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# SPATIO-TEMPORAL VARIABILITY OF SOIL B-GLUCOSIDASE ACTIVITY AT THE ARABLE FIELD SCALE

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*Abstract.* Knowledge about the spatio-temporal variability of soil enzymatic properties is crucial in evaluating their structure-function relationship and their impact on ecosystem functions. The aim of this study was to assess the spatio-temporal variability of soil β-glucosidase (BG) activity against selected physico-chemical properties at the arable field scale. A grid soil sampling (10×10 m) was used to measure the spatial variation of soil properties across a 0.4-ha field. The BG activity was analyzed in soil samples that were collected from the upper 20 cm of Luvisols at 50 locations in April and August 2007 and 2008. Additionally, total organic carbon ($C_{ORG}$), total nitrogen ($N_{TOT}$) concentrations, soil $pH_{KCl}$ as well as texture and water content were determined. The dataset was analyzed using classical statistical and geostatistical methods. Based on the analysis of variance, it was found that the BG activity, $C_{ORG}$, and water content showed significant differences between the four sampling dates. The BG activity showed a high contribution of nugget effect in *sill* (over 50%) and revealed a moderate spatial structure. The range of spatial autocorrelation calculated for the BG activity was between 15.0 and 61.0 m. Less intensive sampling grid should be recommended for soil BG activity in further studies concerning spatial variability in arable field scale. In turn, more frequent sampling must be included in the sampling strategy in order to better understand whether the BG activity always shows permanent spatial patterns in soil or whether it is more randomized.

**Keywords:** β-glucosidase, geostatistics, spatial variability, temporal changes

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## INTRODUCTION

Spatial heterogeneity is one of the characteristic features of the soil environment (Paul 2007). This is also true for agricultural soils, which although subjected to homogenization, show a substantial level of spatial variability with respect to soil chemistry and the activity of extracellular enzymes (Štursova and Baldrian 2011). Most studies of soil properties, also enzymatic properties, are focused on the differences in their spatial structure that are caused by natural and anthropogenic factors. Less attention has been paid to the spatial variation of soil properties in a uniformly managed arable field, where no experimental factors were tested (Peigné *et al*. 2009, Franklin and Mills 2009). Very often, the soils within uniformly managed sites are considered to be homogenous and thus it is assumed that the soil properties are similar. Accordingly, the number of analyses of soil properties is limited to a few measured points in a given area and a composite sample is often assessed to represent the average population in the area sampled. However, the average values of homogenized soil samples often do not accurately reflect the real state of soil ecosystems. That is why attention should be paid to evaluation of spatial variability of soil properties, which is often carried out using geostatistic techniques. Geostatistics provides a set of statistical tools allowing for the description and modeling of spatial patterns, prediction at unsampled locations and the assessment of the uncertainty of these predictions (Goovaerts 2010). Geostatistical methods are applicable in soil science to measure properties that vary continuously in space. This technique is being applied in different fields of soil science to assess the spatial variation in soil physical (Staugaitis and Šumskis 2011, Haruna and Nkongolo 2013), chemical (Zhang *et al*. 2014a, Zhang *et al*. 2014b), microbiological (Goux *et al*. 2012, Jeelani *et al*. 2017) and biochemical properties including enzymatic activity (Aşkin and Kizilkaya 2006, Piotrowska and Długosz 2012, Baldrian 2014).

The soil enzymatic activity also varies temporally. Current studies on the temporal variability in soil enzymes have shown contradictory trends with various studies reporting peaks in different seasons as well as both positive and negative responses to temporal patterns in soil temperature and moisture (e.g. Wallenstein *et al*. 2009, Ladwig *et al*. 2014).

Cellulose, the major constituent of all plant materials and the most abundant polysaccharide on Earth, is a linear biopolymer of glucose molecules, connected by β-1,4-glycosidic bonds. Enzymatic hydrolysis of cellulose to glucose, providing simple sugars for the soil microorganisms, plays an important role in the organic carbon cycle and requires synergistic action of at least three groups mixtures of hydrolytic enzymes including endoglucanases, exoglucanases (cellobiohydrolases), and β-glucosidases (Dashtban *et al*. 2010). The BG enzyme (EC 3.2.1.21; cellobiase) catalyzes the hydrolysis of β-D-glucopyranosides in the final, rate-limiting step in the degradation of cellulose. Since the enzyme

is very sensitive to environmental changes caused by soil management, determination of its spatio-temporal changes might be helpful in soil quality determination (Stott *et al.* 2009). That is why the objectives of the study were (1) to investigate the spatial and temporal variability of the β-glucosidase activity in a arable field scale against selected physicochemical properties and to assess the contribution of random variation in total soil variability, (2) to determine whether the temporal variation affects the spatial pattern variability of soil properties, and (3) to assess the relationship between β-glucosidase activity and some physico-chemical properties on four sampling dates.

## MATERIALS AND METHODS

### *Site description and samples collection*

The study was carried out in a grid patterned 40×90 m area in 80-ha of an agricultural field located near the village of Orlinek (near Mrocza) in the Kuyavian-Pomeranian region, northwest Poland. The soil that was studied was classified as a typical Luvisol (IUSS Working Group WRB 2007), which was composed of 79.3% sand, 14.6% silt and 6.1% clay. Winter wheat (*Triticum aestivum* L.) was cultivated in 2006/2007, and winter rape (*Brassica napus* L.) in 2007/2008. Wheat plants received phosphorus as $(NH_4)_3PO_4$ (200 kg·ha$^{-1}$, 46% of P) and potassium as KCl (200 kg·ha$^{-1}$, 60% of K) in autumn 2006. Winter wheat was fertilized with 300 kg·ha$^{-1}$ of N applied as ammonium nitrate (43% of N) in March 2007 and with 250 kg·ha$^{-1}$ of N used as ammonium-calcium nitrate (N – 27%, MgO – 4%, CaO – 7%) in April 2007. As regards winter rape, phosphorus, potassium and a small amount of nitrogen (approx. 5 kg·ha$^{-1}$) was applied together with sulfur as Tarnogram fertilizer (9% of $P_2O_5$, 19% of $K_2O$ and ammonium N). Most of the nitrogen (280 kg·ha$^{-1}$) was applied in spring 2008 as Polifoska (21% of N) (on April 5) and as Ca $(NO_3)_2$ (32% of N) (on April 21). The farm has no animal production and thus no manure has been applied since 1998. The average monthly air temperature and the sum of rainfall in 2007 was 9.0 °C and 618 mm and in 2008 it was 9.4 °C and 507 mm, respectively. The monthly mean of air temperature and the sum of rainfall in the years of the study were presented earlier (Piotrowska-Długosz *et al.* 2017). Fifty soil samples were collected at the stage of the winter wheat spreading on April 12, 2007 and immediately after the harvest on August 6, 2007. The same amount of samples (50) was collected under winter rape on April 1, 2008 and immediately after the harvest of winter rape on August 2, 2008. The soil samples were collected at the same points each time. Samples were collected from 0 to 27 cm of the topsoil at regular distances (10 m). At each location, approximately ten individual samples were collected randomly from a circular area with a radius

of 2 m from the node point and composite samples were prepared. Field-moist samples were sieved (2-mm mesh) and stored for not less than 2 days in order to stabilize the microbial activity and then were analyzed for BG activity within one week. In order to analyze the physico-chemical properties, the soil samples were air-dried at room temperature and sieved (2-mm mesh).

*Soil properties analysis*

Physico-chemical properties were analyzed according to standard methods outlined in Burt (2004). Each sample was assayed in triplicate. The particle size was defined by the aerometer method combined with the sieving method. Total organic carbon ($C_{ORG}$) and total nitrogen ($N_{TOT}$) content was determined using a Vario Max CN dry combustion CN analyzer. Soil pH in 1 M KCl was determined with a glass membrane electrode in the supernatant of a soil suspension using 1:2.5 mixtures of soil and deionized water left to stand overnight at room temperature. Soil water content was determined gravimetrically for each sample by drying the soil at 105°C for 48 hours. β-glucosidase activity (BG) was measured as described by Eivazi and Tabatabai (1988). Briefly, 1 g of soil was incubated with 4 ml of buffer (MUB, pH 6.0) and substrate (p-Nitrophenol-β-glucoside solution – PGN, 25 mm) in reaction flasks for 1 h under continuous stirring. Concentrations of *p*-nitrophenol were determined by direct sample reading at 400 nm after alkalinisation a Tris/NaOH buffer (pH 10.0) and $CaCl_2$. To prepare the controls, the PGN was added at the end of the incubation before adding the $CaCl_2$ and Tris buffer. One unit of BG activity was defined as mmoles of *p*-nitrophenol released per 1h ($mM pNP \cdot kg^{-1} \cdot h^{-1}$).

*Statistical and geostatistical analyses*

The data set of physico-chemical properties was evaluated using classical statistics such as mean, minimum and maximum, standard deviation. The data of BG activity was additionally determined using kurtosis and skewness as well as coefficient of variation (CV), which is the ratio of the standard deviation (SD) to the mean values times 100. A classification scheme based on CV [%] was used to assess the extent of variability for the soil properties according to Wilding (1985). Values of 0–15%, 16–35% and >36% indicate little, moderate and high variability, respectively. Additionally, linear correlation analysis based on Pearson's coefficients ($p<0.05$) was performed to determine the relationships between the variables.

A semivariogram was determined for BG activity in all sampling dates to find the degree of spatial variability between neighboring observations, and the appropriate model function was fit to the semivariogram. The semivariogram function was calculated according to Goovaerts (2010) and appropriate equita-

tion was shown earlier (Piotrowska and Długosz 2012). A semivariogram consists of three parameters that describe the spatial structure as: $\gamma(h) = Co + C$. *Co* represents the *nugget effect*, which is the local variation occurring at scales lower than the sampling interval, such as a sampling error, fine scale spatial variability and measurement error; $Co + C$ is the *sill* (total variance) and the distance at which the values of one variable become spatially independent. At separation distances larger than the range, the sampled locations are no longer spatially correlated (Shahandeh *et al.* 2005). Furthermore, the ratio of the nugget to sill $[Co/(Co+C)]\cdot 100$ indicates the degree of randomness in the data's spatial variability (Piotrowska and Długosz 2012). This ratio was used in this study to define three classes of spatial dependences for the soil variables. A low ratio (<25%) indicated that a large part of the variance was introduced spatially, thus, implying a strong spatial dependency of the variable. When it was between 25–75%, the soil variable had a moderate spatial dependence. A high ratio (>75%) indicated a weak spatial dependency. If the slope of the semivariogram was zero, the variable was considered to be random or non-spatially autocorrelated (pure nugget).

The criterion for selecting the best fitting models was the mean squared deviation ratio (MSDR), which was calculated from the squared errors and kriging variances (Bishop and Lark 2006). If the model for the variogram is accurate, the MSDR should be close to one (Kerry and Oliver 2007).Values for unsampled locations were interpolated using the kriging technique (Karydas *et al.* 2009) and the kriging estimates were mapped to reveal the overall spatial pattern of the data (Burgos *et al.* 2006).

The classical statistics were evaluated using Statistica v. 9.0, while the geostatistical calculations were done using Isatis software (Geovariance Co.).

## RESULTS

The total organic carbon ($C_{ORG}$) was significantly higher in 2008 than in 2007, while there was no significant difference in this property between April and August in both years of the study. Total nitrogen ($N_{TOT}$) ranged 0.79–0.86 $g\cdot kg^{-1}$ and did not differ significantly depending on the sampling date (Table 1, Tukey's test, $p<0.05$). The reaction ($pH_{KCl}$) of the studied soil was from acid (4.08) to neutral (6.68) and did not differ significantly between sampling dates. The soil studied in 2007 did not differ significantly in water content, with a mean value of 7.6% in April and 8.1% in August. This property studied in 2008 was, however, significantly higher in April (13.2%) than in August (3.8%) (Table 1). The β-glucosidase activity ranged 0.27–0.99 $mMpNP\cdot kg^{-1}\cdot h^{-1}$ showing significant differences between the four sampling dates (Table 2, Tukey's test, $p<0.05$). The highest BG activity was noted in April 2007 and decreased systematically in succeeding sampling dates. The BG activity from all sampling dates showed

a similar, moderate variability (CV=19.5–22.1%), skewness between -0.5 and 0.5 and a negative kurtosis (except April 2008), demonstrating a flat distribution compared to the normal distribution.

TABLE 1. PHYSICO-CHEMICAL PROPERTIES IN STUDIED SOIL ($N$ = 50)

| Soil properties | Year | Month | Mean (±SD) | Range |
|---|---|---|---|---|
| $C_{ORG}$ ($g \cdot kg^{-1}$) | 2007 | April | 7.3 (±0.91) b | 5.5–9.0 |
| | | August | 7.5 (±0.85) b | 5.8–9.0 |
| | 2008 | April | 8.3 (±0.78) a | 6.5–10.6 |
| | | August | 8.6 (±1.45) a | 6.7–13.4 |
| $N_{TOT}$ ($g \cdot kg^{-1}$) | 2007 | April | 0.80 (±0.07) a | 0.68–0.99 |
| | | August | 0.80 (±0.11) a | 0.65–1.12 |
| | 2008 | April | 0.86 (±0.14) a | 0.30–1.08 |
| | | August | 0.79 (±0.11) a | 0.57–1.03 |
| $pH_{KCl}$ | 2007 | April | 4.70 (±0.41) a | 4.11–5.76 |
| | | August | 4.86 (±0.57) a | 4.08–6.68 |
| | 2008 | April | 4.64 (±0.30) a | 4.29–6.12 |
| | | August | 4.69 (±0.34) a | 4.20–6.26 |
| Water content (%) | 2007 | April | 7.6 (±0.91) b | 2.8–9.1 |
| | | August | 8.1 (±0.65) b | 6.8–10.4 |
| | 2008 | April | 13.2 (±0.90) a | 11.3–15.5 |
| | | August | 3.8 (±1.53) c | 4.4–12.9 |
| Sand (%) | 2007 | August | 79.3 (±2.3) | 75–85 |
| Silt (%) | 2007 | August | 14.6 (±2.1) | 11–19 |
| Clay (%) | 2007 | August | 6.1 (±1.3) | 4–9 |

$C_{ORG}$ – organic carbon; $N_{TOT}$ – total nitrogen; different lower case letters for each property indicate significant differences (Tukey's test, $p$<0.05); SD – standard deviation

TABLE 2. STATISTICS OF SOIL BG ACTIVITY ($N$ = 50)

| Year | Month | Min | Max | Mean | SD | CV(%) | Kurtosis | Skeweness |
|---|---|---|---|---|---|---|---|---|
| | | mM*p*NP·$kg^{-1} \cdot h^{-1}$ | | | | | | |
| 2007 | April | 0.42 | 0.99 | 0.70 a | 0.155 | 22.1 | -0.722 | 0.121 |
| | August | 0.32 | 0.79 | 0.56 b | 0.106 | 19.9 | -0.432 | 0.206 |
| 2008 | April | 0.33 | 0.83 | 0.52 b | 0.109 | 20.3 | 0.401 | 0.450 |
| | August | 0.27 | 0.63 | 0.41 c | 0.086 | 19.5 | -0.479 | 0.094 |

Different lower case letters indicate significant differences (Tukey's test, $p$<0.05); SD – standard deviation; CV (%) – coefficient of variation

To characterize the spatial variability of the BG activity determined in 2007, exponential and linear models with the nugget effect were fitted to the calculated semivariograms (Table 3, Figs. 1 a–d). The Gaussian model was the best to characterize the enzyme activity in 2008. The spatial variability of the

BG activity was characterized based on the percentage of the nugget to sill ratio [Co/(Co+C),%]. In all sampling dates, the nugget/sill ratio of the enzyme indicated a moderate spatial variability, with a contribution of *nugget* (Co) in *sill* (Co + C) at a level of 50.0–77.2%. The ranges of spatial autocorrelation that were calculated for the BG activity ranged between 15.0 and 61.0 m.

TABLE 3. PARAMETERS OF BG ACTIVITY VARIOGRAM MODELS

| Year | Month | Model | Nugget (Co) | Sill (Co+C) | Co/(Co+C) [%] | Range (m) | MSDR | Spatial dependence |
|---|---|---|---|---|---|---|---|---|
| 2007 | April | Exp + EN | 0.0110 | 0.0164 | 67.1 | 15.0 | 1.196 | M |
| | August | L + EN | 0.0095 | 0.0123 | 77.2 | – | 1.032 | W |
| 2008 | April | Gaussian | 0.0062 | 0.0124 | 50.0 | 61.0 | 1.046 | M |
| | August | Gaussian | 0.0053 | 0.0088 | 60.2 | 44.0 | 1.104 | M |

Exp – Exponential; L – linear; EN – nugget effect; MSDR – mean squared deviation ratio; M – moderate; W – weak

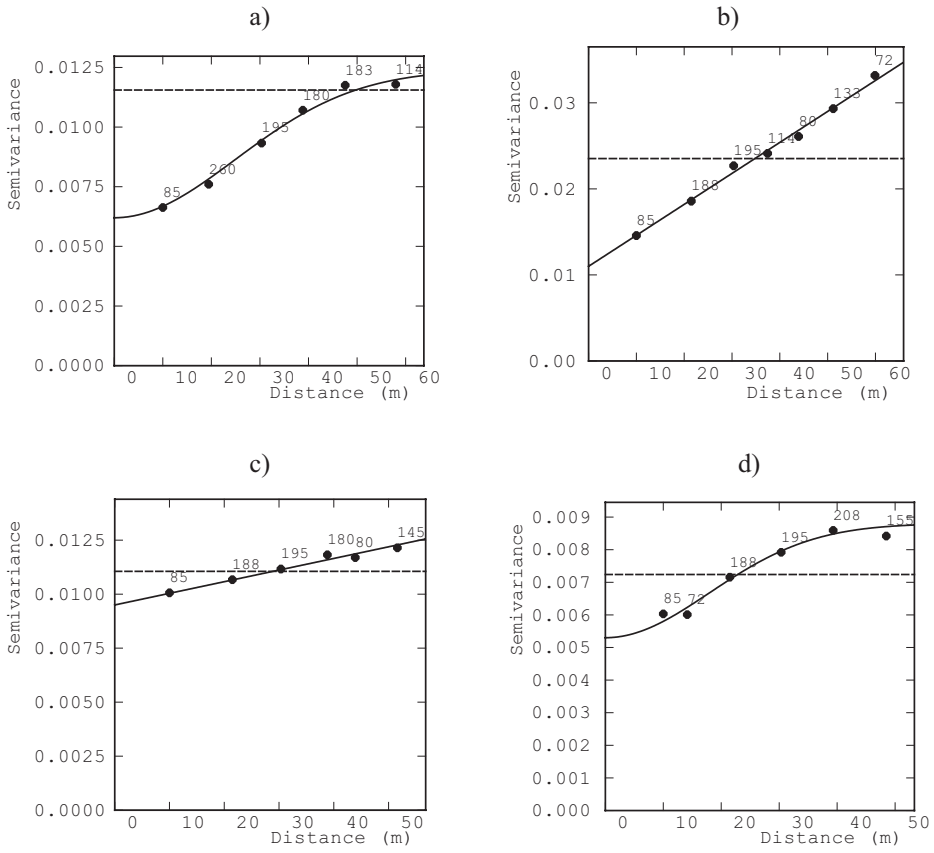


Fig. 1. Experimental semivariograms of β-glucosidase activity in April 2007 (a), August 2007 (b), April 2008 (c), August 2008 (d)

The spatial pattern of the studied properties are presented in Figs. 2a–d. In each figure, darker shading represents the highest values, while light shading is associated with the lowest values. The distribution of BG activity data in particular sampling dates did not share any similarities. The highest BG activity in April 2007 was found at X of 0–20 m and Y of 75–90 m over entire width of the studied area (Fig. 2a). An area with the highest BG activity in August 2007 was placed at X of 80–90 m and Y of 0–40 m as well as at X of 0–40 m and Y of 30–40 m (Fig. 2b). In April 2008 (Fig. 2c), the BG activity increased from the western to eastern part of the field and was the highest at X of 30–70 m and Y of 0–25 m as well as at X of 75–90 m and Y of 0–40 m. The lowest BG activity in August 2008 was located at X of 0–15 m and at Y of 65–80, while between these two bands the values of BG activity were higher and homogenously distributed (Fig. 2d).

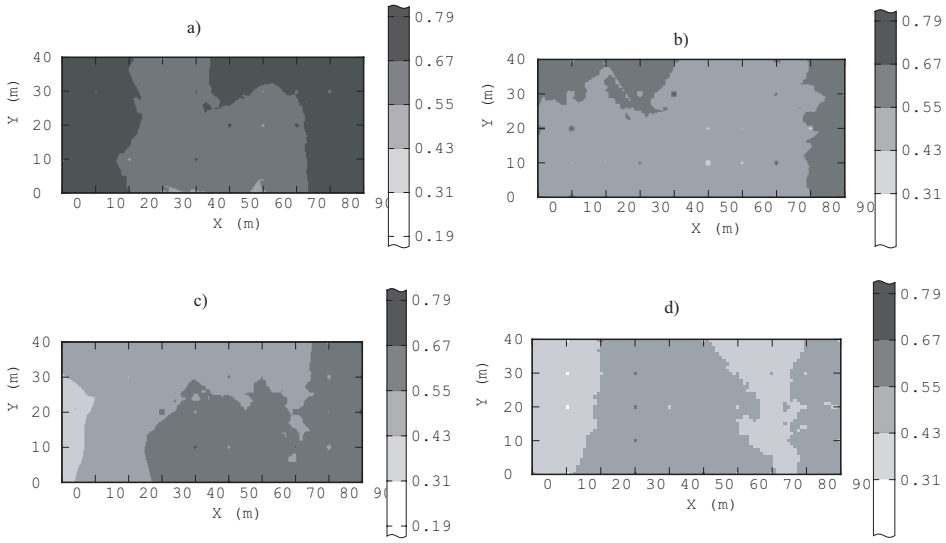


Fig. 2. Spatial distribution of β-glucosidase activity (mMpNP·kg-1·h-1) in April 2007 (a), August 2007 (b), April 2008 (c), August 2008 (d)

The significant correlation obtained among the studied properties were relatively low (Table 4, $p<0.05$). The highest correlation coefficients were shown between $C_{ORG}$ and BG activity (r = 0.604–0.723), while lower between BG activity and $N_{TOT}$ and $pH_{KCl}$ (r = 0.341–0.492). No significant relationship was noted between clay, silt, sand and BG activity, while the enzyme was significantly correlated with water content only in April 2008.

TABLE 4. CORRELATION COEFFICIENTS BETWEEN BG ACTIVITY AND PHYSICO-CHEMICAL PROPERTIES

| Year | Month | $C_{ORG}$ | $N_{TOT}$ | $pH_{KCl}$ | Water content (%) |
|---|---|---|---|---|---|
| 2007 | April | 0.681 | 0.422 | 0.421 | n.s. |
| | August | 0.604 | 0.395 | 0.485 | n.s. |
| 2008 | April | 0.723 | 0.341 | n.s. | 0.41 |
| | August | 0.642 | n.s. | 0.492 | n.s. |

$C_{ORG}$ – organic carbon; $N_{TOT}$ – total nitrogen; given correlations are significant at the 0.05 level; n.s. – not significant

## DISCUSSION

The potential activity of β-glucosidase in studied arable field showed significant seasonal changes patterns and was significantly higher in April than in August in both study years. Seasonal variation of enzymatic activity could be associated with the different stages of plant growth (Dotaniya *et al.* 2014). In our study, increased BG activity was observed during the intensive growth of winter wheat (April 2007) and winter rape (April 2008). The effect of plants in different stages of growth could be related to changes in soil organic matter content caused by different patterns of continuously producing and excreting organic compounds, such as amino acids, organic acids, sugars and vitamins which can increase soil microbial activity (Hinsinger *et al.* 2006). In this study, higher BG activity was probably connected with the existence of higher cellobiose content (and other *β*-glucosides), succeeding product of cellulose decomposition, which induced the BG activity. Indeed, the substrate induction was earlier noted for the BG activity (Saibi *et al.* 2011). Since the cellulase complex acts in a synergistic manner, the product of endoglucanase serves as a substrate to the BG activity (Dashtban *et al*. 2010).

The seasonal pattern in soil enzymatic activities usually reflected the impact of a combination of environmental factors such as soil moisture and temperature among others (Puissant *et al.* 2015). Although both the temperature and soil moisture have been indicated earlier as important factors related to the changes in biological properties (Bell *et al*. 2009), a marked interaction between the temperature and soil moisture data and BG activity was not found on the subsequent sampling dates in this study. The lack of the correlation between soil moisture and soil BG activity (except of April 2008) suggested that factors other than moisture, such as a limited supply of readily oxidizable substrates, and/or a different microbial population could influenced this activity.

The BG activity, which plays a key role in the soil carbon cycling is usually closely connected with the organic C concentration, which was confirmed by other authors (e.g. Böhme and Böhme 2006, Piotrowska and Koper 2010).

According to Wang *et al.* (2017), soil BG activity was closely related to the content of easily soluble organic carbon. Also in this study we found the BG activity to be correlated with soil $C_{ORG}$ and $N_{TOT}$ concentration as well as with soil pH. Soil pH ranging from 4.08 to 6.68 could be a possible source of the variability of soil BG activity in this study. The effect of pH on soil enzyme activity was attributed to the fact that even slight changes in soil pH can disturb the interaction between enzymes and their substrates (Aşkin and Kizilkaya 2006). The hydrogen ion concentration modifies the enzyme reaction site, as well as the stability of immobilized enzymes in the soil matrix (Kang and Freeman 1999).

There is little research concerning the spatial variability of soil BG with use of geostatistics (El-Naggar *et al*. 2010). We used two indices of spatial dependence in our geostatistical analysis of BG activity – the nugget/sill ratio and the range. The analyzed properties indicated a nugget, which represents the random variation usually derived from the inaccuracy of measurements or variations of the properties that cannot be detected in the sample range. The nugget/sill ratio indicates the degree of spatial dependence at the sampling scale and allows for a comparison of the relative size of the nugget effect among different soil properties (Goovaerts 2010). The nugget effect of BG activity data was quite large relative to the sill and this property revealed a moderate or weak spatial structure. The weak spatial dependence indicates an extrinsic variability, for example, due to human activity such as fertilization or tillage (Piotrowska and Długosz 2012). Another parameter of soil spatial variability is the range (m), which is considered to be the distance beyond which observations are not spatially dependent. Generally, samples need to be taken at a distance shorter than the range of the variogram in order to develop reliable maps (Kerry *et al.* 2010). Results obtained in this study (range values between 15 and 61 m) suggested that the strategies that are chosen to create the sampling scheme (every 10 m) for the properties studied were not suitable and less intensive sampling grid should be recommended for this field.

## CONCLUSIONS

1. The analysis of variance showed a significant temporal variability in the soil β-glucosidase activity.
2. Soil BG activity was spatially dependent at the scale of sampling grid and revealed a moderate to high variability that was dependent on various factors, such as management practices, soil structure and texture or the nested location of microorganisms in soil.
3. Less intensive sampling grid should be recommended for soil BG activity in further studies concerning spatial variability in arable field scale.
4. Since the BG activity showed differentiation in the spatial pattern

between sampling dates, which was confirmed by the geostatistical parameters, more frequent sampling throughout the growing season must be included in the experimental planning in order to gain a better understanding of whether the BG activity shows any permanent spatial patterns in soil ecosystem all of the time or is more randomized.

5. The data obtained showed that it is important to take spatial variability into account when developing a sampling strategy. If the sampling is performed in varying patterns from one date to another, considerable spatial variation can be superimposed on the temporal changes.

6. Significant relationship between β-glucosidase activity and organic carbon content, total nitrogen concentration and soil reaction on four sampling dates was noted.

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