

## Spatial variability of soil nitrogen forms and the activity of N-cycle enzymes

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### ABSTRACT

Knowledge about the spatio-temporal variability of soil chemical and biological properties is crucial in evaluating their structure-function relationship and their impact on ecosystem functions. A study was conducted in order to evaluate the spatial variability of nitrogen (N) forms and N-cycle enzymes of a Phaeozem located in the Northwestern Poland. Fifty soil samples were collected every 10 m from the area of 90 × 40 m that was selected from an 80 ha agricultural field after the harvest of winter wheat. The samples were analysed for total nitrogen ( $N_{\text{tot}}$ ); mineral nitrogen ( $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N) and the activities of the N cycle enzymes – urease (UR), nitrate reductase (NR) and arginine deaminase (ADA). The coefficients of variation were low for  $N_{\text{tot}}$  content and UR activity, while a moderate variability was noted for mineral N and ADA activity and a high one was noted for NR activity. Most of the properties revealed a contribution of random variance (nugget effect) in total variability (sill), while only  $N_{\text{tot}}$  and ADA activity were determined by the structural variance. The effective ranges were from 11.9 m for  $\text{NO}_3^-$ -N to more than 50.3 m for  $\text{NH}_4^+$ -N. Kriged maps showed that every property revealed a different distribution on the study area. The significant variability of the studied properties should be taken into account in soil management practices. Additionally, this knowledge would contribute to a better understanding of the nitrogen transformation in soil and functioning of the ecosystem.

**Keywords:** total and mineral N; N-related enzymes; spatial structure; geostatistics

The heterogeneity of soil properties, especially soil nutrients such as mineral nitrogen, is considered to be a problem for the determination of soil sampling strategies and the optimization of soil management (Haberle et al. 2004, Piotrowska and Długosz 2010, Piotrowska 2011). Within one field, differences in status of properties may be induced by different soil types or variations in profile development. Additionally, external factors, e.g. soil fertilization and tillage, significantly influence the soil properties in agricultural soils.

The efficiency of nitrogen use in agricultural fields varies only slightly between 40–50%, which may be attributed to improper nitrogen use management, imbalanced fertilization and losses through leaching, volatilization and immobiliza-

tion (Parama and Munawery 2012). An agricultural system with site-specific management should be more economically and environmentally suitable than a uniform-rate application. Such site-specific nutrient management is based on the prediction of the spatial variability of crop management parameters, soil nutrients and other soil properties (Inamura et al. 2004), which ensures the appropriate application of fertilizers according to the variations in the field that actually exist. As a result it may reduce nutrient losses from production fields, which is of special economic and environmental importance (Cambouris et al. 2008).

There has been a growing interest in the study of the spatial variation of soil properties using geostatistics, as this technique has been used suc-

cessfully in characterizing spatial variations of heavy metals (e.g. Lim et al. 2001), micronutrients (e.g. Liu et al. 2004), biological soil features, such as soil enzymatic activity (Stark et al. 2004, Aşkin and Kizilkaya 2006) and other soil properties (Liu et al. 2008, Staugaitis and Šumkis 2011). Geostatistics provides the basis for quantitatively describing any spatial variation in soil, for estimating soil properties and mapping them and finally for planning a rational sampling scheme (Inamura et al. 2004).

The objectives of this study were (1) to determine the spatial dependency of the total and mineral nitrogen and the activities of the enzymes involved in N transformation in the surface horizon of a Phaeozem and (2) to map the spatial distribution of the studied properties.

## MATERIAL AND METHODS

**Description of the study field and soil sampling.** The research was conducted in a grid-pattern 90 m × 40 m plot of an agricultural field of 80 ha situated in the Cuiavia-Pomerania region, north-western Poland. The studied Phaeozem was a sandy loam soil with total organic carbon ( $C_{org}$ ) ranging from 15.2–26.5 g/kg. Soil reaction varied from slightly acid to neutral ( $pH_{KCl}$  from 6.3–7.0). Prior to the study, the field was cropped with winter rapeseed (*Brassica napus* L.) followed by winter wheat (*Triticum aestivum* L.). Fifty soil samples were collected at regular intervals (10 m × 10 m) from the surface horizon (0–20 cm) after the harvest of the winter wheat (August 2007). The soil samples were passed through a 2 mm sieve and divided into three sub-samples. One set of sub-samples was air-dried for the chemical analyses ( $pH$ ,  $C_{org}$ ), another set was stored at 4°C in order to measure soil enzymatic activity and the last sub-sample was analysed for the mineral nitrogen.

**Soil analysis.** Soil organic C and total N content were assayed using a Vario Max CN dry combustion analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). Soil pH (1 mol/L KCl) was measured using the potentiometric method in 1:2.5 soil:solution suspensions.  $NO_3^-$ -N and  $NH_4^+$ -N were extracted from the field-moist soil with  $K_2SO_4$  and KCl respectively, and nitrate N was determined using phenoldisulphonic acid (Taras 1950), while ammonium N was measured using the indophenol blue method (Stevenson

1982). The activity of soil enzymes (urease, nitrate reductase and arginine deaminase) was assayed as described by Kandeler (1995).

**Statistical and geostatistical analysis.** Basic descriptive statistics were determined for each set of data. The data of all properties, with the exception of NR activity, were normally distributed (Shapiro-Wilk test). Non-normal, skewed NR activities were log-transformed and further analyses were performed with the corrected data. A classification scheme was used to identify the extent of variability of the soil properties based on their CV (%) values for which values of 0–15, 16–35 and > 36% indicate a small, moderate and high variability (Wilding 1985). The classical statistics were evaluated using Statistica v. 9.0 software (StatSoft Inc., Tulsa, USA).

In order to analyse the spatial structure of the data and to calculate semivariograms, the Isatis geostatistical software was used (Geovariance Co., Avon-Fountainbleau, France). Semivariograms were prepared using the following formula (Isaaks and Srivastava 1989):

$$\gamma(h) = 1/2N(h) \sum [z(x_i + h) - z(x_i)]^2$$

Where:  $\gamma(h)$  – experimental semivariogram value at distance interval  $h$ ;  $N(h)$  – number of sample pairs within the distance interval  $h$ ;  $z(x_i)$  and  $z(x_i + h)$  – sample value at two points separated by the distance interval  $h$ .

The mixed spherical/linear or linear models were fitted to the empirical semivariograms. The criterion to select the best fitting models was the mean squared deviation ratio (MSDR) calculated from the squared errors and kriging variances (Webster and Oliver 2001). The spatial variability of the properties was categorized into classes based on the percentage of total variance present as random variance (Cambardella et al. 1994):

$$[Co/(Co + C)] \times 100.$$

Maps displaying the spatial variance of the properties determined were drawn on the basis of the semivariograms.

## RESULTS AND DISCUSSION

**Descriptive statistics of the soil properties.** Normal distribution of the row properties data was confirmed by similar values of mean, median and a significantly lower standard deviation (SD)

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Table 1. Statistics of soil properties ( $n = 50$ )

Property	Min	Max	Mean	Median	SD	Skewness	Kurtosis	CV (%)
$N_{\text{tot}}$	1.52	2.68	1.99	1.95	0.241	0.696	0.409	12.1
$\text{NO}_3^-$ -N	11.2	22.7	17.1	17.1	2.77	-0.028	-0.608	16.2
$\text{NH}_4^+$ -N	6.56	18.0	11.1	9.92	2.65	0.588	-0.335	23.9
UR	10.4	21.5	16.1	16.5	2.37	-0.134	-0.329	14.7
NR	0.053	3.44	0.36	0.22	0.520	4.69	25.9	114.0
ADA	3.15	5.91	4.13	4.04	0.654	0.725	-0.185	15.8

$N_{\text{tot}}$  – total nitrogen (g/kg);  $\text{NO}_3^-$ -N – nitrate N (mg/kg);  $\text{NH}_4^+$ -N – ammonium N (mg/kg); UR – urease (mg  $\text{NH}_4^+$ -N/kg/h); NR – nitrate reductase (mg  $\text{NO}_2^-$ -N/kg/h); ADA – arginine deaminase (mg  $\text{NH}_4^+$ -N/kg/h); SD – standard deviation; CV – coefficient of variation

than the mean, which is an important condition for a normal frequency distribution (Table 1). In the case of NR activity, SD values were much higher than the mean or median, thus confirming a non-normal distribution of the data.

With the exception of the  $\text{NO}_3^-$ -N content and UR activity data, all of the properties studied were positively skewed showing a right-sided asymmetry. The distribution of  $N_{\text{tot}}$  and  $\text{NH}_4^+$ -N concentrations and ADA activity data were moderately skewed. An approximately symmetric distribution was noted for  $\text{NO}_3^-$ -N content and UR activity, while highly skewed data (4.69) were noted only for NR activity. Similarly, in the study of Parkin (1987), the enzymatic activity involved in denitrification was highly skewed and was close to a log-normal distribution. Right-tailed skewness resulted from the presence of a few exceptionally large values in the background of the majority of low values. Additionally, a right-tailed skewness resulted in the mean being larger than median. Kurtosis is a measure of whether the data are peaked or flat relative to the normal distribution (Liang et al. 2008).

With the exception of the NR activity data, most of the properties being studied had kurtosis close to zero, thus suggesting a platykurtic distribution, which was flatter than the normal distribution. Only NR activity data exhibited a high leptokurtic distribution (25.9).

According to Wilding (1985) classification the  $N_{\text{tot}}$  content and UR activity data showed a low variability, while both mineral forms of N and ADA activity data were in the moderate variability class. In general, CV values for total N were usually in low to moderate variability classes ranging 14.2–30.0%, while mineral N forms and the enzymes involved in the N cycle were usually in the moderate to high variability in a broad range of 17.0–63.7% (e.g. Cambardella et al. 1994, Bergstorm et al. 1998, Shahandeh et al. 2005, Aishah et al. 2010). However, our results did not confirm the thesis that chemical properties are less variable than biological ones. In this study, only NR activity had a very high variability ( $CV = 114\%$ ), while two other enzymatic activities were less variable.

**Spatial variability of the properties studied.** In order to characterize the spatial variability of proper-

Table 2. Parameters of variogram models

Property	Model	Sill (Co + C)	Nugget (Co)	Nugget effect Co/(Co + C)	Range (m)	MSDR	Spatial dependence
		(mg/kg) <sup>2</sup>		(%)			
$N_{\text{tot}}$	Sf, L	0.01	–	–	30.0	0.999	–
$\text{NO}_3^-$ -N	Sf, L, NE	8.07	5.3	65.7	11.9	1.000	M
$\text{NH}_4^+$ -N	Sf, L, NE	1.278	0.079	6.8	50.3	1.005	S
ADA	Sf, L	0.442	–	–	32.4	1.002	–
NR	Sf, L, NE	0.269	0.027	10.0	15.9	1.006	S
UR	L, NE	1.293	0.751	58.1	–	1.000	M

The abbreviations are those given under Table 1. Sf – spherical; L – linear; NE – nugget effect; MSDR – mean squared deviation ratio; S – strong; M – moderate

ties studied, linear (UR) or spherical/linear ( $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N,  $\text{N}_{\text{tot}}$ , ADA, NR) models were fitted to the semivariograms calculated (Table 2). Both mineral-N forms and NR and UR activities showed a random variance (nugget variance – Co) in the total variance (sill). Nugget variance represents the experimental error and field variation within the minimum sampling spacing (Aishah et al. 2010). A variable is considered to have a strong spatial dependency if the ratio is less than 25%, moderate if the nugget/sill ratio is between 25–75% and weak if the ratio is greater than 75% (Cambardella et al. 1994). The nugget/sill ratios calculated for nitrate-N content and UR activity were 65.7% and 58.1%, respectively, thus indicating a moderate spatial dependency. This finding suggests that extrinsic factors such as fertilization, tillage and other soil management practices weakened their spatial correlation after long-term cultivation (Aishah et al. 2010). A lower (31.3%) or similar (57.2%) contribution of the nugget effect to total variability in UR activity was noted by Aşkin and Kizilkaya (2006). NR activity and ammonium-N

content data showed a nugget/sill ratio of 6.8% and 10.0%, thus indicating a strong spatial variability, which can be attributed to intrinsic factors such as soil texture and mineralogy (Cambardella et al. 1994).

The spatial range of influence calculated for the properties studied ranged between 11.9–50.3 m (Table 2). Since the range is the maximum distance over which the results are correlated (Bergstorm et al. 1998), those results indicate a rational sampling distance for the properties studied within their effective spatial correlation ranges. Therefore, correctly planning and analysing an adequate number of soil samples is extremely important. If the sampling distance is bigger than the range, the data are no longer spatially correlated and they cannot be analysed geostatistically (Goovaerts 1998). On the other hand, if the sampling scheme is too dense, there is no need to analyse an indispensable amount of samples, which is both time-consuming and increases the cost of the research (Jung et al. 2006).

The main application of geostatistics to soil science has been the estimation and mapping of soil

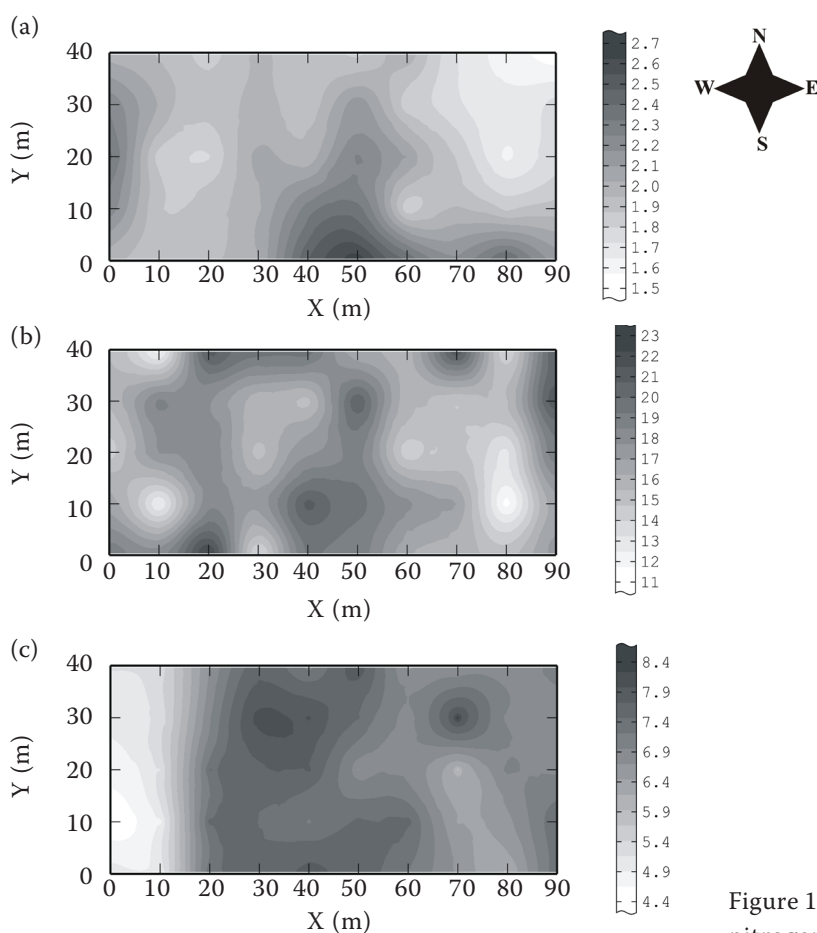


Figure 1. Nitrogen forms concentration: (a) total nitrogen (N); (b) nitrate N; (c) ammonium N

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property values in unsampled areas. In each figure, light shading represents the lowest values, while darker shading is associated with the highest values (Figures 1–2). All of the variables studied differed markedly in spatial distribution. Only the area with the highest nitrate-N in the south of the field (at 40–55 m of length and 0–30 m of width) was associated with the highest content of total N (Figure 1a–b). The concentration of  $\text{NO}_3^-$ -N revealed an irregular distribution, where nests of higher results were irregularly found among lower data sets (Figure 1b). The highest values of UR activity were noted in the triangle situated in the central and eastern parts of the area (Figure 2a). Almost all of the study area showed NR activity values lower than  $0.8 \text{ mg NH}_4^+\text{-N/kg/h}$  (Figure 2b). A band of relatively higher ADA activity data ran vertically from the north to the south of the field at a length of 30–45 m (Figure 2c). Generally the distribution of each of the properties did not share any similarities with the others, which confirmed the lack of

a relationship among them as was revealed by the correlation analysis (data not presented).

In conclusions, results can help in evaluating the contribution of intrinsic factors in the total variability of soil properties, which can mask the influence of soil management practices, such as crop rotation, fertilization and tillage. For some properties (e.g.  $\text{NH}_4^+\text{-N}$ ,  $\text{N}_{\text{tot}}$  concentrations, ADA activity) the sampling intervals should be increased in future studies and that the number of soil samples can be reduced. This is important since the sampling and analysis of soil is time and labour-consuming. Ammonium-N content showed a higher range values, thus indicating a stronger spatial correlation than  $\text{NO}_3^-$ -N and seems to be a better property for maps produced for site-specific N fertilization. However, since mineral N forms are highly temporally variable, more determinations of these properties should be done within the season, prior to the formulation of any valid conclusions concerning site-specific management.

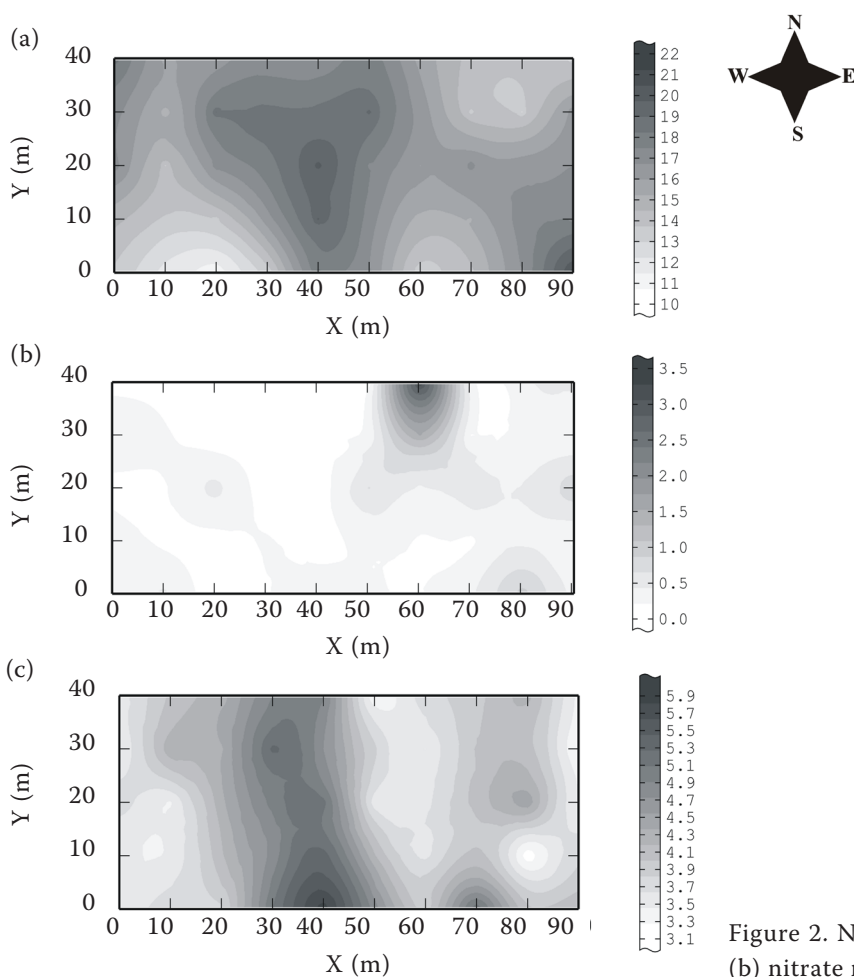


Figure 2. N-cycle enzymes activity: (a) urease; (b) nitrate reductase; (c) arginine deaminase



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