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Evaluation of Factors Influencing the Biomass of Soil Microorganisms and DNA Content

Wolińska Agnieszka, Stępniewska Zofia, Bułaś Aleksandra, Banach Artur

Department of Biochemistry and Environmental Chemistry, Institute of Biotechnology, The John Paul II Catholic University, Lublin, Poland.
Email: awolin@kul.lublin.pl

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ABSTRACT

The aim of the study was the statistical evaluation of the impact of water potential (pF), oxygen availability (ODR) and the way of land use on microbial biomass (MB) and soil DNA content. Soil was extracted from the surface (0 - 20 cm) and subsurface (20 - 40 cm) layers of Mollic Gleysol. Soil material was collected in July 2009 from the village Kosiorów (SE part of Poland), from the two distinct neighbouring areas: agriculturally exploited (AE), and fallow land (FL), which served as the control area. Moisture content was determined for a range of pF values (0, 1.0, 1.5, 2.0), which corresponded to availability of water usable by microorganisms and plant roots. Finally, our results revealed significant ($p < 0.001$) positive relationship between DNA and soil MB content, and negative correlations between soil MB and both pF and ODR. Importantly, MB seemed significantly dependent on the different way of land use, and higher MB content was noted in the soil agriculturally exploited ($p < 0.05$) in contrast to the control area.

Keywords: Microbial Biomass; Soil DNA; Water Potential; Agricultural Activity

1. Introduction

The term of microbial biomass is commonly used to describe the total mass of microorganisms present in soil [1]. The importance of the MB in soil functioning is well recognised [2]. Moreover, MB as an integrative measure of the physiologically active part of the soil microflora is recommended by 8 countries of European Union (e.g., Germany, United Kingdom, Austria, Switzerland), as important factor of soil quality, which is included in soil microbial degradation monitoring program [3].

Soil MB is also considered to be a useful criterion for an early indication of environmental stress [1,4] as variability in microbial communities can precede detectable changes in soil properties. An example is the turnover rate of the MB, which is much faster and takes, e.g., 1 - 5 years, than the turnover rate of total soil organic matter [5]. It is partly due to the large pool of relatively inactive and dormant microorganisms, having the potential to reflect the past [6]. Life in the soil environment is constantly influenced by drying and rewetting cycles, as soils are continually exposed to rainfall, wind and snow [7]. A part of the microbial population dies during each drying-and-wetting cycle resulting in the fluctuation of soil microbial composition [8]. Soil water content as a function of the soil water tension is described by pF curve, which provides information about the ability for water retaining by the soil pores at any given water tension, or conversely, how tightly a water is held between soil aggregates [9]. Therefore, also soil aeration status is strongly depended from pF values. It has been also shown experimentally, that ODR factor satisfactorily reflects the supply of oxygen to the plant roots [10,11]. Oxygen availability is among the most important factors affecting soil microbial activeties [12]. There is a specific gas demand for different soil microbes including bacteria, fungi, and other microorganisms [7]. ODR is affected by several factors. It increases with reducing soil water content or increasing suction up to a certain level and then declines with further depletion of water [13]. Soil environment is the major reservoir of microbial genetic diversity and thus should be particularly protected. The breakthrough in soil biology was the discovery of DNA, which is a carrier of biological information and the best characteristics of every organism. Thus, soil DNA analysis is considered to be important and precise tool towards a better recognition of soil microbial functionality and interrelationships among them.

The size of MB was found to be strongly correlated with content of base cations, base saturation, cation exchange capacity, and organic matter quality [14], as well as with soil bulk density, nutrient contents and phosphatase and invertase activities [15]. However, there is a lack of studies on relationships between MB and such important soil factors like pF or ODR, what may be decisive

for the course of processes responsible for plant development, and soil fertility. Investigation of the effect of human agricultural activity on MB content is also interesting part of the current study. Thus, the aim of the work was the statistical description of the impact of pF, ODR, and human agricultural activities on MB and DNA content in soil.

2. Material and Method

2.1. Soil and Investigated Area Description

The soil used in the experiment was *Mollic Gleysol* (**Table 1**). Soil was sampled in July 2009 from the village Kosiorów, situated in the Wilków community (SE part of Poland) from depths of 0 - 20 and 20 - 40 cm. To make possible an estimation of the effect of different way of land use on MB, soil samples were collected from two neighbouring plots: one of them was agriculturally exploited with systematic fertilization and pasturage (under human activity), whilst the other one was classified as fallow land and used as a control area (without any human impact).

2.2. Assaying of Soil Retention Curves

The instrument used for determining water retention curves was a steel pressure chamber, inside of which a porous plate saturated with water was located. At the bottom, soil samples, continuously exposed to atmospheric pressure, make the hydraulic contact with the porous plate [16]. The chamber was closed and the desired air pressure P was applied, driving away the soil water retained at pressures below P, until equilibrium was reached [16]. Soil samples were collected using plastic containers and placed in an airtight chamber (for 10 days), part of a laboratory set LAB o12 (Soil Moisture Equipment Company, USA), before a pressure was applied. The moisture content was determined via the drying process, for the range of water potentials (0, 1.0, 1.5 and 2.0 pF), corresponding to availability of water usable by microorganisms and plant roots.

2.3. ODR Measurement

After determination of proper pF values, ODR was measured by ODR-meter manufactured by the Institute of Agrophysics, Polish Academy of Soil Sciences (Lublin), using Malicki and Bieganowski [17] method. The ODR technique consists of the measurement of the electric current intensity corresponding to the reduction of oxygen on a platinum cathode placed in the soil and negatively polarized with respect to the reference electrode (calomel). As oxygen is consumed at the microelectrode, more oxygen needs to diffuse radially to the electrode in response to the accumulated gradient. This is analogous to oxygen consumption by respiration of root surface or by microbial respiration. Four platinum wire electrodes (0.5 mm × 4 mm) were placed at the depth of 2 cm and polarized to –0.65 V versus saturated calomel electrode for 4 min. The data were recorded in three replicates, for each sample.

2.4. Microbial Biomass

Soil MB was determined by a fumigation-extraction method using $CHCl_3$ as an agent responsible for the cellular death of microorganisms, according to Joregensen [18] procedure. Concentration of head-space CO_2 released by microorganisms which survived incubation with $CHCl_3$ was measured by a gas chromatograph (Varian CP-3800, equipped with a TCD detector).

2.5. DNA Extraction

Soil DNA was extracted from samples at pF 0 (full water capacity conditions) and pF 2.0 (field capacity conditions), using the GeneMatrix soil DNA isolation kit (EURx 1.4, Poland), according to the manufacturer's instructions. This kit was designed specifically for the rapid isolation of pure, humic-free microbial DNA from soil samples, and guaranteed the proper DNA isolation procedure. Concentration of DNA was determined spectrophotometrically at 260 nm (Shimadzu, UV-1800, Japan).

2.6. Data Analyses

Statistical analyses were performed by means of Statistica 8.0 software (STATSOFT, USA). One-way ANOVA test was used to investigate significant ($p < 0.05$) effect of pF, ODR, DNA content on soil MB quantity. Results of the significance differences analyses are presented in **Table 2** only. In **Figures 1-4** the average values with standard deviations are demonstrated.

Table 1. Basic characteristics of the soil.

Place	Depth (cm)	Granulometric composition (%, dia in mm)				pH (H_2O)	TOC (%)
		1 - 0.1	0.1 - 0.02	0.02 - 0.002	<0.002		
Agriculturally exploited (AE)	0 - 20	87	8	3	2	6.7	22.5
	20 - 40	90	7	2	1	6.4	1.4
Fallow land (FL)	0 - 20	91	6	3	0	6.2	20.1
	20 - 40	95	3	2	0	6.5	1.3

Table 2. Statistically significant relationships between MB and analyzed parameters, N = 12.

Object investigated	Depth (cm)	pF	ODR	c DNA
AE	0 - 20	–0.68*	–0.20 n.s.	0.97**
	20 - 40	–0.53*	–0.52 n.s.	0.99***
FL	0 - 20	–0.94***	–0.83***	0.86*
	20 - 40	–0.74**	–0.59*	0.89*

*, **, ***—indicate significance at the $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; n.s.—not significant difference.

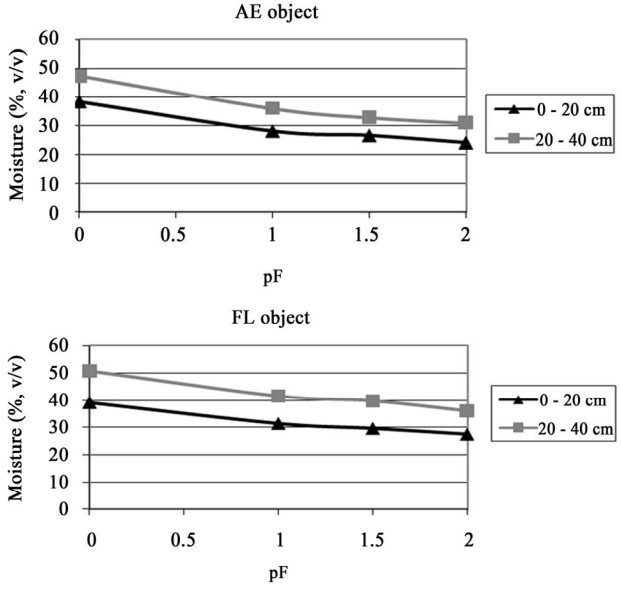

Figure 1. The relationship between soil water content (% v/v) and pF values. The curves are related to two depths (0 - 20; 20 - 40 cm) of the investigated soil areas.

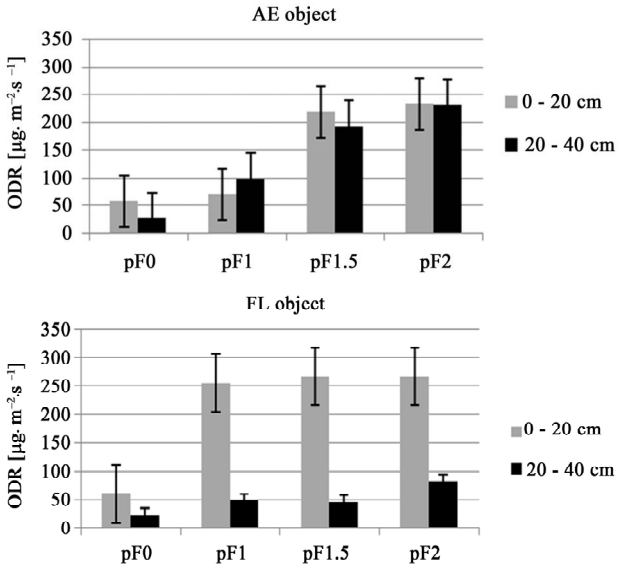

Figure 2. Variability of ODR values as an effect of water potential at two depths of the investigated soil areas. Average values with standard deviations are presented.

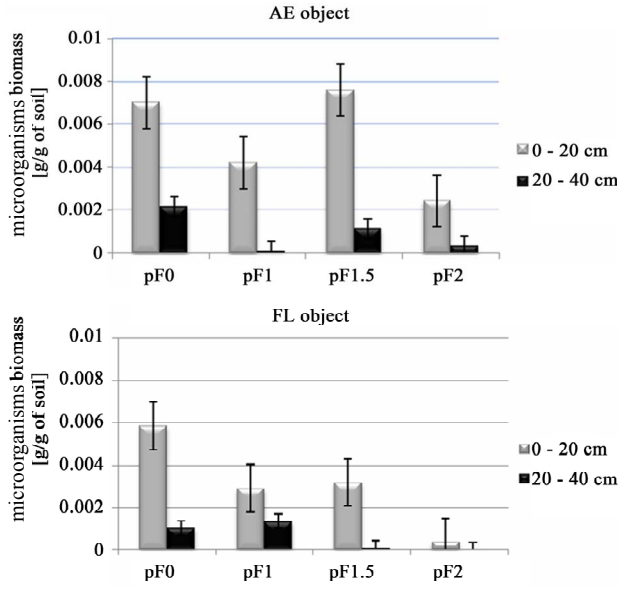

Figure 3. Variability of MB levels as an effect of water potential at two depths of the investigated soil areas. Average values with standard deviations are presented.

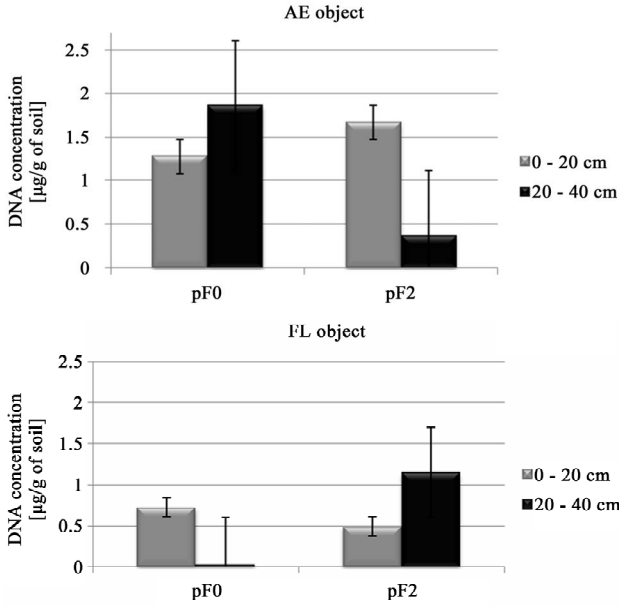

Figure 4. DNA content at two depths of the investigated soil areas as an effect of water potential. Average values with standard deviations are presented.

3. Results and Discuss

3.1. Soil Retention Abilities

An incubation of the soil samples under different controlled moisture conditions altered significantly ODR, MB and DNA concentration. The relationships between soil water content (%, v/v) and pF for the two layers of *Mollic Gleysol*, for both tested areas (AE and FL) are presented in **Figure 1**.

Generally, the investigated soils demonstrated similar abilities to retain water, even though soil from the control area (FL) displayed slightly higher (c.a. 3% more than soil agriculturally exploited) capacity for water keeping. This might be connected with beginning of decay process on AE object, what resulted in looseness of soil structure and higher intensity of mineralization and humification of the soil organic compounds. Moreover, higher ability of holding water in the subsurface layers were noted, and equalled 34% - 47% v/v and 41% - 50% v/v in AE and FL objects, respectively. Similar capability of *Mollic Gleysol* for water maintaining was also indicated [19]. The soil-water interactions are greatly or extremely important to soil fertility and therefore are the subject of interest to agricultural engineers and farmers. Furthermore, information about water holding capacity is important for agronomic and hydrologic characteristic of soils. It expresses, how much water can be stored in the soil for plant use during periods without rain or irrigation [20].

This provides an indication of soil sensitivity to drought and could be used to calculate the probability of occurrence of deep drainage or groundwater recharge process. It was also reported that macropore continuity is very important to the aeration status of the soil, thus the effect of soil compaction on other aeration properties depends on soil hydro-physical status [13,21].

3.2. Oxygen Availability in AE and FL Objects

Based on the performed measurements it was found, that pF constitutes a significant factor ($p < 0.001$) determining ODR levels in the soil environment. Soil desiccation, occurring in the direction from pF 0 to pF 2.0, was the reason of stimulation of ODR (**Figure 2**).

The oxygen availability in relation to the soil water potential indicates, that ODR values at surface layer of AL object fluctuated from 50 to 233 μg $O_2/(m^2 \cdot s)$ at pF 0 and pF 2.0, respectively. At deeper layer (20 - 40 cm) of agriculturally exploited *Mollic Gleysol* ODR ranged between 26 till to 240 μg $O_2/(m^2 \cdot s)$, for as follows pF 0 and pF 2.0. Stronger variety of ODR values in the FL object was observed, we noted values between 60 and 266 μg $O_2/(m^2 \cdot s)$ as in the surface layer, whilst in the subsurface 80% decrease of ODR was found and registered values oscillated from 23 to 81 μg $O_2/(m^2 \cdot s)$, for pF 0 and pF 2.0, respectively. We assume that way of land use and sys- tematically applied ploughing at AE object may be the reason of higher ODR level as well in surface as in sub- surface layer. FL on the other hand, despite the fact of comparable level of ODR in surface layer, in the subsur- face was characterized by rapid reduction of oxygen availability, below 35 μg $O_2/(m^2 \cdot s)$, which is the minimum level of ODR necessary for proper root growth [11,22]. This might be caused by lack of human agricultural activities, as e.g. ploughing may contribute in soil ventilation improvement. On the contrary, [19] Walczak *et al*. (2001) noted tendency for higher oxygen availability in the deeper layers, rather than in surface of the *Mollic Gleysol*, whereas [23] Stępniewska *et al*. (2003) observed analogous trend in the *Eutric Cambisol*.

It may be caused either by methodical limitations, as the water barriers or water films present on the surface of electrode could be broken off [9], or by the differences in granulometric composition of analyzed soil samples (**Table 1**), as the fact, that large granulation favourable for forming of aeration pores was noted in the subsurface layers.

3.3. Soil Microbial Biomass

Soil MB content was also strongly influenced by pF conditions and the way of land use (**Figure 3**). The significantly higher values (0.0077 and 0.0058 g/g of soil) were stated in the surface layers at full water capacity conditions (pF 0) for AL and FL objects, respectively, as compared to the deeper layers of soil. In subsurface layers the reduction of MB content, c.a. 5 times, was observed.

The highest values of MB estimations in AE object were undoubtedly connected with total organic carbon (TOC) content, what favoured the microorganisms' abundance by supplying sources of energy necessary for activity of soil biota and growing crops. This observation is consistent with other studies [24-26]. Registered almost 85% reduction of MB in the subsurface layers come out of distribution of microorganisms in the soil profile, since microorganisms are mostly confined to the surface soil layer owing to better aeration and greater nutrient availability. Anthropogenic activities and soil management in particular, are mostly responsible for disturbing the chemico-physical and biological equilibrium of soil [27]. A particularly serious problem is the decrease in the organic matter content of agricultural soils, which may endanger soil fertility and enhance erosion. The MB, as a small fraction of soil organic matter, is a source and sink of nutrients and controls soil organic matter mineralization [27,28] Fisk and Fahey (2001), analogically at current study noted higher content of MB (by 20% - 30%) at agricultural areas in response to fertilization, than at fallow ones. Also, other findings pointed out a distinct relationship between soil fertility and soil MB, suggesting that MB measurements provide a valid estimate of soil quality [14,29].

3.4. DNA Content

Similarly to MB distribution of DNA concentrations in surface layers were 2 times higher in AE object (**Figure 4**), than in control soil (not under cultivation) at pF 0. Even more differentiation in subsurface layers were stated, where DNA level reached 12 fold higher values in AE, in

relation to FL area. The usage of GeneMatrix soil DNA isolation kit let us to receive 0.4 - 1.8 µg/g and 0.5 - 1.2 µg/g of soil DNA concentrations in AE and FL objects, respectively and revealed that the quality (fragment size and purity) of the extracted DNA was generally very good. However, one should always realize that extraction of DNA from soil samples is never 100% efficient and can vary from a few µg to almost 200 µg DNA per g dry weight of soil. Most of authors, however, reported that the obtained values ranged from 1 to about 50 µg of total DNA per g dry weight of soil depending on the method applied and soil sample studied [30,31].

3.5. Statistical Relationships between Parameters Analyzed

Statistical relationships between MB and investigated parameters (pF, ODR, DNA content, way of land use) described by correlations coefficient (r) are presented in **Table 2**. Significant influence ($p < 0.05$) of tested parameters on MB was found. The positive relationships between MB and DNA content, and negative correlations between MB and soil physical factors like pF and ODR were revealed.

Both our and other studies demonstrated that MB can be highly sensitive to environmental factors. Although, study by [32] Singh and Yadava (2006) confirmed negative correlation between MB and soil moisture, even so prior to our study, rather little attention has been paid to the influence of pF, ODR, DNA content and way of land use on soil MB. Therefore, our work was focused on these relationships and determination of statistical correlations. The positive relationships between MB and DNA content suggest the domination of intercellular DNA form at both AE and FL objects, and MB is considered to be a good factor relating to the total mass of microorganisms present in soil. Our results are supported by the findings of [33] Blagodatskaya *et al*. (2003), who noted that DNA content correlated strongly with the total MB in *Paleosol* soils from Southern Urals ($R^2 = 0.97$), as well as by work of [34] Hartmann *et al*. (2005), who described analogical correlation ($r = 0.91^{***}$). Nevertheless, the interpretation of our results has been challenging because of the lack of enough publications in available literature concerning the determination of r coefficients as a goodness of fit between investigated soil factors and MB. However, determined relationships demand further investigations and selection of the other soil types, for better explanation and confirmation of the obtained correlations.

4. Conclusion

Importantly, the way of land use significantly ($p < 0.05$) influenced on MB and DNA content, and higher content of MB and DNA concentration were noted in the AE ($p < 0.05$), in contrast to the FL area. Significant ($p < 0.05$) positive relationships between soil MB and DNA concentration were revealed, whereas pF and ODR were negatively correlated to MB. Oxygen availability values of 30 - 255 µg $O_2/(m^2 \cdot s)$ and 20 - 80 µg $O_2/(m^2 \cdot s)$ in the AE and FL objects, respectively, suggested higher oxygen availability in the subsurface layer of AE area ($p < 0.05$), what might be connected with human agricultural practices, e.g. traditional regular ploughing, which significantly improves soil aeration status.

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