil degradation processes

Received: 8 March 2024; Revised: 27 June 2024; Accepted: 3 July 2024; Available online: 11 August 2024

င**ါ** BY

e−mail: stanislaw.lyszczarz@urk.edu.pl

^{©2024} The Author(s). http://creativecommons.org/licenses/by/4.0

(Krasowicz *et al*., 2011). Monitoring the impact of land use on the properties of soil helps to maintain the proper condition of the soil over the long term (Liu *et al*., 2003). Monitoring the temporal and spatial variation of soil properties facilitates understanding of the evolution of soil properties and adjustment of the management methods over time (Jin *et al*., 2021). Agricultural management practices such as fertilization, irrigation, mechanization and the return of straw to the soil significantly alter the soil environment (Niu *et al*., 2021). In general, arable soils contain significantly less organic matter which translates into a poorer structure and a lower amount of microorganisms. Soil fertility and productivity depend on soil organic matter (SOM) which is a reservoir of nutrients and is essential in the nutrient cycle (Steiner *et al*., 2007). It also improves the physical, chemical and biological properties of soils (Bhattacharya *et al*., 2010). The way the soil is used has an impact on microorganisms and microbiological processes by changing the quantity and quality of plant debris reaching the soil which is the primary source of SOM (Błońska *et al*., 2017).

As a result of the type of management method, the soil structure and nutrient content may change, which in turn leads to changes in the content of C, N and P (Tang *et al*., 2022). According to these authors, intensive agricultural practices change the soil porosity and texture and lead to clear differences in the C, N and P stoichiometry. C:N:P stoichiometry studies allow an understanding of the balance of chemical elements within ecological relationships (Sterner and Elser, 2002). The soil C:N and C:P ratios reflect the rate of decomposition of organic matter and the rate of nutrient mineralization (Zhang *et al*., 2015). The stoichiometry of microbial soil biomass shows changes in the microbial composition as a result of changes in environmental conditions (Fanin *et al*., 2013). The flow of C, N and P as a result of mineralization or immobilization is controlled by soil microorganisms (Bünemann *et al*., 2012). Understanding the microbial stoichiometry of soil biomass may be important in assessing processes occurring in forest ecosystems (Heuck *et al*., 2015). The activity of soil enzymes is commonly recognized as a sensitive indicator of changes occurring in soil as a result of land use (Błońska *et al*., 2017). Soil microorganisms produce extracellular eco−enzymes for more efficient nutrient acquisition which translates as part of C, N and P cycles (Luo *et al*., 2017). Agricultural soils are often subject to fertilization which impacts the amount and diversity of microorganisms and consequently, enzymatic activity (Morugán−Coronado *et al*., 2022). Based on Qin *et al*. (2021), enzyme stoichiometry can be an effective indicator of the impact of agricultural use. Therefore, according to Lasota *et al*. (2022) the enrichment of forest soils with biochar resulted in changes in enzymatic stoichiometry. C:N:P stoichiometry is a useful tool that reflects the nutrient cycle in mountain ecosystems, and previous research has shown that in the case of species with higher ecological requirements stoichiometry can indicate nutrient limits (Lasota *et al*., 2021).

The aim of this research was to determine the C:N:P stoichiometry of different land use types. The research covered soils in the forest−agriculture land use transect. Soil samples were collected in forest and agricultural areas and on the borders between forest and agricultural areas. In this study, the relationship between eco−enzyme stoichiometry, microbial biomass stoichiometry and its potential regulators was explored. The following hypotheses were tested: (i) the effect of land use and the provision of different amounts and quality of detritus on the stoichiometry of C:N:P of soil, (ii) the limiting element for soil microbial biomass and eco−enzyme activity is C and N and to a lesser extent P content, (iii) the eco−enzyme stoichiometry mirrors diverse resource conditions, whereas the soil microbial biomass C:N:P stoichiometry is less informative for gauging resource availability along the transect, and (iv) the forest ecosystem provides a valuable quality and quantity of organic matter shaping the favourable microbial and enzymatic stoichiometry of the soil.

Traditional soil assessment methods often focus on physical and chemical parameters. The eco− enzymatic stoichiometry used in the study as well as microbial biomass allows a more precise assessment of these changes leading to the identification of predictive indicators. This perspec− tive is crucial for understanding the impact of land use intensification and anthropo−pressure on soil ecosystems. The information obtained from this study would be important for monitoring nutrient resources in forest and agricultural soils.

Materials and methods

STUDY AREA AND SOIL SAMPLING. The experiment was conducted in the area of the Rybnik Forest District located in the south of Poland (Fig. 1) The area is characterised by an average annual rainfall of 705 mm and an average annual temperature of 8.4°C. The climate and soil parent material are homogeneous across the transects. The study area of Rybnik Forest District was dominated by Brunic Arenosols and Cambisols (WRB, 2022) with unified soil texture (sandy loam) formed on glacial moraines. The soil assessment was carried out on the basis of an analysis of these profiles and additional information obtained from the Forest Inspectorate. The selected subtype was confirmed when the soil description was made while conducting the fieldwork. The soils in the study transects were described as Brunic Cambic Arenosol. The name of the soil group is based on the literature (Świtoniak *et al*., 2016). All transects were located on the same soil type and did not differ in texture.

Soil samples for laboratory analysis were obtained at the beginning of the spring and vege− tation season in May 2019. The forest area studied was a mixed pine−oak [70% Scots pine *Pinus*

Fig. 1.

Scheme of the study and the sampling point locations in the transect

sylvestris L. and 30% sessile oak *Quercus petraea* (Matt.) Liebl.] stand with an age of 80 years and a tree count of about 550 trees per hectare. The agricultural area of the surveyed transects was an area where wheat was grown.

For the experiment, six transects 200 m in length running through a forest use area, an eco− tone, and an agricultural use area were established (Fig. 1). Study material was collected every 50 m, where the midpoint of each transect was located at the ecotone. Soil samples were collected from the humus horizon (0−15 cm) after exposing the organic horizon in the forest use soils and the ecotone zone. The depth of this soil horizon is rich in decomposed organic material and plays a key role in nutrient cycling, microbial activity and overall soil fertility. Soil analysis from this depth allowed valuable information on soil composition and condition to be established, particularly in forested areas and ecotones where organic matter dynamics are significant. Due to variations in management practices, agricultural soils lacked the upper organic layer typically found in forest soils.

LABORATORY ANALYSIS. Freshly collected soil samples were dried and then sieved through a 2 mm mesh sieve. The particle size distribution was analyzed using a laser diffraction method (Analysette 22, Fritsch, Idar−Oberstein, Germany). C and N content of soil samples was determined using an elemental analysis apparatus (LECO CNS TrueMac Analyzer Leco, St. Joseph, MI, USA). After digestion of the mixture with concentrated nitric acid and perchloric acid in a 3:1 ratio and using an analyzer (ICP−OES ThermoiCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, U.K.), the content of P was determined. At the molecular level C:N, C:P and N:P ratios were calculated. Using fluorogenic labeled substrates, the enzyme activities of β -glucosidase (BG), N-acetyl- β -−D−glucosaminidase (NAG), and phosphatase (PH) were determined (Pritsch *et al*., 2004; Turner, 2010; Sanaullah *et al*., 2016) using a multidetector plate reader (Biotek) with excitation at 355 nm and emission at 460 nm. Eco−enzyme stoichiometry was calculated using BG:NAG, BG:PH and NAG:PH for C:N, C:P and N:P ratios in the soil (Lasota *et al*., 2021). A fumigation and extraction method (Jenkinson and Powlson, 1976; Vance *et al*., 1987) was used to determine the C, N and P content of microbial biomass.

STATISTICAL ANALYSIS. Pearson correlation coefficients were calculated to summarize the ratio of C:N:P stoichiometry of the studied soils. Principal component analysis (PCA) was used to evaluate the relationship between C:N:P stoichiometry and land use. Analysis of variance was used to assess differences between mean values of soil properties. Differences with a value of $p < 0.05$ were considered statistically significant. R statistical software (R Core Team, 2020) and R Studio (RStudio Team, 2020) were used to perform the statistical analysis.

Results

PHYSICOCHEMICAL PROPERTIES. The soils in the transect differed significantly in their physico− chemical and biochemical properties (Table 1). Significantly higher C content was recorded both in forest soils and soils of the edge point. In the case of agricultural soils, the C content was seven times lower than in forest soils (10.69% and 1.46%, respectively). Forest soils and soils of the edge point were characterized by a significantly higher content of N. In forest soils, the N content was five times higher than in agricultural soils (0.53% and 0.10%, respectively). The examined soils did not differ significantly in phosphorus content (Table 1). Lower content of phosphorus was recorded in soils of the edge point and agricultural soils.

BIOCHEMICAL PROPERTIES. The investigated soils differed in their enzymatic activity. Significantly higher activity of BG, NAG and PH was noted in the soils of forest areas compared to the agri−

cultural soils (Table 1). Agricultural soils were characterized by several times lower activity of all the tested eco−enzymes compared to the forest soils. The significantly highest microbial biomass of C, N and P was recorded in the forest soils compared to the agricultural soils. Microbial biomass of C was three times higher than in the agricultural soils. Microbial nitrogen biomass was more than twice as high in the forest soils as compared to the agricultural soils, and microbial phosphorus biomass was more than four times higher (Table 1). The activity of all tested eco− enzymes and microbiological biomass strongly positively correlated with the content of C and N (Fig. 2). The activity of BG and PH as well as microbiological biomass of N and P positively correlated with the phosphorus content (Fig. 2).

PHYSICOCHEMICAL AND BIOCHEMICAL STOICHIOMETRY PROPERTIES AND CORRELATIONS. A sig− nificantly higher C:N ratio was recorded in the forest soils and in the soils of the edge point (22.8 and 21.8, respectively) compared to the agricultural soils (17.6) (Fig. 3). In the case of the C:P and N:P ratios, the forest and edge point soils were characterized by significantly higher values compared to the agricultural soils. The C:P ratio was five times lower in the agricultural soils, and the N:P ratio was four times lower (Fig. 3). In the case of enzymatic stoichiometry,

Mean $\pm SD$; F – forest, FA – edge point, A – arable land; carbon and nitrogen content [%], P – content (mg.kg⁻¹), BG – β-glucosidase (mol MUB g⁻¹ dry soil h⁻¹), NAG – N-acetyl-β-D-glucosaminidase (mol MUB g⁻¹ dry soil h⁻¹), PH – phosphatise (mol MUB g⁻¹ dry soil h⁻¹), MBC – microbial biomass carbon (µg·kg⁻¹), MBN – microbial biomass nitrogen (μ g·kg⁻¹), MBP – microbial biomass phosphorus (μ g·kg⁻¹), small letters in the upper index of the mean values indicate significant differences between different land management types

Fig. 2.

Table 1.

Relationships between the C, N, P content and biochemical characteristics

 $*$ *p*<0.05, BG – β-glucosidase, NAG – N-acetyl-β-D-glucosaminidase, PH – phosphatase, MBC – microbial biomass carbon, MBN – microbial biomass nitrogen, MBP – microbial biomass phosphorus

BG – B-glucosidase, NAG – N-acetyl-β-D-glucosaminidase, PH – phosphatase, MBC – microbial biomass carbon, MBN – microbial biomass nitrogen, MBP – microbial biomass phosphorus, F – forest, FA – edge point, A – arable land

statistically significant differences were determined in the examined soils (Fig. 3). In the case of the BG:NAG ratio, lower values were recorded in the forest soils and the soils of the edge point. The BG:PH ratio did not differ significantly between the studied soils. A significantly lower NAG:PH ratio was recorded in the agricultural soils compared to the forest soils and soils of the edge point. In the case of the microbiological stoichiometry of the biomass, no significant differences were found in the studied soils (Fig. 3).

The C:N ratio was positively correlated with the C:P, N:P and NAG:PH ratio and nega− tively with BG:NAG (Fig. 4). The C:P ratio was positively correlated with the N:P, NAG:PH ratio and negatively with BG:NAG. In the case of the N:P ratio, an additional correlation was noted with NAG:PH and a negative correlation with BG:NAG (Fig. 4).

Factors 1 and 2 distinguished by the PCA explained a total of 61.43% of the variance in the C:N:P stoichiometry (Fig. 5). Factor 1 is related to the molar C:N:P ratio and factor 2 is related

Fig. 5.

The projection of variables on a plane of the first and second PCA factor BG – β-glucosidase, NAG – N-acetyl-β-D-glucosaminidase, PH – phosphatase, MBC – microbial biomass carbon, MBN – microbial biomass nitrogen, MBP – microbial biomass phosphorus, F – forest, FA – edge point, A – arable land

to the stoichiometry of the microbial biomass. The conducted PCA analysis also confirmed the distinctiveness of the soils of the studied transects. The PCA analysis showed that the agricul− ture soils are distinct.

Discussion

The results demonstrated that land use and the provision of different quantities and qualities of detritus significantly influenced the stoichiometry of C:N:P in soil confirming our hypothesis (i). The highest C:N:P ratio was recorded in forest soils of 531:23:1, a lower one in soils of the edge point of 500:22:1, and the lowest in agricultural soils of 103:6:1. The differences in the C:N:P ratio are the result of statistically significant differences in the content of C and N in the soils of the forest−agriculture transect. Significantly higher C and N content was found in forest soils and soils of the edge point as compared with the agricultural soils. The high C content was related to forest stands with a large share of pine. The type of land use affects the quantity and quality of SOM (Yang *et al*., 2016; Gawęda *et al*., 2019). Changing the management method to agriculture reduces the organic C stock by up to 75% depending on the geographic location (Lal, 2004). Gawęda *et al*. (2019) reported a clear trend to increasing the accumulation of C in post−agricultural soils forested with birch. Significantly higher C:N, C:P and N:P ratios in forest soils are the effect of the quality of the delivered plant litter. Trees provide different types of organic matter as the soil is filled with aboveground and underground woody biomass as well as herbaceous plants which results in the rate of nutrient release (Young, 1989). Trees have extensive root systems and the roots are the main source of C (Jones *et al*., 2004). The amount of nutrients released as a result of the death of roots may be greater than the decay of the litter (Yuan *et al*., 2010). Owing to the differences in the biomass delivered to the soil in the transect results in various types of humus development. As a result of soil acidification by trees, mor type humus is formed which is characterized by a high C:N ratio resulting from the slow decomposition process (Błońska *et al*., 2021). Pardon *et al.* (2017) when assessing the effect of tree rows on soil properties found a significantly higher content of C and N in soils in the vicinity of trees which results from the input of tree litter and the impact of throughfall water. In our research, the soils of the edge point showed a significantly higher content of C and N compared to agricultural soils which is directly related to the presence of trees and their supply of organic matter.

Our findings confirmed that C and N are the limiting elements for soil microbial biomass and eco−enzyme activity with P being less significant thus supporting the hypothesis (ii). The study confirmed that the limiting element for soil microbial biomass and eco−enzyme activity is C and N and to a lesser extent P content in the transects. Additionally, the stoichiometry of eco− enzymes reflected different resource conditions while soil microbial biomass C:N:P stoichiometry had less utility in the assessment of availability of resources in the transect. P content was not significantly differentiated in the forest−agriculture transect. Contrary to the content of C and N, there were no significant differences in the content of P between forest and agricultural soils. The activity of all the tested eco−enzymes correlated stronger with the content of C and N compared to the content of P.

The eco−enzyme stoichiometry was observed to effectively reflect varying resource condi− tions, whereas soil microbial biomass stoichiometry of C:N:P has less impact for measuring resource availability along the transect which validates the hypothesis (iii). The relationship between the activity of BG, NAG and PH with the content of C and N was the effect of dif− ferences in the quality and quantity of SOM in the forest−agriculture transect. Changes in the activity of soil enzymes are caused by vegetation which is mainly root turnover, root exudation and aboveground detrital inputs (Kotroczó *et al*., 2014). Previous studies indicate a relationship between enzymatic activity and the fractional composition of SOM (Wani, 2021). SOM consists of a mixture of a labile fraction that decomposes quickly and a stable fraction that is stored in soil for millennia (Kalambukattu *et al*., 2013). An increased amount of plant debris reaches the forest soil which affects the amount of the light and heavy fraction of SOM and, consequently, translates into the activity of microorganisms and their enzymes (Błońska *et al*., 2020). According to He *et al*. (2021) soils with a higher content of soil organic carbon (SOC), especially the labile fraction, are characterized by a higher activity of microorganisms. Diversification of microbiota

promotes the activity of soil eco−enzymes. This study research also confirmed a strong relation− ship between the content of C in soils used in various ways with microbial biomass. According to Wang *et al*. (2022) SOC predicts soil microbial biomass C, N and P at a global scale.

The data also confirmed that forest ecosystems offer high−quality and substantial quantities of organic matter which shape favourable soil microbial and enzymatic stoichiometry, thereby supporting the hypothesis (iv). Based on our results, it can be concluded that by using the activity of eco−enzymes and microbial biomass content of C, N and P, it is possible to observe changes in the content of SOM resulting from the type of management method. Both the forest soils and soils of the edge point were characterized by a significantly lower BG:NAG ratio and a signifi− cantly higher NAG:PH ratio compared to agricultural soils. The eco−enzymatic stoichiometry differentiates forest soils from agricultural soils which is result of a higher content of C, N and P in forest soils. Our experiment showed that the microbial stoichiometry of the biomass did not reflect the effect of land use type. Any significant differences in the biomass microbial stoichiom− etry between agricultural and forest soils were not noticed.

Microhabitats may have influenced nutrient availability and microbial activity. Differences in moisture content, vegetation cover and open canopy areas may affect soil properties which may explain the lack of significant differences in microbial biomass stoichiometry between agricultural and forest soils. Therefore, more detailed studies are needed which should include a thorough analysis of the soil environment.

Conclusions

This research study has confirmed the possibility of using stoichiometry to assess the impact of land use on soils. Molar stoichiometry and eco−enzymatic stoichiometry significantly distinguished forest soils from agricultural soils. The stoichiometry of microbial biomass turned out to be less sensitive in the assessment of changes in soils caused by land use type. The amount and quality of the supplied organic matter influenced the stoichiometry of the studied soils. The stoichiometry of the studied soils confirmed the higher availability of nutrients in forest soils. The quantity and quality of organic matter supplied have a significant impact on shaping the eco−enzymatic and microbial stoichiometry of soils suggesting the need for agricultural practices that promote balanced SOM cycling such as perennial agriculture, compost application or intercropping. These approaches can help to maintain nutrient availability in soils which can contribute to maintaining soil health and fertility.

Authors' contributions

Conceptualization – J.L., E.B.; methods – E.B.; investigation – J.S., S.Ł.; formal analysis – S.Ł., E.B.; writing−original draft preparation – J.L., S.Ł, E.B.; writing−review and editing – J.L., S.Ł, E.B.; visualization – S.Ł.

Conflicts of interest

The authors declare no conflict of interest. The financial supporters had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manu− script, or in the decision to publish the results.

Funding source

Article was financed by a subvention from the Ministry of Science and Higher Education of the Republic of Poland for the University of Agriculture in Krakow (SUB:040012:D019).

References

- **Bhattacharyya, R., Prakash, V., Kundu, S., Srivastva, A.K., Gupta, H.S., Mitra, S., 2010.** Long term effects of fertilization on carbon and nitrogen sequestration and aggregate associated carbon and nitrogen in the Indian sub−Himalayas. *Nutrient Cycling in Agroecosystems*, 86 (1): 1−16. DOI: [https://doi.org/10.1007/s10705−009−9270−y.](https://doi.org/10.1007/s10705-009-9270-y)
- **Błońska, E., Lasota, J., Vasconcelos da Silva, G.R.V., Vanguelova, E., Ashwood, F., Tibbett, M., Watts, K., Lukac, M., 2020.** Soil organic matter stabilization and carbon−cycling enzyme activity are affected by land man− agement. *Annals of Forest Research*, 63 (1): 71−86. DOI: https://doi.org/10.15287/afr.2019.1837.
- **Błońska, E., Lasota, J., Zwydak, M., 2017.** The relationship between soil properties, enzyme activity and land use. *Forest Research Papers*, 78: 39−44. DOI: [https://doi.org/10.1515/frp−2017−0004.](https://doi.org/10.1515/frp-2017-0004)
- **Błońska, E., Piaszczyk, W., Staszel, K., Lasota, J., 2021.** Enzymatic activity of soils and soil organic matter stabi− lization as an effect of components released from the decomposition of litter. *Applied Soil Ecology*, 157: 103723. DOI: https://doi.org/10.1016/j.apsoil.2020.103723.
- **Bünemann, E.K., Oberson, A., Liebisch, F., Keller, F., Annaheim, K.E., Huguenin−Elie, O., Frossard, E., 2012.** Rapid microbial phosphorus immobilization dominates gross phosphorus fluxes in a grassland soil with low inorganic phosphorus availability. *Soil Biology and Biochemistry*, 51: 84−95. DOI: https://doi.org/10.1016/j.soilbio.2012.04.012.
- **Fanin, N., Fromin, N., Buatois, B., Hättenschwiler, S., 2013.** An experimental test of the hypothesis of non−home− ostatic consumer stoichiometry in a plant litter−microbe system. *Ecology Letters*, 16 (6): 764−772. DOI: [https://](https://doi.org/10.1111/ele.12108) [doi.org/10.1111/ele.12108.](https://doi.org/10.1111/ele.12108)
- **Gawęda, T., Błońska, E., Małek, S., 2019.** Soil organic carbon accumulation in post−agricultural soils under the influence birch stands. *Sustainability*, 11 (16): 4300. DOI: https://doi.org/10.3390/su11164300.
- **Gawęda, T., Małek, S., Błońska, E., Jagodziński, A.M., Bijak, S., Zasada, M., 2021.** Macro−and micronutrient contents in soils of a chronosequence of naturally regenerated birch stands on abandoned agricultural lands in Central Poland. *Forests*, 12 (7): 956. DOI: https://doi.org/10.3390/f12070956.
- **He, L., Lu, S., Wang, C., Mu, J., Zhang, Y., Wang, X., 2021.** Changes in soil organic carbon fractions and enzyme activities in response to tillage practices in the Loess Plateau of China. *Soil and Tillage Research*, 209: 104940. DOI: https://doi.org/10.1016/j.still.2021.104940.
- **Heuck, C., Weig, A., Spohn, M., 2015.** Soil microbial biomass C:N:P stoichiometry and microbial use of organic phosphorus. *Soil Biology and Biochemistry*, 85: 119−129.
- **IUSS Working Group WRB, 2022.** World Reference Base for Soil Resources. In International Soil Classification System for Naming Soils and Creating Legends for Soil Maps, 4th ed. Vienna: International Union of Soil **Sciences**.
- **Jenkinson, D.S., Powlson, D.S., 1976.** The effects of biocidal treatments on metabolism in soil−V: A method for measuring soil biomass. *Soil Biology and Biochemistry*, 8 (3): 209−213. DOI: https://doi.org/10.1016/j.soilbio.2015.02.029.
- **Jin, J., Wang, L., Müller, K., Wu, J., Wang, H., Zhao, K., Berninger, F., Fu, W., 2021.** A 10−year monitoring of soil properties dynamics and soil fertility evaluation in Chinese hickory plantation regions of southeastern China. *Scientific Reports*, 11 (1): 1−13. DOI: [https://doi.org/10.1038/s41598−021−02947−z.](https://doi.org/10.1038/s41598-021-02947-z)
- **Jones, D.L., Hodge, A., Kuzyakov, Y., 2004.** Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist*, 163 (3): 459−480. DOI: [https://doi.org/10.1111/j.1469−8137.2004.01130.x.](https://doi.org/10.1111/j.1469-8137.2004.01130.x)
- **Kalambukattu, J.G., Singh, R., Patra, A.K., Arunkumar, K., 2013.** Soil carbon pools and carbon management index under different land use systems in the Central Himalayan region. *Acta Agriculturae Scandinavica*, *Section B−Soil and Plant Science*, 63 (3): 200−205. DOI: https://doi.org/10.1080/09064710.2012.749940.
- **Kotroczó, Z., Veres, Z., Fekete, I., Krakomperger, Z., Tóth, J.A., Lajtha, K., Tóthmérész, B., 2014.** Soil enzyme activity in response to long−term organic matter manipulation. *Soil Biology and Biochemistry*, 70: 237−243. DOI: https://doi.org/10.1016/j.soilbio.2013.12.028.
- **Krasowicz, S., Oleszek, W., Horabik, J., Dębicki, R., Jankowiak, J., Stuczyński, T., Jadczyszyn, J., 2011.** Rational management of the soil environment in Poland. *Polish Journal of Agronomy*, 7: 43−58.
- **Lal, R., 2004.** Soil carbon sequestration impacts on global climate change and food security. *Science,* 304 (5677): 1623−1627. DOI: https://doi.org/10.1126/science.1097396.
- **Lasota, J., Babiak, T., Błońska, E., 2022.** C:N:P stoichiometry associated with biochar in forest soils at historical charcoal production sites in Poland. *Geoderma Regional*, 28: e00482. DOI: https://doi.org/10.1016/j.geodrs.2022.e00482.
- **Lasota, J., Małek, S., Jasik, M., Błońska, E., 2021.** Effect of planting method on C:N:P stoichiometry in soils, young silver fir (*Abies alba* Mill.) and stone pine (*Pinus cembra* L.) in the upper mountain zone of Karpaty Mountains. *Ecological Indicators*, 129: 107905. DOI: https://doi.org/10.1016/j.ecolind.2021.107905.
- **Liu, X., Han, X., Song, C., Herbert, S.J., Xing, B., 2003.** Soil organic carbon dynamics in black soils of China under different agricultural management systems. *Communications in Soil Science and Plant Analysis*, 34 (7−8): 973−984. DOI: [https://doi.org/10.1081/CSS−120019103.](https://doi.org/10.1081/CSS-120019103)
- **Luo, L., Meng, H., Gu, J.D., 2017.** Microbial extracellular enzymes in biogeochemical cycling of ecosystems. *Journal of Environmental Management*, 197: 539−549. DOI: https://doi.org/10.1016/j.jenvman.2017.04.023.
- **Morugán−Coronado, A., Pérez−Rodríguez, P., Insolia, E., Soto−Gómez, D., Fernández−Calvińo, D., Zornoza, R., 2022.** The impact of crop diversification, tillage and fertilization type on soil total microbial, fungal and bacterial abundance: A worldwide meta−analysis of agricultural sites. *Agriculture, Ecosystems and Environment*, 329: 107867. DOI: https://doi.org/10.1016/j.agee.2022.107867.
- **Niu, X., Liu, C., Jia, X., Zhu, J., 2021.** Changing soil organic carbon with land use and management practices in a thousand−year cultivation region. *Agriculture, Ecosystems and Environment*, 322: 107639. DOI: [https://doi.org/](https://doi.org/10.1016/j.agee.2021.107639) [10.1016/j.agee.2021.107639.](https://doi.org/10.1016/j.agee.2021.107639)
- **Pardon, P., Reubens, B., Reheul, D., Mertens, J., De Frenne, P., Coussement, T., Janssens P., Verheyen, K., 2017.** Trees increase soil organic carbon and nutrient availability in temperate agroforestry systems. *Agriculture, Ecosystems and Environment*, 247: 98−111. DOI: https://doi.org/10.1016/j.agee.2017.06.018.
- **Pritsch, K., Raidl, S., Marksteiner, E., Blaschke, H., Agerer, R., Schloter, M., Hartmann, A., 2004.** A rapid and highly sensitive method for measuring enzyme activities in single mycorrhizal tips using 4−methylumbellif− erone−labelled fluorogenic substrates in a microplate system. *Journal of Microbiological Methods*, 58 (2): 233−241. DOI: https://doi.org/10.1016/j.mimet.2004.04.001.
- **Qin, L., Freeman, C., Jia, X., Zhang, Z., Liu, B., Zhang, S., Jiang, M., 2021.** Microbial enzyme activity and sto− ichiometry signal the effects of agricultural intervention on nutrient cycling in peatlands. *Ecological Indicators*, 122: 107242. DOI: https://doi.org/10.1016/j.ecolind.2020.107242.
- **R Core Team, 2020.** R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available from: [https://www.R−project.org.](https://www.R-project.org)
- **R Studio Team, 2020.** R Studio: Integrated Development for R. Studio. Boston: PBC. Available from: http://www.rstudio.com/.
- **Ranger, J., 2018.** Forest soils: Characteristics and sustainability. In: J. Berthelin, Ch. Valentin, J.Ch. Munch, eds. Soils as a key component of the critical zone 1: Functions and services, 1, pp. 163−186. DOI: [https://doi.org/10.1002/](https://doi.org/10.1002/9781119438069.ch7) [9781119438069.ch7.](https://doi.org/10.1002/9781119438069.ch7)
- **Sanaullah, M., Razavi, B.S., Blagodatskaya, E., Kuzyakov, Y., 2016.** Spatial distribution and catalytic mecha− nisms of −glucosidase activity at the root−soil interface. *Biology and Fertility of Soils*, 52 (4): 505−514. DOI: [https://](https://doi.org/10.1007/s00374-016-1094-8) [doi.org/10.1007/s00374−016−1094−8.](https://doi.org/10.1007/s00374-016-1094-8)
- **Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., de Macędo, J.L.V., Blum, W.E., Zech, W., 2007.** Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil*, 291 (1): 275−290. DOI: [https://doi.org/10.1007/s11104−007−9193−9.](https://doi.org/10.1007/s11104-007-9193-9)
- **Sterner, R.W., Elser, J.J., 2002.** Ecological stoichiometry: The biology of elements from molecules to the biosphere. Princeton: Princeton University Press, 464 pp.
- **Świtoniak, M., Kabała, C., Charzyński, P., 2016.** Propozycja anglojęzycznych nazw jednostek Systematyki gleb Polski. (Proposal of English equivalents for the soil taxa names in the Polish Soils Classification). *Soil Science Annual*, 67 (3): 103−116. DOI: [http://dx.doi.org/10.1515/ssa−2016−0013.](http://dx.doi.org/10.1515/ssa-2016-0013)
- **Tang, X., Hu, J., Lu, Y., Qiu, J., Dong, Y., Li, B., 2022.** Soil C, N, P stocks and stoichiometry as related to land use types and erosion conditions in lateritic red soil region, south China. *Catena*, 210: 105888. DOI: [https://doi.org/](https://doi.org/10.1016/j.catena.2021.105888) [10.1016/j.catena.2021.105888.](https://doi.org/10.1016/j.catena.2021.105888)
- **Turner, B.L., 2010.** Variation in pH optima of hydrolytic enzyme activities in tropical rain forest soils. *Applied and Environmental Microbiology*, 76 (19): 6485−6493. DOI: [https://doi.org/10.1128/AEM.00560−10.](https://doi.org/10.1128/AEM.00560-10)
- **Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987.** An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, 19 (6): 703−707. DOI: [https://doi.org/10.1016/0038−0717\(87\)90052−6.](https://doi.org/10.1016/0038-0717(87)90052-6)
- **Wang, Z., Zhao, M., Yan, Z., Yang, Y., Niklas, K.J., Huang, H., Mipam, T.D., He, X., Hu, H., Wright, S.J., 2022.** Global patterns and predictors of soil microbial biomass carbon, nitrogen, and phosphorus in terrestrial ecosystems. *Catena*, 211: 106037. DOI: https://doi.org/10.1016/j.catena.2022.106037.
- **Wani, S.A., 2021.** Assessment of changes in soil organic carbon fractions and enzyme activities under apple growing ecosystems in temperate North−Western Himalayas. *Resources, Environment and Sustainability*, 6: 100036. DOI: https://doi.org/10.1016/j.resenv.2021.100036.
- **Yang, L., Luo, P., Wen, L., Li, D., 2016.** Soil organic carbon accumulation during post−agricultural succession in a karst area, southwest China. *Scientific Reports*, 6 (1): 1−8. DOI: https://doi.org/10.1038/srep37118.
- **Young, A., 1989.** Agroforestry for soil conservation. Wallingford: CAB International, International Council for Research in Agroforestry, 276 pp.
- **Yuan, Z.Y., Chen, H.Y., 2010.** Fine root biomass, production, turnover rates, and nutrient contents in boreal forest ecosystems in relation to species, climate, fertility, and stand age: Literature review and meta−analyses. *Critical Reviews in Plant Sciences*, 29 (4): 204−221. DOI: https://doi.org/10.1080/07352689.2010.483579.
- **Zhang, W., Zhao, J., Pan, F., Li, D., Chen, H., Wang, K., 2015.** Changes in nitrogen and phosphorus limitation during secondary succession in a karst region in southwest China. *Plant and Soil*, 391 (1): 77−91. DOI: [https://doi.org/](https://doi.org/10.1007/s11104-015-2406-8) [10.1007/s11104−015−2406−8.](https://doi.org/10.1007/s11104-015-2406-8)

Streszczenie

Ekoenzymatyczna i mikrobiologiczna stechiometria użytkowania gleb leśnych i rolniczych z klimatu umiarkowanego: studium przypadku z południowej Polski

Intensyfikacja upraw i nawożenie gleb użytkowanych rolniczo zmniejszają liczbę i różnorodność mikroorganizmów glebowych odpowiedzialnych za obieg węgla organicznego, azotu ogólnego i fosforu ogólnego. W badaniach określono stechiometrię C:N:P dla różnych sposobów użyt− kowania gruntów wpływających na właściwości gleby. Zbadano związek między stechiometrią enzymów, stechiometrią biomasy drobnoustrojów i potencjalnymi regulatorami tych parametrów, w tym właściwościami gleby. Przetestowano następujące hipotezy: 1) wpływ użytkowania terenu jest widoczny w stechiometrii C:N:P gleby; 2) elementem ograniczającym biomasę drobno− ustrojów glebowych i aktywność ekoenzymów jest zawartość C i N, w mniejszym stopniu P; 3) stechiometria ekoenzymów odzwierciedla różne warunki zasobowe, podczas gdy stechiometria C:N:P biomasy drobnoustrojów glebowych jest mniej przydatna w ocenie dostępności zasobów w transekcie. Uzyskane informacje są istotne w monitorowaniu zasobów składników odżywczych w glebach leśnych i rolniczych. Badania przeprowadzono w Nadleśnictwie Rybnik w południowej Polsce (50°05'55"N, 18°32'42"E). Badaniami objęto gleby w transekcie leśno-ekotonowo-rolniczym, a próbki glebowe pobrano na obszarach leśnych i rolnych oraz na granicy obszarów leśnych i rol− nych (ryc. 1). Wyznaczono 6 transektów o długości 200 m biegnących przez obszar użytkowania leśnego, ekoton i obszar użytkowania rolniczego. Próbki gleby pobierano co 50 m, przy czym punkt środkowy każdego transektu znajdował się na skraju ekotonu. W próbkach gleby oznaczono zawartość C, N i P, aktywność ekoenzymatyczną i biomasę drobnoustrojów (tab. 1). Badania potwierdziły możliwość wykorzystania stechiometrii do oceny wpływu użytkowania gruntów na gleby. Potwierdzono hipotezy mówiące, że rodzaj użytkowania gruntów ma istotny wpływ na ilość i jakość materii organicznej w glebie. Najwyższy stosunek C:N:P odnotowano w glebach leśnych (531:23:1), niższy w glebach obrzeży (500:22:1), a najniższy w glebach rolniczych (103:6:1) (ryc. 2). Dysproporcje te wynikają z istotnych różnic w zawartości C i N pomiędzy glebami transektu leśno−rolniczego. Znacząco wyższe stężenia C i N występowały w glebach leśnych i obrzeżnych w porównaniu z glebami rolniczymi. W przypadku gleb rolniczych zawartość węgla była 7−krotnie niższa niż w glebach leśnych (odpowiednio 10,69% i 1,46%) (ryc. 3). Istotnie wyższe stosunki C:N, C:P i N:P w glebach leśnych są efektem jakości dostarczanej ściółki roślinnej. Zarówno gleby leśne, jak i gleby na obrzeżach charakteryzowały się znacznie niższym stosun− kiem BG:NAG i znacznie wyższym stosunkiem NAG:PH w porównaniu z glebami rolniczymi. Drzewa dostarczają różnych rodzajów materii organicznej, gleba jest wypełniona nadziemną i podziemną biomasą drzewną, a także roślinami zielnymi, co skutkuje szybkością uwalniania składników odżywczych. Wnioski te sugerują, że rodzaj użytkowania gruntów ma istotny wpływ na ilość i jakość materii organicznej w glebie. Stechiometria molowa i stechiometria ekoenzy− matyczna znacząco odróżniały gleby leśne od gleb rolniczych (ryc. 4). Aktywność enzymatyczna gleb leśnych jest powodowana przez wyższe zawartości glebowego węgla dostarczanego przez roślinność, głównie przez rozkład korzeni, wysięk korzeniowy i nadziemne wkłady detrytusowe. Stechiometria biomasy drobnoustrojów okazała się mniej czuła w ocenie zmian w glebach spo− wodowanych sposobem użytkowania. Ilość i jakość dostarczanej materii organicznej wpływała na kształtowanie stechiometrii badanych gleb (ryc. 5). Stechiometria badanych gleb potwier− dziła większą dostępność składników pokarmowych w glebach leśnych.