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THE EFFECT OF ENVIRONMENTAL FACTORS ON TOTAL SOIL DNA CONTENT AND DEHYDROGENASE ACTIVITY

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Abstract: The aim of the study was a statistical evaluation of the impact of selected soil factors – water potential (pF), total organic carbon content (TOC) and land use – on the total DNA content and dehydrogenase activity (DHA) in Mollic Gleysol. Additionally, we wanted to establish the interrelations between two of the most important biological parameters in soli: activity of intracellular dehydrogenases and total DNA content. Soil originating from the surface layer of the control site displayed higher DHA (c.a. by 57%) and DNA content (c.a. by 25%) as compared to an cultiavted meadow. Our results also indicate a significant (p <0.05) positive relationship between the soil DNA content and DHA. Importantly, intensive and systematical agricultural soil usage resulted in the reduction of its DHA and DNA content.

Key words: Dehydrogenase activity; DNA content; soil water potential; total organic carbon; way of land use

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INTRODUCTION

Life in the soil environment is inevitably connected with drying and rewetting cycles, as soils are continually exposed to rainfall, wind and snow cover (Wolińska and Stępniewska, 2011). The availability of water is considered the major factor determining microbial activity in soil (Geisseler et al., 2011). Drying increases soil water potential and in most cases hampers microbial and enzymatic activities. Repeated flooding and drainage of the soil results in oxic-anoxic shifts, which entail temporal and spatial changes in the soil processes, microbial communities and, consequently, activities of soil enzymes. Soil enzymatic activity is closely related to microbial biomass and activity, as it catalyzes biochemical reactions and influences nutrient cycling in the soil environment (Burns, 1982). It has been suggested that the enzymatic activity of soli integrates information about the microbial status and soil physicochemical conditions, and is therefore regarded to be a sensitive sensor of ecosystem/environmental stress and changing

soil properties (Aon et al., 2001; Hueso et al., 2012; Yuan and Yue, 2012).

Among the many types of soil enzymes, dehydrogenases (EC 1.1.1.) are particularly important, since they exist only inside viable microbial cells (they do not accumulate extracellularly in the soil) and thus provide reliable information about soil biology, fertility and productivity. As a result, DHA is often used as an indicator of overall microbial activities (Frąc and Jezierska-Tys, 2011; Wolińska and Stępniewska, 2011; Hueso et al., 2012; Yuan and Yue, 2012). Dehydrogenases play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from organic substrates to inorganic acceptors. Thus, the determination of DHA in soil provides a large amount of information about its biological characteristics. The term "dehydrogenase" refers to group of enzymes capable of catalyzing biochemical processes under various environmental conditions. Dehydrogenases are present in the cells of some aerobic microbiota; however, this enzyme is more effectively produced by anaerobes (Brzezińska et al., 1998, 2000; Włodarczyk, 2000). In other words, soil DHA strongly increases under anaerobic conditions (Brzezińska et al., 1998; Stępniewski et al., 2000; Wolińska and Stępniewska, 2011). Therefore, knowledge of the factors influencing soil biology, especially soil microorganisms and soil enzymes, is fundamental to sustainable environmental management.

Nucleic acids are another ubiquitous soil compounds. Most of the information about soil microorganisms' ecology and diversity is lodged in the genetic material (DNA) occurring in this complex environment (Wolińska et al., 2011). Moreover, soil DNA analysis is considered an important and precise tool for the characterization of soil microbial functionality (Wolińska et al., 2011). In this work, we examined the effects of pF, TOC and different ways of land use on soil DNA content and DHA level. Based on laboratory findings, we hypothesized there should be a close relationship between the activity of intracellular dehydrogenases and DNA content in the soil environment. In addition, we hypothesized that some of the important environmental factors, such as pF and TOC, as well as land use (agricultural activity), which affect DHA, should also influence DNA content in the soil environment. Thus, we wanted to establish the interrelation between the two most important soil biological parameters: activity of intracellular dehydrogenases and total DNA content. This is an important problem, as literature data referring to these connections are ambiguous.

Table 1. The basic properties of the investigated soil.

Area	Depth (cm)	Granulometric composition (% diameter in mm)				pH (H_2O)
		1-0.1	0.1-0.02	0.02-0.002	< 0.002	
Agriculturally exploited (AE)	0-20	93	2	1	4	7.62
	20-40	94	1	2	3	7.00
	40-60	95	1	1	3	6.71
Fallow land (FL)	0-20	92	2	3	3	6.56
	20-40	92	3	1	4	6.59
	40-60	95	1	1	3	6.57

MATERIALS AND METHODS

Study site and soil collection

The soil used in the experiment was Mollic Gleysol, collected in the three field replicates and at three depths (0-20, 20-40 and 40-60 cm) from the village Kosiorów, situated in the Wilków community in eastern Poland (51° 13′ N 21° 53′ E). Our site was a former floodplain area along the Chodelka River, a tributary of the middle part of the Vistula River, which has demonstrated an increased frequency of summer flooding during the last decade. What is more, this location has been proposed as a storage basin to take back-flowing water from the Vistula during peaks of discharge (Banach et al., 2009). We selected two study areas: one of them was agriculturally exploited (AE) with systematic fertilization and pasturage (under human activity), whilst the other one was classified as fallow land (FL), and used as a control area (without any human impact). More particularly, the first meadow was cultivated for haymaking, fertilized with nitrogen, phosphorus and potassium at unknown dosage, and moved twice a year, in the spring and summer seasons (Banach et al., 2009). The second grassland was decidedly less fertilized, was not moved, but was grazed. Both locations were representative for the described area and comprised species-rich grasslands dominated by *Deschampsia cespitosa* L. and *Holcus lanatus* L. (Banach et al., 2009). The basic characteristic of Mollic Gleysol is shown in Table 1.

Determination of the soil water-retention ability

The instrument used to determine soil water-retention abilities (pF curves) was a steel pressure chamber, with a porous plate saturated with water inside. Soil samples were transferred to plastic cylinders (h = 5 cm, V = 100 cm^3) and placed on a plate inside the stainless-steel pressure chamber, containing a porous plate saturated with water at the bottom, in order to obtain a hydraulic contact between the sample and the porous plate (Pires et al., 2005). The laboratory set LAB o12 (Soil Moisture Equipment Company, USA) was used. Pressure was applied for the following pF values: 0, 1.0, 1.5 and 2.0, which correspond to a values linked to a range of microorganisms and plant roots (Harris, 1981). The pF values were characterized by the following water-retention capacity: 55% v/v (pF 0); 40% v/v (pF 1.0); 35% v/v (pF 1.5) and 25% v/v (pF 2.0).

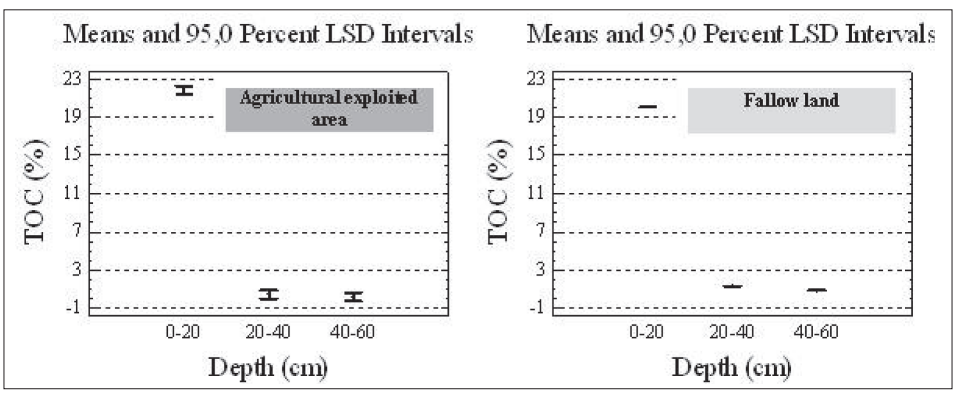

Fig. 1. Distribution of TOC content in the investigated soil material (n = 9, p <0.0001).

Measurement of TOC

TOC was determined using an automatic carbon analyzer TOC-V_{CSH} SSM 5000A (Shimadzu, Japan). Soil samples were pulverized and dried prior to analysis. Each sample was analyzed separately (in three replications) for its total organic carbon. The soil sample (150 mg) was combusted at 900°C in a column containing a platinum and cobalt oxide catalyst. Under these conditions, all carbon compounds were converted into carbon dioxide form and detected by an infrared detector.

DHA assay

Soil DHA was tested using 2.3.5-triphenyltetrazolium chloride (TTC), according to the protocol of Casida et al. (1964). According this method, specific dyes, such as triphenyl tetrazolium chloride that can specify the flow of electrons, are useful indicators of electron transport system (ETS) activity. With the reduction of a colorless, water-soluble substrate (TTC) by dehydrogenases, an insoluble product with red color (triphenylformazan – TPF) is formed. TPF can be easily quantified calorimetrically. Absorbance (λ = 485 nm) was measured using a UV-1800 (Shimadzu, Japan) spectrophotometer. DHA was expressed as μg TPF g^{-1} min^{-1}. All measurements were done in triplicate and calculated on the basis of the oven-dried (105°C) soil mass.

Table 2. Correlations between TOC, pF, DNA content and DHA in Mollic Gleysol with different types of land use (n=36, $p < 0.05$).

Area	Factor	DNA[b]	TOC[c]	pF
AE	DHA[a]	0.38*	0.49**	- 0.79***
FL		0.47**	0.58**	- 0.84***

*p <0.05, **p <0.01, ***p <0.001. [a] μg TPF g^{-1} min^{-2} ; [b] mg g^{-1}; [c] %.

Soil DNA extraction procedure

Soil DNA was extracted using the procedure by Sambrook and Russel (2001), with small modifications. Total nucleic acids were extracted from 1 g of soil (in three replications) with the addition of 10 ml of extraction buffer (100 mM Tris-Cl pH 8.0, 100 mM EDTA pH 8.0, 1.5 M NaCl) and 2 g of 0.1 mm glass beads (Sigma-Aldrich) in a 25 ml stainless steel grinding jar. The cells in the supernatant were pelleted and subjected to lysis using GES solution (5 M guanidine thiocyanate, 100 mM EDTA, 0.5% sarkosyl (pH 8)). DNA was purified using an ice-cold solution of ammonium acetate (7.5 M) and a chloroform: isoamyl (24:1) mixture. Cell debris were removed by centrifugation. DNA was precipitated at -20°C with isopropanol for 2 h, pelleted by centrifugation at 17 500×g for 30 min, rinsed 5 times with 70% v/v ethanol and resuspended in 30 ml of ultrapure DNAse-free water. The DNA concentration in each purified extract was quantified by UV spectroscopy (UV-1800 Shimadzu, Japan) at 260 nm, and expressed as mg DNA g^{-1} dry soil.

Statistical analysis

Analyses of variance were conducted by Statistica 9.0 program (StatSoft, Inc. USA) to test differences among the tested soil parameters. One-way ANOVA, post hoc Tukey's and Pearson tests were performed to find significant differences between the investigated factors. The differences were considered significant at $p < 0.05$.

RESULTS

TOC content in the soil profiles

TOC content demonstrated a decreasing trend with the increase in soil depth (Fig. 1). The highest concentration of TOC occurred in the surface

layer of Mollic Gleysol, amounting to 22.47% and 20.09% for AE and FL, respectively. In the subsurface part of the soil profiles (20-40 cm), the TOC content in the AE and FL sites was strongly reduced by 98.1% and 93.3% (in comparison to the surface), respectively. The lowest TOC concentration in the subsoil part of Mollic Gleysol was noted, and was only 0.21% and 0.87% for AE and FL, respectively. Comparison of the two investigated sites with respect to TOC content demonstrated slightly higher but not significant ($p > 0.05$) TOC values at the AE site.

Response of soil DHA to different pF values

Incubation of the soil samples under different controlled moisture conditions significantly altered both DHA level and DNA concentration. Our findings confirmed that DHA is strongly affected by water potential (Fig. 2), as we noted the highest DHA values (0.0016 and 0.0036 μg TPF g^{-1} min^{-2}) for AE and FL, respectively, in the surface layers and at full water capacity conditions (pF 0). Moreover, DHA in the surface part of the FL site was 55.5% higher than in the AE site. This might suggest a negative effect of human agricultural impact on soil enzymatic activity. Meanwhile, the lowest values of DHA characterized the deepest part of Mollic Gleysol profile (40-60 cm), ranging from 0.00016 to 0.00019 μg TPF g^{-1} min^{-2}, for AE and FL, respectively. Differences in DHA between surface (0-20 cm) and subsoil (40-60) layers were 92.5 and 94.7% lower for AE and FL, respectively. In pF 2.0 conditions. DHA demonstrated a different trend in comparison to the other tested pF values, as we found its maximum in the subsurface layer, both in the AE and FL area. These differentiations were more noticeable in the FL site, where the DHA level in the subsurface layer was 20% higher than in the surface part ($p < 0.05$).

Response of DNA content to different pF values

DNA concentration was also found to be the highest (3-4 mg g^{-1}) in the surface part of both investigated meadows (Fig. 3). The most favorable moisture conditions for DNA presence was pF 1.0. However, the subsurface layer of Mollic Gleysol was DNA-rich; the concentrations in the top layer were 1.5-1.7 mg g^{-1} at the AE, and 1.25-1.75 mg g^{-1} at the FL site, at pF ranging from 0-1.5. At pF 2.0, the DNA content increased with soil depth ($p > 0.05$) and the highest content was found in the subsoil. It can be concluded that the soil DNA level is affected by the water content, and that its content decreases with the decrease in soil moisture, which means that higher soil moisture provides better conditions for DNA presence in a soil environment.

Relationships between investigated factors

Finally, using statistical tools the correlations between DHA, DNA content, TOC and pF were determined (Table 2). Significant positive correlations ($p < 0.05$) for DHA and DNA were found, which was confirmed by the r coefficient values ($r = 0.38^*$) and ($r = 0.47^{**}$) for AE and FL, respectively. Proportional correlations were also noted in the case of DHA and TOC content. An inversely proportional significant ($p < 0.0001$) relationship was found in the case of DHA, DNA and pF, which means that DHA increased with water supply, reaching maximum values at pF 0. The determined relationships show that the increase in TOC and DNA content results in an increase in DHA in the Mollic Gleysol profile, independent of land use. Thus, our hypothesis about the close relationship between the activity of DHA and DNA presence in the soil environment has been verified.

DISCUSSION

TOC distribution in the soil profile

TOC distribution determined in the current study displayed a decreasing trend with soil profile depth. A similar TOC distribution in a Mollic Gleysol profile was earlier described by Turski and Witkowska-Walczak (2004) and Wolińska et al. (2012a, 2012 b). Most authors reported that the quality of carbon is particularly important because it affects the supply of energy for both enzyme production and microbial growth (Fontaine et al., 2003; Frąc and Jezierska-Tys, 2011; Wolińska and Stępniewska, 2011; Yuan and Yue, 2012). Soil containing a higher proportion of TOC provides more substrate to support microbial biomass (Yuan and Yue, 2012) and thereby soil DHA. In the opinion of many authors (Stępniewski et al., 2000; Włodarczyk, 2000; Frąc and Jezierska-Tys, 2011; Yuan and Yue, 2012), soil enzymes are biological indicators of the quality, fertility and productivity of soils, as well as of the anthropogenic impact. Nannipieri et al. (2002) as well as Frąc and Jezierska-Tys (2011) were of the opinion that soil enzymatic activity (particularly DHA) may be an early and sensitive indicator of the degree of soil degradation and can be used for the estimation of anthropogenic factors affecting soil quality.

Response of soil DHA to different pF values

Changes in soil moisture status can also markedly affect the magnitude of the soil intercellular enzymes activity because many soil microorganisms are known to be intolerant to low soil moisture content (Harris, 1981). In the present study, limitation of water (pF 2.0) seemed to reduce both DHA level and DNA content in relation to the full water capacity conditions (pF 0). It was also demonstrated that DHA is strongly impacted by the water content and that its activity decrease with decreasing soil moisture (Nayak et al., 2007; Pascual et al., 2007; Wolińska and Stępniewska, 2011). The tendency of soil DHA to increase under anaerobic conditions was observed in model experiments (by incubating soils under soil flooding) as well as under natural field conditions (Stępniewski et al., 2000; Brzezińska et al., 2001).

Response of soil DNA content to different pF values

DNA concentration determined in the current study also displayed spatial distribution in the soil profile. An analogical DNA trend was earlier noted in the loess soil profile (Wolińska et al., 2012b). The general content of DNA varies in relation to soil type, soil conditions, microbial abundance, type of soil cultivation, climate, etc. Wolińska et al. (2011) demonstrated higher soil DNA content in full water capacity conditions; however, in the range pF 0-3.2 the differences were not significant (p >0.05). Results from the current study indicated that DNA content was highest at pF 1.0 and the lowest at pF 2.0. These observations are in agreement with results obtained previously by Schimel et al. (2007) and Finlay and Esteban (2009).

Relationships between investigated factors

We found positive significant (p <0.05) relationships between DNA concentration, TOC content and soil DHA. Although studies by Sheu et al. (2008), Acosta-Martinez et al. (2010) and Wolińska et al. (2012b) confirmed positive correlations between DNA and TOC, prior to our study little attention has been paid to the statistical description of the relationship between soil DHA and DNA content. Some of the authors de

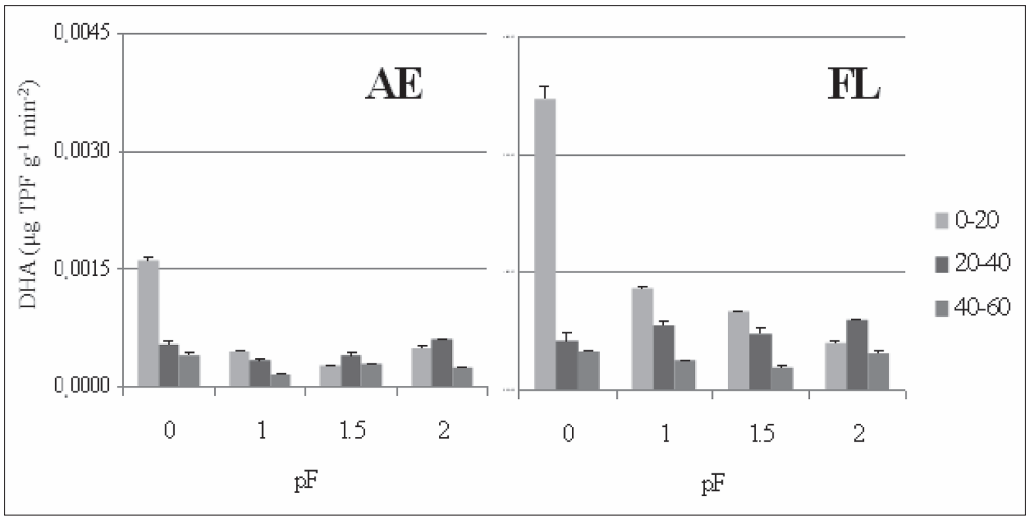

Fig. 2. Relationship between DHA level and different values of pF in agricultural exploited (AE) and fallow land (FL). Average values with standard deviations are presented.

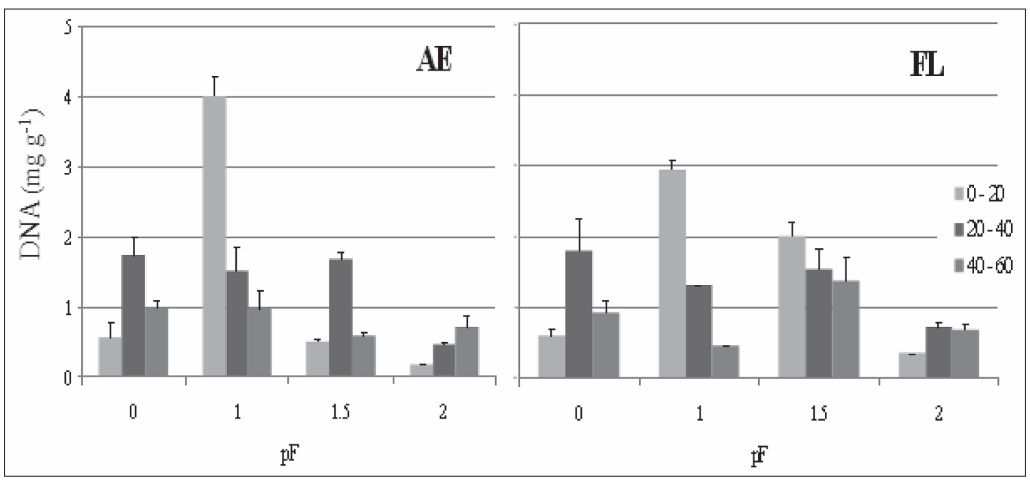

Fig. 3. Relationship between DNA concentration and different values of pF in agricultural exploited (AE) and fallow land (FL). Average values with standard deviations are presented.

scribed only positive correlations between DHA and soil microbial biomass and indicated that higher values of DHA in soils are connected with a higher abundance of bacteria (Baskar et al., 2003; Furczak and Joniec, 2007; Nayak et al., 2007). Nevertheless, the interpretation of our results has been a challenge, because even though only a few of the mentioned studies have examined the effects of TOC and pF on the soil DNA content, there is still a lack of data concerning the statistical determination of the correlation between soil factors and DNA concentrations. Consequently, more research on other soil types and under different moisture conditions is needed to complete the understanding soil DNA dependence on physical and biological parameters.

CONCLUSION

Our study showed that there is a close relationship between the activity of intracellular dehydrogenases and the presence of DNA in soli, pF, TOC and agricultural activity. It was demonstrated that biological factors (DHA and DNA content) displayed higher values in the soil samples taken from the control site (non-cultivated). It was also found that DHA positively correlated (p <0.05) with DNA (to date, similar interdependencies have not been clearly described in the literature) and TOC, but negatively correlated with pF. Finally, we assumed that agricultural activity limits soil fertility through the reduction in its DHA level (which was observe to be 51.3% in the surface layers), when compared to control soil.

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Conflict of interest disclosure: There is no conflict of interest

REFERENCES

Acosta-Martinez, V., Burrow, G., Zobezk, T.M. and *V.G Allen* (2010). Soil microbial communities and function in alternative systems to continuous cotton. *Soil Sci. Soc. Am. J.* **74**, 1181-1192.

Aon, M.A., Cabello, M.N., Sarena, D.E., Colaneri, A.C., Franco, M.G., Burgos, J.L. and *S. Cortassa* (2001). Spatio-temporal patterns of soil microbial and enzymatic activities in an agricultural soil. *Appl. Soil Ecol.* **18**, 239-254.

Banach, A.M., Banach, K., Visser, E.J., Stępniewska, Z., Smits, A.J.M., Roelofs, J.G.M. and *L.P.M. Lamers* (2009). Effects of summer flooding on floodplain biogeochemistry in Poland; implications for increased flooding frequency. *Biogeochemistry* **92**, 247-262.

Baskar, S., Baskar, R. and *A. Kaushik* (2000). Role of microorganisms in weathering of the Konkan-Goa laterite formations. *Curr. Sci.* **85**, 1129-1134.

Brzezińska, M., Stępniewska, Z. and *W. Stępniewski* (1998). Soil oxygen status and dehydrogenase activity. *Soil Biol. Biochem.* **30**, 1783-1790.

Brzezińska, M., Stępniewska, Z., Stępniewski, W. and *M. Pasztelan* (2000). Dehydrogenase and catalase activity in *Eutric Histosol* as affected by soil temperature and water content. *Acta Agrop.* **35**, 31-40.

Brzezińska, M., Stępniewski, W., Stępniewska, Z., Przywara, G. and *T. Włodarczyk* (2001). Effect of oxygen deficiency on soil dehydrogenase activity in a pot experiment with triticale cv. Jago vegetation. *Int. Agrop.* **15**, 145-149.

Burns, R.G. (1982). Enzyme activity in soil: location and possible role in microbial ecology. *Soil Biol. Biochem.* **14**, 423-427.

Casida, L., Johnson, J. and *D. Klein* (1964). Soil dehydrogenase activity. *Soil Sci.* **98**, 371-376.

Finlay, B.J. and *G.F. Esteban* (2009). Oxygen sensing drives predictable migrations on a microbial community. *Environ. Microbiol.* **1**, 81-85

Fontaine, S., Mariotti, A. and *L. Abbadie* (2003). The priming effect of organic matter: a question of microbial competition. *Soil Biol. Biochem.* **35**, 837-843.

Frąc, M. and *S. Jezierska-Tys* (2011). Agricultural utilisation of dairy sewage sludge: its effect on enzymatic activity and microorganisms of the soil environment. *Afr. J. Microbiol. Res. 5*, 1755-1762.

Furczak, J. and *J. Joniec* (2007). Preliminary study of sludge effect on soil microbial activity of a *Podzolic* soil under willow culture. *Int. Agrop.* **21**, 39-48.

Geisseler, D., Horwat, W.R. and *K.M. Scow* (2011). Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. *Pedobiologia* **54**, 71-78.

Harris, R.F. (1981). Effect of water potential on microbial growth and activity. In: *Water Potential Relations in Soil Microbiology* (Eds. J.F. Parr, W.R. Gardner, and L.F. Elliot), 23-91, Madison, WI.

Hueso, S., Garcia, C. and *T. Hernandez* (2012). Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. *Soil Biol. Biochem.* **50**, 167-173.

Nayak, D.R., Babu, J. and *T.K. Adhya* (2007). Long-term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition. *Soil Biol. Biochem.* **39**, 1897-1906.

Pascual, I., Antolin. M.C., Garcia, C., Polo. A. and *M. Sanchez-Diaz* (2007). Effect of water deficit on microbial characteristics in soil amended with sewage sludge or inorganic fertilizer under laboratory conditions. *Biores. Tech.* **98**, 29-37.

Pires, L.F., Bacchi, O.O. and *K. Reichardt* (2005). Soil water retention curve determined by gamma-ray beam attenuation. *Soil Till. Res.* **82**, 89-97.

Sambrook, J. and *D.W. Russel* (2001). Molecular cloning a laboratory manual. Cold Spring Harbour Laboratory Press, New York. NY, **11**, 6-11.

Scheu, C., Wu, C.Y., Chen, S.C. and *C.C. Lo* (2008). Extraction of DNA from soil for analysis of bacterial diversity in transgenic and no transgenic Papaya sites. *J. Agric. Food Chem.* **56**, 11969-11975.

Schimel, J., Balser, T.C. and *M. Wallenstain* (2007). Microbial stress-response physiology and its implications for ecosystem function. *Ecology* **88**, 1386-1394.

Stępniewski, W., Stępniewska, Z., Gliński, J., Brzezińska, M., Włodarczyk, T., Przywara, G., Varallay, G. and *K. Rajkai* (2000). Dehydrogenase activity of some hungarian soils as related to their water and aeration status. *Int. Agrop.* **14**, 341-354.

Turski, M. and *Witkowska-Walczak, B*. 2004. Physical properties of *Luvisols* derived from silt loam formations under forest and arable cropping. *Acta Agrop.* **101**, 5-51.

Włodarczyk, T. (2000). Some aspects of dehydrogenase activity in soils. *Int. Agrop.* **14**, 365-376.

Wolińska, A., Stępniewska, Z., Bułaś, A., and *A. Banach* (2012a). Evaluation of factors influencing the biomass of soil microorganisms and DNA content. *Open J. Soil Sci. 2*, 64-69.

Wolińska, A., Stępniewska, Z., Wołoszyn, A. and *M. Rzewuska* (2012b). Interrelations between DNA concentrations and physicochemical parameters in the loess soil profile. *Acta Agrop.* **19**, 437-448.

Wolińska, A. and *Z. Stępniewska* (2011). Microorganisms abundance and dehydrogenase activity as a consequence of soil reoxidation process. In: *Soil Tillage and Microbial Activities,* 7 (Ed. M. Miransari), 111-143, Research Signpost, Kerala. India.

Wolińska, A., Stępniewska, Z., Wołoszyn, A., Pytlak, A. and *A. Dziuba* (2011). Means of molecular nucleic acids analysis in soil investigation. *Acta Agrop.* **194**, 7-60.

Yuan, B.C. and *D.X. Yue* (2012). Soil microbial and enzymatic activities across a chronosequence of Chinese pine plantation development on the loess plateau of China. *Pedosphere* **22**, 1-12.