Vol. 62, 2016, No. 6: 250–255 *Plant Soil Environ.*

doi: 10.17221/158/2016-PSE

Soil moisture as a factor affecting the microbiological and biochemical activity of soil

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ABSTRACT

The purpose of this research has been to identify relationships between soil moisture and the growth and development of microorganisms, their diversity and the activity of soil enzymes. Four soils with different texture were analysed. Air-dry soils were watered up to the moisture content corresponding to 20, 40 and 60% of the maximum water capacity (MWC) and subsequently were submitted to determinations of the counts of soil microorganisms, colony development index and ecophysiological diversity index for bacteria, actinomycetes and fungi. In addition, the response of seven soil enzymes to soil humidity was examined. It was found that the most optimum soil moisture for the development of organotrophic bacteria was the one at the level of 20% of MWC. For *Azotobacter* spp. bacteria and actinomycetes, the 40% MWC soil moisture level was optimum, while fungi developed the best at the soil moisture level of 60% of MWC. In turn, the activity of soil dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase, β-glucosidase and arylsulfatase was the highest in soil with 20% of MWC. The principal component analysis showed that the soil moisture determined the microbial and biochemical soil activity to a much lesser degree than did the soil type.

Keywords: microbial communities and activity; microbiota; enzymatic activity; ecophysiological biodiversity

The thermal and hydrologic regime is the key factor responsible for the microbial and biochemical soil properties (Giacometti et al. 2013). The water content in soil affects the physiological state of microorganisms and plants (Walker et al. 2003). In summer, respiration and water fluctuations are greater in wet than in dry soils. According to Silva et al. (2008), respiration depends more strongly on moisture than on temperature. Well-moist soils hold more functionally diverse microbial communities. However, excessive soil moisture may lead to a lower biomass of microorganisms (Silva et al. 2008, Unger et al. 2009), mostly due to the formation of oxygen conditions that are unfavourable to aerobic bacteria, both gram-positive and gram-negative ones, and to mycorrhizal fungi (Unger et al. 2009). Drought can also disturb the homeostasis of soil (Kim et al. 2008). In addition, water is essential for maintaining the catalytically active state of soil enzymes (Jiang and Zhang 2002).

In view of the above considerations, it is extremely important to determine correlations between the soil moisture content and soil microbial activity. To prevent error due to the spatial scale describing the heterogeneity of soils, four soils different in texture as well as in the organic carbon and nitrogen content were selected for our research. The rationale behind this selection of soils lies in the conclusions to analysis made by numerous researchers, who have suggested that the microbial and biochemical activity of soil is closely connected with its physical and chemical properties (Lagomarsino et al. 2012, Nannipieri et al. 2012). Another reason was the fact that soil's biological properties affect soil fertility and crop yielding potential (Kucharski and Jastrzębska 2006, Wyszkowska et al. 2007). Hence, our objective has been to determine dependences between soil moisture and the growth and development of microorganisms, their physiological diversity and activity of soil enzymes.

Supported by the Polish Ministry of Science and Higher Education, Project No. N N305 2258 33.

doi: 10.17221/158/2016-PSE

Table 1. The composition of the grain size soils

Soil type	Percentage of fraction (d)		
	2.00 ≥ d > 0.05 mm	0.05 ≥ d > 0.002 mm	d ≤ 0.002 mm
Sand	92.47	7.07	0.46
Loamy sand	85.18	13.82	1.00
Sandy loam	51.27	45.36	3.37
Silty loam	34.96	60.21	4.83

MATERIAL AND METHODS

Soil properties. Four proper soils (Eutric Cambisol) with different grain-size composition were chosen for our study. They originated from the Research Station in Tomaszkowo (NE Poland, 53.7161°N, 20.4167°E). Soil samples were taken from the arable humus soil horizon. According to the grain-size classification developed by the World Reference Base of Soil Resources (2014), the sampled soils represented the following types of texture: sand (S); loamy sand (LS); sandy loam (SL) and silty loam (SiL). Having selected the soils and identified their properties (Tables 1 and 2), the subsequent stage of the research was performed under strictly controlled conditions, at the Department of Microbiology, the University of Warmia and Mazury in Olsztyn (NE Poland).

Experimental design. The experiments were carried out in three replications, under laboratory conditions. Glass beakers, each 150 mL in capacity, were filled with 100 g dry matter (DM) batches of soil (S, LS, SL and SiL), previously passed through a 2 mm mesh sieve. Experiment was repeated in four series: (1) air-dry (a.d.) soil; (2) 20%; (3) 40% and (4) 60% of the maximum water capacity of soil. Having obtained the required soil moisture by adding distilled water to soil, the beakers were covered with perforated foil and incubated in an incubator, for 16 weeks, at the temperature of 25°C. The soil moisture content was monitored twice weekly.

Soil microorganisms. On two occasions during the whole experiment (at week 4 and 16), counts of organotrophic bacteria, *Azotobacter* spp. bacteria, actinomycetes and fungi were determined by the plate method in soil samples from each repetition, in consecutive replications. The media used in this experiment were identical to those employed in the study by Wyszkowska et al. (2007). Microorganisms were cultured at the temperature of 28ºC. Colonies of bacteria belonging to *Azotobacter* spp. were counted after 2 days, organotrophic bacteria and actinomycetes – after 7 days, and fungi – after 5 days. In order to determine the colony development (CD) index and ecophysiological (EP) index, cultures of respective dilutions with the medium were counted every day for 10 consecutive days. Afterwards, the index of colony development CD was derived from the formula:

$$CD = [N_1/1 + N_2/2 + N_3/3..... N_{10}/10] \times 100$$

Where: N_1, N_2, N_3,.... N_{10} – stand for a proportional count of colonies grown at day 1, 2, 3, ..., 10 (Sarathchandra et

Table 2. Physicochemical properties of the soil

Soil type	C_{org}	N_{tot}	K_w	Na_w	Ca_w	Mg_w	pH_{KCl}	HAC	EBC	CEC	BS (%)
	(g/kg DM soil)		(mg/kg DM soil)					($mmol_+$/kg DM soil)			
Sand	3.9	0.3	66	41	599	26	6.6	6.5	35.5	42.0	84.5
Loamy sand	6.7	0.61	168	46	812	28	6.3	14.4	49.2	63.6	77.3
Sandy loam	9.9	1.14	168	57	2214	50	6.8	5.2	131.4	136.6	96.2
Silty loam	9.9	1.38	196	53	3556	74	7.0	6.5	197.2	203.7	96.8

org – organic; tot – total; w – replaceable; DM – dry matter; HAC – hydrolytic acidity; EBC – sum of exchangeable cations; CEC – cation exchange capacity; BS – base saturation

doi: 10.17221/158/2016-PSE

al. 1997). The index of ecophysiological diversity EP was calculated from the equation:

$$EP = -\Sigma(p_i \times \log p_i)$$

Where: p_i – number of colonies of microorganisms on a given day divided by the number of all colonies (De Leij et al. 1993).

Soil enzymes. On the same days when counts of microorganisms were taken, the activity of the following soil enzymes was determined in individual soil samples, in three replications: dehydrogenases (EC 1.1) – by the Lenhard method modified by Öhlinger (1996), catalase (EC 1.11.1.6), urease (EC 3.5.1.5), arylsulfatase (EC 3.1.6.1), β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) – according to Alef et al. (1998). The following were used as substrates: 2,3,5-triphenyl tetrazolium chloride (TTC) for dehydrogenases, hydrogen peroxide for catalase, 4-nitrophenyl phosphate disodium (PNPNa) for phosphatases, urea for urease, p-nitrophenyl β-D-glucopyranoside (PNG) for β-glucosidase and potassium 4-nitrophenylsulfate (PNS) for arylsulfatase. The activity of enzymes was expressed in the following units (in 1 kg DM of soil/h): μmol of triphenyl-formazan (TPF) for dehydrogenases, mol O_2 for catalase, mmol N-NH_4^+ for urease, mmol of ρ-nitrophenyl (PNP) for acid phosphatase, alkaline phosphatase, β-glucosidase and arylsulfatase. Determinations of the activity of all enzymes, except catalase, were accomplished on a Perkin-Elmer Lamda 25 spectrophotometer (Massachusetts, USA).

Physico-chemical properties of soil. Prior to the experiment, soil samples were tested with a Mastersizer 2000 laser particle size analyser (Malvern, Worcestershire, UK) to determine particle size distribution in soils; soil pH was determined by the potentiometric method in KCl solution of the 1 mol/L concentration; hydrolytic acidity (HAC) and exchangeable base saturation were measured by the Kappen method (Carter 1993), total nitrogen content was assessed with the method by the Kjeldahl, potassium, calcium and magnesium content was measured by ASA and organic carbon (C_{org}) content was determined by the Tiurin method (Nelson and Sommers 1996). The results of the HAC and EBC measurements served to calculate cation exchange capacity (CEC) and base saturation (BS) from the formula:

$$CEC = EBC + HAC;$$

$$BS = (EBC/CEC) \times 100.$$

Statistical analysis. ANOVA analysis of variance was performed using Statistica 10.0 software (StatSoft 2015). Homogenous groups were distinguished with the Tukey's test, at $P = 0.01$. The Pearson's simple correlation coefficients were calculated between dependent and independent variables. The results were also submitted to the principal component analysis (PCA). In addition, the η^2 coefficient was calculated using the ANOVA analysis of variance. This coefficient identifies the contribution of individual variables to values of dependent variables.

Results derived from microbiological and biochemical analyses are presented as means from 2 dates because our analysis of the η^2 coefficients revealed that the soil incubation time did not have a significant effect on counts of microorganisms, their diversity or the activity of enzymes.

RESULTS AND DISCUSSION

The influence of soil moisture on the soil microbial and biochemical activity does not form a simple relationship (Hueso et al. 2011, 2012, Geisseler et al. 2012). The reason is that soil moisture is never stable in the natural environment. Our experiment proves that both microbiological and biochemical properties of all the tested soils depended on the specific level of moisture in soil. Detailed and significant dependences between the grain-size distribution of soil and its moisture content were revealed by the PCA, which included in the first two principal components such factors as the dispersion of the counts of microorganisms and the activity of enzymes (Figure 1). The horizontal axis explains 76.15% of the total variance of the variables, while the vertical axis pertains to 11.47% of the said variance. Around the axis representing the first component variable there are vectors which correspond to the original variables of the analysed microorganisms and enzymes. All the values of the vectors, representing both counts of microorganisms and activities of enzymes shaped by the first principal component are negative, ranging from –0.994 for dehydrogenases to –0.766 for alkaline phosphatase.

A projection of the cases onto the plane of the factors proves that the optimum moisture in soil for the development of actinomycetes was 40% of the MWC, while 20% MWC in lighter soils and

doi: 10.17221/158/2016-PSE

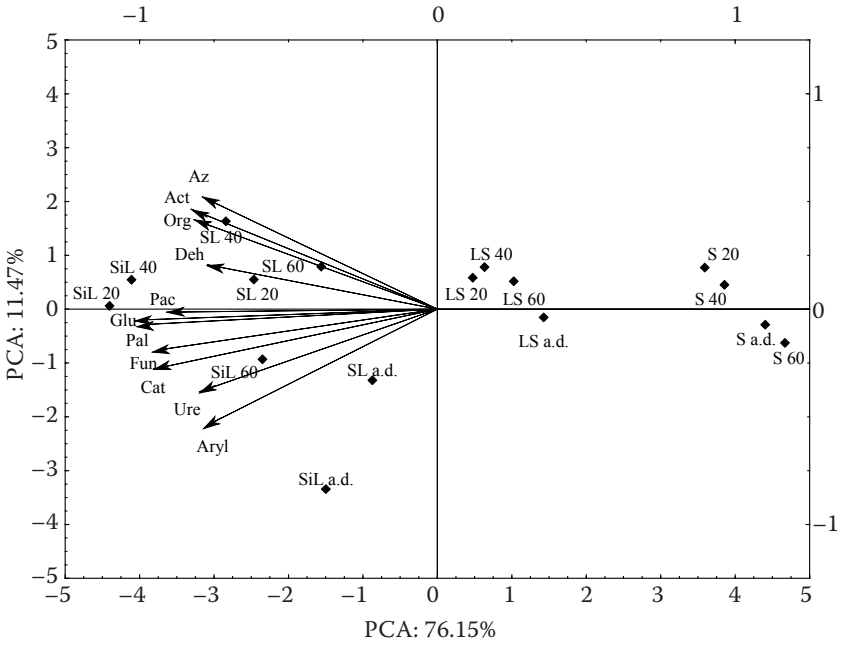

Figure 1. Counts of soil microorganisms and activity of soil enzymes in soils with different soil moisture (PCA). Org – organotrophic bacteria; Az – Azotobacter; Act – actinomycetes; Fun – fungi; Deh – dehydrogenases; Ure – urease; Pac – acid phosphatase; Pal – alkaline phosphatase; Glu – β–glucosidase; Aryl – arylsulfatase; Cat – catalase; S – sand; LS – loamy sand; SL – sandy loam; SiL – silty loam; soil moisture: a.d. – air-dry, 20, 40, 60 – % maximum water capacity (MWC)

40% in more compact soils were optimal for the development of bacteria. Fungi were most numerous in silty loam of the moisture content equal to 20% MWC; in sandy loam and sand – at 40% MWC and in loamy sand – at 60% MWC. The activity of soil dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase, β-glucosidase and arylsulfatase was the highest in soils with the moisture content of 20% to 40% MWC. The PCA clearly demonstrates that the highest counts of microorganisms and the highest enzymatic activity occurred in soils with a higher content of colloidal silt. Our analysis of the distribution of particular cases, reflecting the soil microbiota, implicates that the type of soil formation differentiated counts of microorganisms and activity of enzymes more strongly than soil moisture. This is certainly associated with the content of organic carbon and nitrogen in such soils as well as their sorption capacity (Table 2). In conclusion, optimal soil moisture content – irrespective of the content of colloidal silt, organic carbon or nitrogen – is approximately 20% MWC for the development of organotrophic bacteria, 40% MWC for *Azotobacter* spp. bacteria and actinomycetes, and 60% MWC for fungi (Table 3).

In turn, the activity of soil dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase, β-glucosidase and arylsulfatase was the highest in soils with the moisture content around 20% MWC (Table 4). This was most probably due to the enhanced development of microorganisms and reflected the oxygen conditions in soil. Hueso et al. (2011) and Geisseler et al. (2012) also demonstrated higher enzymatic activity in soils with low moisture content.

Table 3. Impact of soil moisture on the count of soil microorganisms

Soil moisture (% MWC)	Organotrophic bacteria (10^9)	*Azotobacter* (10^3)	Actinomycetes (10^9)	Fungi (10^7)
	10^n CFU/kg soil DM			
Air-dry	11.57^{d}	9.84^{c}	9.17^{c}	56.41^{d}
20	23.09^{a}	16.43^{ab}	12.27^{b}	75.84^{b}
40	21.44^{b}	16.52^{a}	15.08^{a}	71.67^{c}
60	14.01^{c}	11.21^{b}	9.25^{bc}	79.25^{a}
r	0.13	0.16	0.14	0.82*

The same letters in the columns indicate homogeneous groups. MWC – maximum water capacity. *$P \leq 0.01$ between soil moisture and count of microorganisms, $n = 19$

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Table 4. Impact of soil moisture on the colony development and the ecophysiological diversity indexes of microorganisms

Soil moisture (% MWC)	Colony development			Ecophysiological diversity		
	Org	Act	Fun	Org	Act	Fun
Air-dry	33.78^{c}	26.80^{bc}	33.11^{c}	0.89^{a}	0.90^{a}	0.82^{a}
20	37.86^{b}	30.33^{a}	37.38^{b}	0.83^{b}	0.87^{b}	0.65^{c}
40	38.26^{ab}	27.85^{b}	41.23^{a}	0.82^{bc}	0.89^{ab}	0.68^{bc}
60	38.48^{a}	24.80^{c}	39.82^{ab}	0.80^{c}	0.90^{a}	0.69^{b}
r	0.84*	–0.48	0.87*	–0.93*	0.35	–0.60*

The same letters in the columns indicate homogeneous groups. MWC – maximum water capacity. *$P \leq 0.01$ between soil moisture and count of microorganisms, $n = 19$. Org – organotrophic bacteria; act – actinomycetes; fun – fungi

The soil moisture content, by altering conditions for soil microbiota, causes changes in the structural diversity and activity of microorganisms (Kim et al. 2008). The diversity of organotrophic bacteria and fungi was higher in air-dry soils than in the wet ones. However, the diversity of actinomycetes was similar in all soil samples, irrespective of their moisture. Also Schjønning et al. (2011) showed that dry soils were characterized by a greater diversity of microorganisms than irrigated ones. However, there are also studies (Kim et al. 2008) which demonstrate that the diversity of bacteria is not enriched when soil is watered. In our experiment, colonies of microorganisms grew faster from wet than from air-dry soils, which is evidenced by higher CD values in the former group of soils (Table 4). This finding is particularly important because the more rapidly growing microorganisms there are in soil, the more easily fresh organic matter is degraded (Zaborowska et al. 2015). However, such microorganisms are less stable in the environment than those which grow more slowly. And it is the slow-growing microorganisms that are responsible for maintaining the homeostasis of soil (Borowik and Wyszkowska 2016). It is worth mentioning that soil microorganisms are generally well-adaptable to changing air and water conditions in a soil pedon (Walker et al. 2003).

The results presented in this paper unequivocally demonstrate that the soil moisture content is an exceptionally significant factor that changes the biological activity of soil (Ojeda et al. 2013). Both excessively dry and wet soil may lead to a decrease in the biomass of microorganisms (Landesman and Dighton 2010), mostly by creating conditions unfavourable to aerobic gram-positive and gram-negative bacteria as well as to mycorrhizal fungi. Excess of water in the soil environment due to flooding or periodically heavy rainfalls is particularly threatening to aerobic bacteria (Walker et al. 2003). For soil microbiology, it is important to determine the optimum moisture content of pedon because it is the soil microbiota that is responsible for the rate of organic matter transformation as well as the detoxication of mineral and organic xenobiotic substances.

Table 5. Impact of soil moisture on the activity of soil enzymes

Soil moisture (% MWC)	Deh (μmol TFF)	Cat (mol O_2)	Ure (mmol N-NH_4)	Glu	Pac	Pal	Aryl
				(mmol PNP)			
				(kg/soil DM/h)			
Air-dry	8.111^{d}	0.256^{b}	1.642^{c}	0.714^{d}	1.099^{d}	1.215^{d}	0.209^{b}
20	9.442^{a}	0.282^{a}	1.794^{a}	0.869^{a}	1.358^{a}	1.754^{a}	0.287^{a}
40	9.241^{b}	0.257^{b}	1.791^{b}	0.840^{b}	1.237^{b}	1.729^{b}	0.170^{c}
60	8.447^{c}	0.234^{c}	1.446^{d}	0.749^{c}	1.144^{c}	1.483^{c}	0.144^{d}
r	0.164	–0.592*	–0.465	0.135	0.016	0.400	–0.969*

Deh – dehydrogenases; Cat – catalase; Ure – urease; Pac – acid phosphatase; Pal – alkaline phosphatase; Glu – β–glucosidase; Aryl – arylsulfatase. Other explanation as in Table 3

doi: 10.17221/158/2016-PSE

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Received on February 26, 2016
Accepted on May 5, 2016

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