Spatial differentiation
of total nitrogen content and the activity
of N-transforming enzymes in a soil

ZRÓŻNICOWANIE PRZESTRZENNE ZAWARTOŚCI AZOTU OGÓŁEM ORAZ AKTYWNOŚCI ENZYMÓW PRZEMIAN AZOTU W GLEBIE

Abstract: The objective of this study was to evaluate and compare the spatial differentiation of total N (N\textsubscript{TOT}) content and urease (UR), nitrate reductase (NR) and arginine deaminase (ADA) activities in the surface horizon of Luvisol and Phaeozem of the Pomerania and Cuiavia region. 50 soil samples from both study areas were collected in April 2007 in a square sampling grid (90 × 40 m). The results were evaluated with the use of geostatistical methods. Spatial variability of the investigated parameters was evaluated by using empirical semivariograms with adjusted theoretical mathematical model of variograms. Raster maps of the studied properties were drawn. The concentration of chemical properties (TN, TOC, pH\textsubscript{KCl}) and the activity of UR and ADA was significantly higher in Phaeozem compared to Luvisol. Only the nitrate reductase activity was similar in samples of both types of soils. To characterise the spatial variability of the properties studied, spherical or mixed (spherical/linear) models with or without the nugget effect (only NR activity in Luvisol), were fitted to the calculated semivariograms. Total N content, NR activity in Phaeozem and ADA activity in Luvisol were in the strong variability class (the nugget effect < 25 %), while UR activity in both soil types and ADA activity in Phaeozem were situated in the moderate variability class (the nugget effect between 25 and 75 %). The ranges of influence calculated for properties studied ranged from 9.0 to 17 m. The raster maps showed that the distribution of each variable had a different pattern on the area studied. A specific variable was distributed in both topsoils in a different way.

Keywords: Luvisol, Phaeozem, spatial variability, total-N, urease, nitrate reductase, arginine deaminase activity

The amount of N contained in soils in organic forms by far exceeds that which is present in soluble inorganic forms (NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+}) [1]. Organically bound nitrogen in

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soil undergoes complex biochemical transformations as the result of which plant available mineral nitrogen forms are produced [2]. On every stage of these processes specific soil enzymes take part. They are produced by microorganisms and act exclusively intracellularly or are secreted to the soil environment and act extracellularly [3, 4]. Some of the enzymes are produced by plants in response to the presence of a specific substrate. Decomposition of urea to NH₃ (and CO₂) is brought about through the action of urease, an enzyme found practically in all soils [1]. Plants revealed the urease activity in the presence of urea, while they did not show it when the ammonium nitrate was the source of nitrogen [5]. Similarly, dissimilatory nitrate reductase is an adaptive enzyme produced only in the presence of nitrates as the substrate [6]. Nitrate reductase is the rate limiting enzyme in the initial process of denitrification (reduction of NO₃⁻ to NO₂⁻) [7]. At present one of the most often used indicators of soil biological activity is the ability of soil to ammonification of arginine. The process occurs only intracellularly and is associated with microbial biomass activity [8]. The level of the activity of specific enzymes involved in the N-cycle determines the intensity of soil nitrogen compounds transformation and could be the indicator of the nitrogen availability [9, 10].

Although a lot of attention has been devoted in the literature to the transformation of soil nitrogen and enzymes taking part in these processes in the agricultural space [11–13], most of research is carried out as laboratory experiments or in microplots systems under precisely controlled conditions. Very few studies however have concentrated on the spatial variability of those properties in the field scale, what is of a great theoretical and practical significance [14, 15]. Soil enzymatic activity, similarly to other biological properties, shows a high spatial variability, determination of which allows to estimate the real changes of the soil environment. The research of soil properties on the given area (eg arable field) is often done in one or a few points and on the basis of some results the decisions regarding soil utilization are undertaken.

The aim of the study was to determine the spatial variability of total nitrogen content and the activities of enzymes involved in the N transformation in the surface horizon of Luvisol and Phaeozem using geostatistical tools.

Material and methods

The research was carried out in an 80-ha agricultural field located at the village of Orlinek near Mrocza in the Pomerania and Cuiavia region, northwest Poland. The two areas of 90 × 40 m were selected for the research within the arable field. One area was covered with typical Luvisol and the second one with Phaeozem [16]. Chosen soils were situated as near as possible to avoid the influence of other factors on properties studied. In spite of the same material parent both soils were characterized by different morphological structure and other physicochemical, microbiological and biochemical properties. Winter wheat (Triticum aestivum L.) was cultivated after winter rape (Brassica napus L.) as the forecrop. Fifty soil samples were collected on each area at the stage of the winter wheat spreading on 12 April 2007 at regular intervals (10 m) from the 0–20 cm top layer.

Each sample consisted of 10 individual sub-samples taken randomly from a circle area with a radius of 2 m from the node point. Field-moist samples were sieved
(< 2 mm) and stored at 4 °C for not less than 2 days in order to stabilize microbial activity and then were analyzed for enzymatic activity within two weeks. Soil samples were analyzed for physical and chemical properties after air-drying at room temperature and sieving (< 2 mm).

Arginine deaminase activity (ADA) was measured using the Kandeler [17] method. After the addition of an aqueous L-arginine solution (11.5 M), soil samples were incubated for 3 h at 37 °C. The same procedure was followed for the control as for the enzyme assay but the control samples were stored immediately at –20 °C. After the incubation ammonium released by ADA was extracted with 2M KCl. For photometric analysis at 630 nm the filtrate was mixed with sodium phenolate solution (0.12 M), sodium nitropruside solution (0.17 M) and sodium hypochlorite (0.005 M NaOCl in 0.125 M NaOH) and allow to stand for 30 minutes at room temperature for colour development [18].

Soil urease activity was assayed as described by Kandeler and Gerber [19]. Briefly, 1 g of moist soil was incubated with 4 cm³ of borate buffer (pH 10.0) and 0.5 cm³ of urea solution for 2h at 37 °C. After the incubation ammonium released by UR was extracted with 2 M KCl. After filtering the resulting suspension, the concentration of ammonium ions were determined. To assess the ammonium content the filtrate was mixed with the Na salicylate/NaOH solution and the sodium dichloroisocyanide and allow to stand at room temperature for 30 minutes prior the measuring the optical density at 690 nm.

Assimilatory nitrate reductase activity (NR) was determined according to Kandeler [17]. Field moist soil samples were incubated for 24 hours at 25 °C under waterlogged condition with 0.9 mM 2,4-DNP (dinitrophenol) solution, substrate (25 mM KNO₃) and destilled water. Controls were prepared the same way but they were incubated at –20 °C. After incubation 10 cm³ of 4 M KCl solution was added to both samples and controls, the contents of test tubes were mixed briefly and filtered immediately. For spectrophotometric analysis 5 cm³ of filtrates, 3 cm³ of ammonium chloride buffer (0.19 M, pH 8.5) and 2 cm³ of colour reagent (sulfanilamide and N-(1-naphthyl)-ethylenediamine hydrochloride) was added to the test tubes, mixed, and allowed to stand for 15 minutes at room temperature. Extinction of samples and controls was measured at 520 nm against the reagent blank.

All enzymatic assays were performed in triplicate. The data were corrected for oven-dry (105 °C) moisture content. One unit of arginine deaminase and urease activities were defined as the number of mg of product released by 1 kg of dried soil at 37 °C per 1 hour (mg N-NH₄⁺ · kg⁻¹ · h⁻¹) while values of NR activity was expressed as mg N-NO₂⁻ · kg⁻¹ · h⁻¹.

Physicochemical properties were determined according to standard methods and each sample was analyzed in triplicate. A particle-size was carried out using the Cassagrande’s method as modified by Proszynski; sand fraction content was determined using the sieving method; the pH in 1 M KCl was measured using the potentiometric method in 1 : 2.5 soil: solution suspensions; total organic carbon (TOC) and total nitrogen (TN) contents were determined using a dry combustion CN analyzer (Vario Max CN).
The spatial structure of properties studied has been characterized by using some geostatistical tools. A semivariogram was determined for each studied parameter in order to characterize the degree of spatial variability between neighboring samples, and the appropriate model function was fit to the semivariogram. Three basic parameters of semivariogram: sill, nugget effect and range were calculated [20]. The spatial variability of the properties studied was categorized into classes based on the percentage of total variance present as random variance: \( \frac{C_0}{(C_0 + C)} \cdot 100 \), as proposed by Cambardella et al [21].

In order to choose the best models adjusted the empirical variograms, a cross-validation procedure was used. The criterion to select the best fitting models was the mean squared deviation ratio (MSDR) calculated from the squared errors and kriging variances [22, 23]. Finally, the best-fit semivariograms were used to model the spatial distribution of variables by punctual kriging [24] and the maps illustrating the spatial variance of the parameters were drawn. The geostatistical calculations were done using Isatis software (Geovariance Co.).

**Results and discussion**

Analysis of variance showed that the concentration of chemical properties (TN, TOC, \( \text{pH}_{\text{KCl}} \)) and the activity of UR and ADA was significantly higher in Phaeozem compared with Luvisol \( (P < 0.05) \). The \( \text{pH}_{\text{KCl}} \) values ranged from 4.11 to 5.76 in Luvisol and from 6.45 to 7.13 in Phaeozem, respectively. Organic carbon content in Luvisol ranged 5.51–9.0 g \( \cdot \) kg\(^{-1} \) with mean value of 7.27 g \( \cdot \) kg\(^{-1} \), while in Phaeozem TOC concentration amounted for 13.1–25.1 g \( \cdot \) kg\(^{-1} \) with mean 18.7 g \( \cdot \) kg\(^{-1} \). The basic statistical parameters of the TN content and enzymatic activity (mean, minimum, maximum) are presented in Fig. 1. Average ADA activity in Phaeozem was more than three times higher

![Fig. 1. Basic statistical parameters of variables studied (n = 50): L – Luvisol, P – Phaeozem, UR – urease, NR – nitrate reductase, ADA – arginine deaminase activity](image-url)
than that in Luvisol, while the UR activity overpassed the other one six times. Only the nitrate reductase activity data were not significantly different in both soil types.

The spatial variability of the soil properties studied differed significantly in the pattern of variation, which was shown in particular by the semivariogram parameters and kriged maps drawn (Table 1, Figs 2–3). To characterize the spatial variability of the properties, linear (UR activity), spherical (NR activity in Luvisol) or mixed (TN, ADA in Phaeozem) models with or without the nugget effect were fitted to calculated semivariograms.

Table 1

<table>
<thead>
<tr>
<th>Property</th>
<th>Soil type</th>
<th>Model</th>
<th>Nugget (Co)</th>
<th>Sill (Co + C)</th>
<th>Co/(Co+C) [%]</th>
<th>Range [m]</th>
<th>MSDR</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN</td>
<td>Luvisol</td>
<td>SF, L</td>
<td>0.0003</td>
<td>0.016</td>
<td>20.4</td>
<td>15</td>
<td>1.05</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Phaeozem</td>
<td>SF, L</td>
<td>0.0085</td>
<td>0.043</td>
<td>19.8</td>
<td>12</td>
<td>0.89</td>
<td>S</td>
</tr>
<tr>
<td>UR</td>
<td>Luvisol</td>
<td>L, NE</td>
<td>0.120</td>
<td>0.173</td>
<td>69.4</td>
<td>—</td>
<td>0.92</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Phaeozem</td>
<td>L, NE</td>
<td>0.635</td>
<td>1.835</td>
<td>34.6</td>
<td>—</td>
<td>1.00</td>
<td>M</td>
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<tr>
<td>NR</td>
<td>Luvisol</td>
<td>SF</td>
<td>—</td>
<td>0.0195</td>
<td>—</td>
<td>17</td>
<td>1.01</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Phaeozem</td>
<td>L, NE</td>
<td>0.005</td>
<td>0.020</td>
<td>0.25</td>
<td>—</td>
<td>1.16</td>
<td>—</td>
</tr>
<tr>
<td>ADA</td>
<td>Luvisol</td>
<td>L, NE</td>
<td>0.041</td>
<td>0.375</td>
<td>10.9</td>
<td>—</td>
<td>1.17</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Phaeozem</td>
<td>SF, L</td>
<td>0.094</td>
<td>0.130</td>
<td>72.3</td>
<td>9</td>
<td>1.00</td>
<td>M</td>
</tr>
</tbody>
</table>

a SF – spherical, L – linear, NE – nugget effect, b MSDR – mean squared deviation ratio, c SD – spatial dependence, S – strong, M – moderate, TN – total nitrogen content [g/100 kg–1]; UR – urease activity (mgN-NH4+ kg–1 h–1); NR – nitrate reductase activity [mgN-NO2 kg–1 h–1], ADA – arginine deaminase activity [mgN-NH4+ kg–1 h–1].

The spatial variability of the variables studied was categorized into two classes based on the percentage of total variance (sill) presents as a random variance [Co/Co + C, %] (Table 1). The above ratio is an important index for investigating spatial structures and enables comparison of the relative size of the nugget effect among soil properties. When

Fig. 2. Experimental semivariograms of (a) TN content in Luvisol, (b) TN content in Phaeozem
Fig. 3. Experimental semivariograms of (a) ADA activity in Luvisol, (b) ADA activity in Phaeozem, (c) NR activity in Luvisol, (d) NR activity in Phaeozem, (e) UR activity in Luvisol, (f) UR activity in Phaeozem.
the ratio was less than 25%, the variable had a strong spatial dependence; if the ratio was between 25–75%, the variable had a moderate dependence; otherwise, the variable was considered randomly correlated (pure nugget effect) [21]. Nitrate reductase activity in Phaeozem and ADA activity in Luvisol showed a nugget/sill ratio of 0.25 and 10.9% respectively, indicating a strong spatial variability, which can be influenced by variability in natural factors, such as soil texture and mineralogy [21]. Nugget semivariance for UR activity measured in both soil types was large compared with total variance (69.4 and 34.6% of \( \text{sill} \)), suggesting a moderate spatial structure. Similarly, moderate contribution of nugget effect to total variance in UR activity (31.3%) was noted by Aşkin and Kizilkaya [14]. Urease activity in the same Luvisol determined in August 2007 showed a lower \([\text{Co}/(\text{Co} + \text{C}), \%]\) ratio of 19.8 suggesting that spatial structure of a given property has been changing in time [15]. The results indicated that only 27.7% of the ADA activity in Phaeozem was due to structural variance and the random variability accounted for more than 70% (Table 1). A strong class of spatial variability was noted in both soils for TN content. Some other studies have shown similar contribution of nugget variance to total variance (\( \text{sill} \)) in TN variability [25–27]. Total N content determined in the same Luvisol in August 2007 showed however moderate spatial variability [15].

The ranges of influence calculated for the variables studied ranged between 9 and 17 m (Table 1, Figs 2–3). Since the range is the maximum distance over which results are correlated [28] the sampling scheme for the properties studied (10 m) was suitable. If the sampling distance is bigger than the range, the data will no longer be spatially correlated, and as a result the geostatistics cannot be used [29]. The same property can vary significantly within the range due to the sampling distance; usually the longer sampling distance the higher range of influence. For example the range of influence for urease activity was 19 km when the soil was sampled at 4.5 km intervals [30], it reached 125 m when samples were collected in the distance of 15 m [14]. Different range values were noted for total nitrogen contents: 19.2–25 m [27], 42.5 m [31], 50 m [25] and between 208 and 650 m [26].

Spatial distribution of soil properties in both soil types are shown in Fig. 4a–f. Higher values have been noted for each property in Phaeozem as compared to Luvisol. In each figure, a light shading represents the lowest values, while a darker one is associated with the highest values. A band of relatively higher TN content in Phaeozem ran vertically from the north to the south of the field at 40–60 m of the field length, whereas TN concentration in Luvisol was spatially more uniform (Fig. 4a and b). The higher part of the Luvisol area was covered by TN values ranging from 0.8 to 1.1 g·kg\(^{-1}\). Lower values tended to be located at 0–45 m of the field length and 20–40 m of area width. The lowest values of ADA in Luvisol were noted in the centre of the field on the whole field width, while higher values were obtained in west and east part of the area studied (Fig. 4c). The highest ADA activity in Phaeozem was shown at 20–50 m of length and the whole width, while the lowest values were obtained at 0–10 and 50–70 m of the field length (Fig. 4d). Urease activity in Luvisol was similar all over the area except for lower values in the transect extended along the western part of the field (Fig. 4e).
Urease activity in Phaeozem was clearly higher in the centre and in the south-east corner of the field (Fig. 4f).

Many studies have been devoted to examining the relationship between enzymatic activity and soil physic-chemical properties and results have varied with positive, negative, or no correlations being reported [32, 33, and many others]. In this study no significant correlation coefficients were found between total N content and N-cycle enzymes, except for ADA activity in Luvisol (Table 2).

This fact was earlier explained by McGill and Cole [34] who stated that the enzymes involved in N mineralization are less responsive to changes in N demand than the P-mineralizing enzymes are to P demand. According to those authors nitrogen in
**Table 2**

Correlation matrix (*n* = 50)

<table>
<thead>
<tr>
<th></th>
<th>pH&lt;sub&gt;KCl&lt;/sub&gt;</th>
<th>TOC</th>
<th>ADA</th>
<th>NR</th>
<th>UR</th>
<th>TN</th>
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<tbody>
<tr>
<td>TN</td>
<td>— a</td>
<td>0.778</td>
<td>0.496</td>
<td>—</td>
<td>—</td>
<td>x</td>
</tr>
<tr>
<td>UR</td>
<td>0.385</td>
<td>0.569</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NR</td>
<td>—</td>
<td>—</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ADA</td>
<td>0.374</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TOC</td>
<td>—</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>pH&lt;sub&gt;KCl&lt;/sub&gt;</td>
<td>x</td>
<td>—</td>
<td>—</td>
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<tr>
<th></th>
<th>pH&lt;sub&gt;KCl&lt;/sub&gt;</th>
<th>TOC</th>
<th>ADA</th>
<th>NR</th>
<th>UR</th>
<th>TN</th>
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<tbody>
<tr>
<td>TN</td>
<td>—</td>
<td>0.913</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>x</td>
</tr>
<tr>
<td>UR</td>
<td>—</td>
<td>0.343</td>
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<td>—</td>
<td>x</td>
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<td>x</td>
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<td>ADA</td>
<td>0.409</td>
<td>—</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TOC</td>
<td>—</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>pH&lt;sub&gt;KCl&lt;/sub&gt;</td>
<td>x</td>
<td>—</td>
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</tr>
</tbody>
</table>

TN – total nitrogen content (g·kg<sup>-1</sup>), UR – urease activity (mgN-NH<sub>4</sub>·kg<sup>-1</sup>·h<sup>-1</sup>); NR – nitrate reductase activity (mgN-NO<sub>2</sub>·kg<sup>-1</sup>·h<sup>-1</sup>), ADA – arginine deaminase activity (mgN-NH<sub>4</sub>·kg<sup>-1</sup>·h<sup>-1</sup>), TOC – organic carbon content (g·kg<sup>-1</sup>), a – not significant, *** Correlation is significant at the 0.001 level, ** Correlation is significant at the 0.001 level, * Correlation is significant at the 0.01 level.

Organic matter is bound between carbon atoms in a varied configuration, and inorganic N can only be released through multi-step pathways involving a set of enzymes that selectively eliminate particular types of C-N bonds. Moreover, the early products of the decomposition of organic nitrogen compounds often have fates other than those of a complete mineralization. From among studied enzymes only UR activity was significantly related to TOC, which has often been found in other studies [14, 28]. The significant relationship between enzymatic activity and organic C is likely due to higher C levels supporting greater microbial biomass and activity [33]. Additionally, increasing organic matter content provides a better environment for stabilizing and protecting enzymatic proteins in soil [35].

**Conclusions**

Despite the areas selected for the research showed a high surface homogeneity, confirmed by a preliminary morphological and chemical study, the research showed a high spatial variability of soil properties studied within the same area. Higher ranges and spatial variability of the data were shown by soil properties of Phaeozem compared with Luvisol. Investigation of spatial variability of both chemical and biological parameters in the field scale are both of theoretical and practical significance. They
allow to estimate real changes of soil properties as the results of different agrotechnical procedures, what is efficacious in a better management of soil resources. Because of a high spatial distribution of the data decisions regarding soil utilization based on the research of soil properties on the given area done in one or a few points and on the basis of some results seems not to be adequate and can lead to under- or over-estimation of the real values.

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References

ZRÓZNICOWANIE PRZESTRZENNE ZAWARTOŚCI AZOTU OGÓŁEM ORAZ AKTYWNOŚCI ENZYMÓW PRZEMIAN AZOTU W GLEBIE

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Abstrakt: Celem badań było określenie zmienności przestrzennej zawartości N-ogółem (TN) oraz aktywności ureazy (UR), nitroreduktazy (NR) i poziomu deaminacji argininy (ADA) w poziomie powierzchniowym gleby płowej oraz czarnej ziemi regionu Pomorza i Kujaw. W kwietniu 2007 r. z obu obszarów pobrano po 50 próbek glebowych z punktów zlokalizowanych w sztywnej siatce kwadratów (90 × 40 m). Wyniki zmienności
przestrzennej badanych parametrów określono za pomocą empirycznych variogramów oraz map rastrowych. Zawartość parametrów chemicznych (TN, TOC, pH_kCl) oraz aktywność UR i ADA były większe w czarnej ziemi w porównaniu do gleby płowej. Jedynie aktywność nitroreduktazy była zbliżona w obu typach badanych gleb. Zmienność przestrzenną badanych parametrów przedstawiono za pomocą sferycznych lub mieszanych (sferyczno-liniowych) modeli semivariogramów. Zawartość N ogółem, aktywność NR w czarnej ziemi oraz ADA w glebie płowej znajdowały się w niskiej klasie zmienności (wariancja samorodka < 25%) natomiast aktywność UR w obu typach gleb oraz ADA w czarnej ziemi zaliczono do średniej klasy zmienności. Zakresy autokorelacji badanych zmiennych wynosiły od 9 do 17 m. Mapy przestrzennego rozmieszczenia wyników badanych zmiennych wykazały, że rozmieszczenie wartości każdej z nich wykazywało inny kierunek. Ponadto wartości danej cechy były odmiennie rozmieszczone w obu typach gleb.

**Słowa kluczowe:** gleba płowa, czarna ziemia, zmienność przestrzenna, N-ogółem, ureaza, nitroreduktaza, poziom deaminacji argininy