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Full Length Research Paper

Characterization of microbial community in the selected Polish mineral soils after long term storage

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The effect of 19-years storage period at air-dried condition (4°C) and impact of soils rewetting on microbial presence were studied. The topsoil (0 to 20 cm) of *Mollic Gleysol*, *Eutric Cambisol*, *Rendzina Leptosol*, *Orthic Podzol* and *Eutric Fluvisol* were used in the experiment. It was found that 10-days of soil incubation at full water capacity conditions and room temperature is enough for soil microbial regeneration. The moisture content was determined for a range of water potential (pF) values: 0; 1.5; 2.2; 2.7 and 3.2, corresponding to available water and representing different water availabilities for microorganisms and plant roots. According to the results, soil moisture content significantly increased (P<0.001) the abundance of the total number of bacteria and most probable number (MPN) of ammonia oxidizing bacteria (AOB). Molecular analysis (16S rRNA) shows the dominance of *Betaproteobacteria* genera with the main representatives of *Nitrosomonas*, *Nitrosospira*, *Delftia*, *Comamonas* and *Pseudomonas*, as well as exponent species of *Firmicutes* genera: *Clostridium* and *Ruminococcus*.

Key words: 16S rRNA gene analysis, soil water potential, microorganisms abundance, soil rewetting.

INTRODUCTION

Soil is a complex and dynamic biological system, and still (in 2013) it is difficult to determine the composition of microbial communities in soil (Nannipieri et al., 2003). We should realized that one gram of soil may harbor up to 10 billion microorganisms of possibly thousands of different species (Torsvik and Øvreås, 2002; Schloss and Handelsmann, 2006). As less than 1% of the soils bacteria are cultivated under laboratory conditions (Schloss and Handelsmann, 2006; Janssen, 2006), soil ecosystems are, to a large extent, uncharted. Consequently, soil is a very heterogeneous system that comprises a variety of microhabitats with different physicochemical gradients and discontinuous environmental conditions (Grundmann et al., 2001; Torsvik and Øvreås, 2002; Nannipieri et al., 2003). Each soil micelle surrounded by hydrate surface comprises an individual biotope- the separate environment for microorganisms life. But above all, soils are biologically active: not only are they habitat for living organisms, they are formed by these organism and without their presence their development is hindered (Havlicek, 2012).

Soil microorganisms must adapt to microhabitats and live together in consortia with more or less sharp boundaries, interacting with each other and with other biota. Bacterial activities have been reported to be unevenly distributed in soil, leading to the concept of hot spots that are linked to local, transient available C for microbial growth and activity (Grundmann et al., 2001; Nannipieri et al., 2003; Frąc and Jezierska-Tys, 2011). Hot environments are between the supporting life extreme niches that appear to have maintained some degree of special biotechnological interest (Grundmann et al., 2001; Tomova et al., 2010). The two main locations for active bacteria are believed to be soil pores within surrounding water film, in regions of preferential flow, or alternatively entrapped within soil matrix (Grundmann et al., 2001). Analysis of the spatial distribution of bacteria at microhabitat levels demonstrated that more than 80% of the bacteria were located in micropores (2 to 20 µm) of stable micro-aggregates (Torsvik and Øvreås, 2002; Sey

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et al., 2008). Such microhabitats offer the most favorable conditions for microbial growth with respect to water and substrate availabilities, gas diffusion and protection against predation (Torsvik and Øvreås, 2002). Some results proved that a high diversity of bacteria belonging to the *Acidobacterium* division and *Prostheobacter* were present in small particles, whereas large particles harbored microorganisms belonging to the *Alfaproteobacteria* (Torsvik and Øvreås, 2002).

Over the past two decades, molecular methods, especially 16S rRNA gene sequencing, have become very popular to help identify unknown bacteria (Torsvik and Øvreås, 2002; Nannipieri et al., 2003; Janssen, 2006). In turn, this has led to community analysis using total community DNA extracted from the environment. Polymerase chain reaction (PCR)-based fingerprinting techniques give a higher resolution and provide information about changes in the whole community structure (Torsvik and Øvreås, 2002).

Life in the soil environment is constantly connected with drying and rewetting cycles. Liesack et al. (2000) indicated that alternate flooding and drainage of the soils cause spatial and temporal changes of the soil microbial communities and processes. Water is a critical resource and its availability regulates microbial activity within the soil matrix, thus periods of water limitation may affect microbial communities through starvation (Gleeson et al., 2008). A study by Jones and Lennon (2010) found that microbial communities are structured by species responses to environmental variables that fluctuate through time. Dormancy is one trait that allows species to contend with temporal variability of environmental conditions, and is considered to be a common life history strategy among micorbes (Jones and Lennon, 2010). Rapid rewetting of a dry soil causes microorganisms to undergo osmotic shock, possibly inducing cell lysis and a release of intracellular solutes (Gleeson et al., 2008; Iovieno and Bååth, 2008). Majority of the anaerobic microbes form spores or resting stages, whereas, the asporogenous facultative anaerobes modify metabolism to withstand the toxic stress (Das and Dangar, 2008; Gleeson et al., 2008). Evidently, about 34% anaerobic bacteria can survive up to 2 years in the dry (toxic) period of the flooded soils (Liesack et al., 2000).

According to Gleeson et al. (2008), ammonia oxidizing bacteria (AOB) and nitrifying bacteria are a microbial functional groups influenced by a variety of environmental factors, including water content, that dictate community parameters, that is, numbers, diversity and activity *in situ*. Gleeson et al. (2008) noted also that AOB are well adapted to surviving extreme drought and become active within minutes of rewetting dry soils. These observations were the reason why in the current study, we concentrated on determining AOB and nitrifying groups of soil microorganisms.

Even though, a series of publications claim that ancient DNA from plants, animals, and microbes- even viable bacterial cells, can survive in amber, halite, soft tissue and sediments for up to several hundred million years (Johnson et al., 2007; Hebsgaard and Willerslev, 2009), the knowledge on biodiversity in terrestrial conditions is still scanty. These studies suggest also that nucleic acids can persist over geological timescales (DNA sequences >1 million years old). The long-term survival of bacteria sealed in permafrozen sediments for up to million years have also recently been investigated (Johnson et al., 2007). The study showed evidence of bacteria surviving in samples up to 500,000 years which make this the oldest independently authenticated DNA to date obtained from diable cells. Nevertheless, viable microbial cells were recovered from Siberian permafrost as old as 3 million years (Hebsgaard and Willerslev, 2009).

However, currently the investigations concentrated on biological life in the soil after its long time of storage (about 20 years) are strongly limited. Does it mean that there is no biological life in those types of soils? And what about microbial spores which have potential to be active even after long term of dormancy? Thus, the objectives of the current study were: (1) to determinate selected groups of the soil microorganisms (AOB, nitrifying and general bacteria) which are activated at first after long period storage as a result of soil rewetting, and (2) to determine microorganism's abundance in the different water content conditions.

MATERIALS AND METHODS

Soil description

The Institute of Agrophysics of the Polish Academy of Sciences in Lublin has a collection of soils from territory of Poland. The five soil samples used in the current experiment, were taken in 1990 from the topsoil (0 to 30 cm) of *Mollic Gleysol* (Kolno, 22°42'E, 52°28'N), *Eutric Cambisol* (Tarnowo, 16°44E, 52°27'N), *Rendzina Leptosol* (Bezek, 23°20'E, 50°51'N), *Orthic Podzol* (Kolnica, 17°20'E, 50°45'N), *Eutric Fluvisol* (Zawadka, 21°23'E, 49°54'N), and collected in the Soil Bank resources. Since 1990 till to 2009 (when the samples were taken for our investigations), the soils were stored in darkness, under air-dried conditions in the special, unified containers. The temperature was c.a. 4°C and the room was air-conditioned. The basic characteristics of the soil samples are presented in Table 1.

Determination of soils retention abilities

Soil samples (five investigated types) were collected using plastic containers (height of 4.5 cm, diameter of 2.9 cm), and pre-incubated at the state of flooding for 10 days at 20°C. After that, they were placed in an airtight chamber, part of a laboratory set LAB o12 (Soil Moisture Equipment Company, USA) before pressure was applied. The instrument for determining water curves is a steel pressure chamber, inside of which a porous plate saturated with water is located. The bottom was continuously exposed to atmospheric pressure, soil samples were disposed in a way that ensured hydraulic contact between the sample and the porous plate (Pires et al., 2005). The chamber was closed and a desired air pressure *P* was applied to it, driving away the soil water retained at pressures below *P,* until equilibrium is reached (Pires et al., 2005). The moisture content was determined via the drying process, for

Table 1. The main characteristics of investigated soil materials.

Type of soil	Bank no.	Granulometric composition (%, diameter in mm)							Bulk density (g/cm^3)	pH (in H_2O)	Organic C (g/kg)
		Rock + gravel	1-0.1	0.1-0.05	0.05-0.02	0.02-0.005	0.005-0.002	loam			
Mollic Gleysol	208	4	51	17	10	12	4	2	1.43	7.58	1.43
Eutric Cambisol	308	8	64	12	8	7	1	0	1.55	6.25	0.47
Rendzina Leptosol	563	6	49	7	18	14	4	2	1.40	7.95	0.89
Orthic Podzol	701	4	60	11	5	6	4	10	1.43	7.34	1.06
Eutric Fluvisol	967	0	17	14	25	18	14	12	1.25	5.98	1.41

Table 2. Primer sequences used for PCR.

Name of primer	Primer sequence 5'-3'	Target group	Reference
27f	AGAGTTTGATC(AC)TGGCTCAG	Universal	Osborne et al., 2006
301f	GACTGGGACTTCTGGCTGGACTGGAA	AOB	Norton et al., 2002
CTO189f	CCGCCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGAGRAAAGCAGGGGATCG	Nitryfying bacteria	Kowalchuk et al., 1997
1492r	GGYTACCTTGTTACGACTT	Universal	Frank et al., 2008
302 r	TTTGATCCCCTCTGGAAAGCCTTCTTC	AOB	Norton et al., 2002
CTO654r	CYTTGTAGTTTCAAACGC	Nitryfying bacteria	Kowalchuk et al., 1997

the following water potentials: 0, 1.5, 2.2, 2.7 and 3.2 pF values, corresponding to the range of water available and useful for microorganisms and plant roots.

Microbial abundance

The five investigated soil samples (5 g each, with pF value: 0, 1.5 and 3.2) were suspended separately in 50 ml 0.85% NaCl. The c.f.u. (colony form unit) of the total heterotrophic bacteria were enumerated after 14 days growth on a nutritive agar medium (content [g l^{-1}]: peptone 5.0, beef extract 3.0, agar-agar 12.0) at 25°C. The AOB were recorded after 7 days of incubation on 1% water-peptone medium (content [g l^{-1}]: casein 10.0, NaCl 5.0, Na_2HPO_4 1.5, KH_2PO_4 9.0) at 26°C, whereas the nitrifying bacteria community were determined on Winogradsky's medium (content [g l^{-1}]: distilled water 1.0, $(NH_4)_2SO_4$ 2.0, K_2HPO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, NaCl 2.0, $FeSO_4 \cdot 7H_2O$ 0.4), and the colonies were visualized (pink colour) after 14 days, by flooding the tubes with sulphanillic acid reagent (sulphanillic acid, 8.0 g l^{-1} acetic acid (5 M) and α-naphthayl amine, 5.0 g l^{-1} acetic acid (5 M); 1:1 v/v). Population of microbes growing on solid mediums were expressed as c.f.u./g dry soil, and on liquid mediums as a MPN/g dry soil.

DNA extraction and PCR amplification

Total soil DNA was extracted (from each five soil samples investigated, after its pre-incubation in flooded state) using the GeneMatrix isolation kit (EURx 1.4, Poland), according the manufacturer's instructions. This kit was designed especially for the rapid isolation of pure, humic-free microbial DNA from environmental samples, and it was certain that the isolated DNA is proper for successful PCR amplification of: bacteria, fungi, protozoa, algae, etc.

PCR amplification was based on the method described by Agnelli et al. (2004). The primer set used in this experiment is shown in Table 2. PCR reactions were performed in 50 µl volumes, using PCR Master-Mix (Fermentas), 1 µl of each primer (10 µM), 1 U of Taq DNA polymerase (Fermentas), 5 µl $MgCl_2$, 1 µl BSA (500 µg ml^{-1}), 1 µl 10 mM dNTP's and 3 µl of DNA extracted. The PCR was performed with a BioRad MJ Mini Personal thermocycler with the following reaction conditions: 94°C for 90 s, followed by 33 cycles at 95°C for 20 s, 56°C for 30 s, 72°C for 45 s, and a final extension step at 72°C for 7 min. The PCR products, 5 µl sub-samples, were examined by electrophoresis on 1× TAE agarose gel (1% w/v) with appropriate DNA size standards (Mass Ruler™, DNA Ladder Mix, Fermentas) to confirm the size and approximate quantity of the generated amplicons. PCR products were visualized with ethidium bromide (0.25 µg l^{-1}). The sequencing processes were performed in the Laboratory of DNA Sequencing and Oligonucleotide

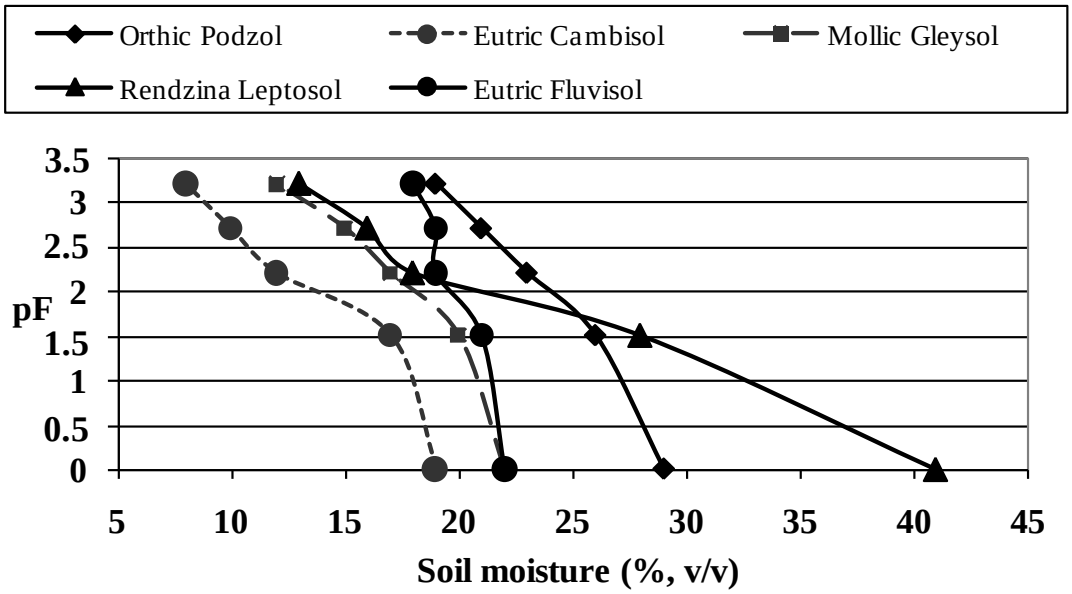

Figure 1. The relationship between soil water content and water potential value– pF curves from the five types of soils investigated.

Synthesis (Institute of Biochemistry and Biophysics, Warsaw, Poland). The received sequences were compared with the closest relatives in the NCBI Gen-Bank database by BLAST program.

Statistical analysis

Statistical analysis was done with Statgraphics 3.0 software (STATSOFT USA). The effect of water potential on microorganisms' abundance was verified by means of multivariate analysis of variance (ANOVA) with Tukey's *post hoc* procedure. When the assumption of ANOVA was not met, the mentioned effect was verified using non-parametric Kruskal–Wallis ANOVA by ranks and detailed pair wise comparisons with U Mann-Whitney's procedure.

RESULTS AND DISCUSSION

Prepared pF-curves, illustrating the relations between soil water content and pF value for the investigated soil types are presented in Figure 1. Soils used in the current study displayed different ability for water retention, thus full water capacity (pF 0) ranged from 19 to 42% v/v, meanwhile pF 3.2 scoped between 8 and 22% v/v of water content (Figure 1). Among soils investigated, *Rendzina Leptosol* demonstrated the highest (14 to 42% v/v), while *Eutric Cambisol* the lowest (8 to 19% v/v) capability of retaining water (for pF 0 and 3.2, respectively). Very high ability for water retention (23 to 29% v/v) was shown by *Mollic Gleysol*, meanwhile *Eutric Fluvisol* and *Orthic Podzol* had similar abilities to retain water (14 to 22% v/v) for as follows: pF 0 and 3.2.

In this study, we examined the short-term effects of soil rewetting on abundance of general soil bacteria, nitrifying and AOB response on different soil water content. Changeability of microorganisms abundance on different values of pF is shown in Figure 2. Water is essential for microbial survival and activity. Thus, we noted the highest microorganisms number (especially AOB group) at full water saturated conditions (pF 0). Significant differences in soil microorganisms abundance ($P<0.01$), as a result of changeable pF values was noted in the case of the total number of bacteria and MPN of AOB. In the case of MPN of nitrifying bacteria, there were no registered fundamental effect of pF on its abundance ($P>0.05$), even though slight reduction of its number with soil drying (pF 1.5) was observed.

By treating each of the soil type individually (Figure 3), it was shown that the highest abundance of the total number of bacteria occurred in *Mollic Gleysol* (21×10^6/g). The other representatives of investigated soils were characterized by similar level of general number of bacteria (2 to 8 × 10^6/g). The second high numerous microorganisms community at investigated soils was AOB, reaching the maximum level of its abundance (11×10^5/g) in *Rendzina Leptosol*. However, quite high number of AOB also in *Eutric Cambisol* (7 × 10^5/g) was noted. *Mollic Gleysol*, *Orthic Podzol* and *Eutric Fluvisol* displayed the same abundance of AOB (1.8 to 2 × 10^5/g). The number of nitrifying bacteria population among soils investigated was highly differentiated. The highest abundance of nitrifies (20 × 10^4/g) in *Mollic Gleysol* and *Rendzina Leptosol* was found. Lowest MPN of nitrifying bacteria was seen in *Eutric Cambisol* (6 × 10^4/g), meanwhile the lowest abundance (0.5 to 1.8 × 10^4/g) was noted in *Orthic Podzol* and *Eutric Fluvisol*, respectively.

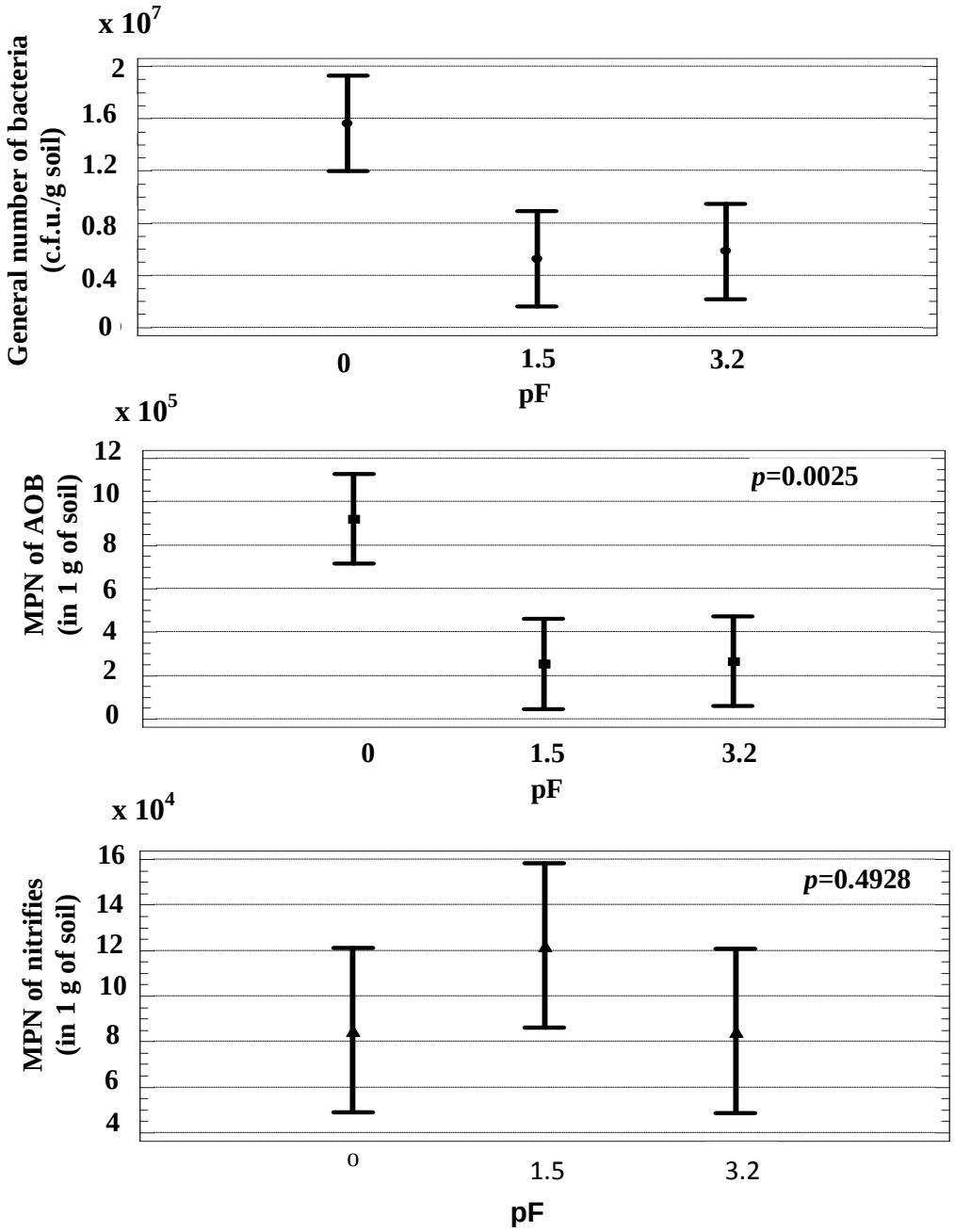

Figure 2. Total number of bacteria, most probable number of ammonia oxidizing bacteria and most probable number of nitrifying bacteria, occurring in 1 g of soil at pF 0; 1.5; 3.2. Results for 5 investigated soil types (n = 15). Mean values with 95% LSD intervals are presented.

In order to determine the soil microorganisms groups activated at first after long period of storage as an effect of soil rewetting, PCR reaction and amplified products sequencing were performed. Positive result of PCR was achieved in the case of each of the five soil types investigated. PCR products vary in length from 1300 to 1500 bp. Comparative analyses of the 16S rRNA sequences revealed the phylogenetic types that compose the soil bacterial community.

The phylogenetic division with closest database match (%) is summarized in Table 3. Only representative sequences with the highest database match (96 to 99% identity) are presented. In the soil types investigated, representatives of genus *Pseudomonas* dominated in the community, as their presence with 96 to 99% identity was noticed in *Mollic Gleysol*, *Eutric Cambisol* and *Eutric Fluvisol*. The other soil types, *Rendzina Leptosol* and *Orthic Podzol* were inhabited by microorganisms

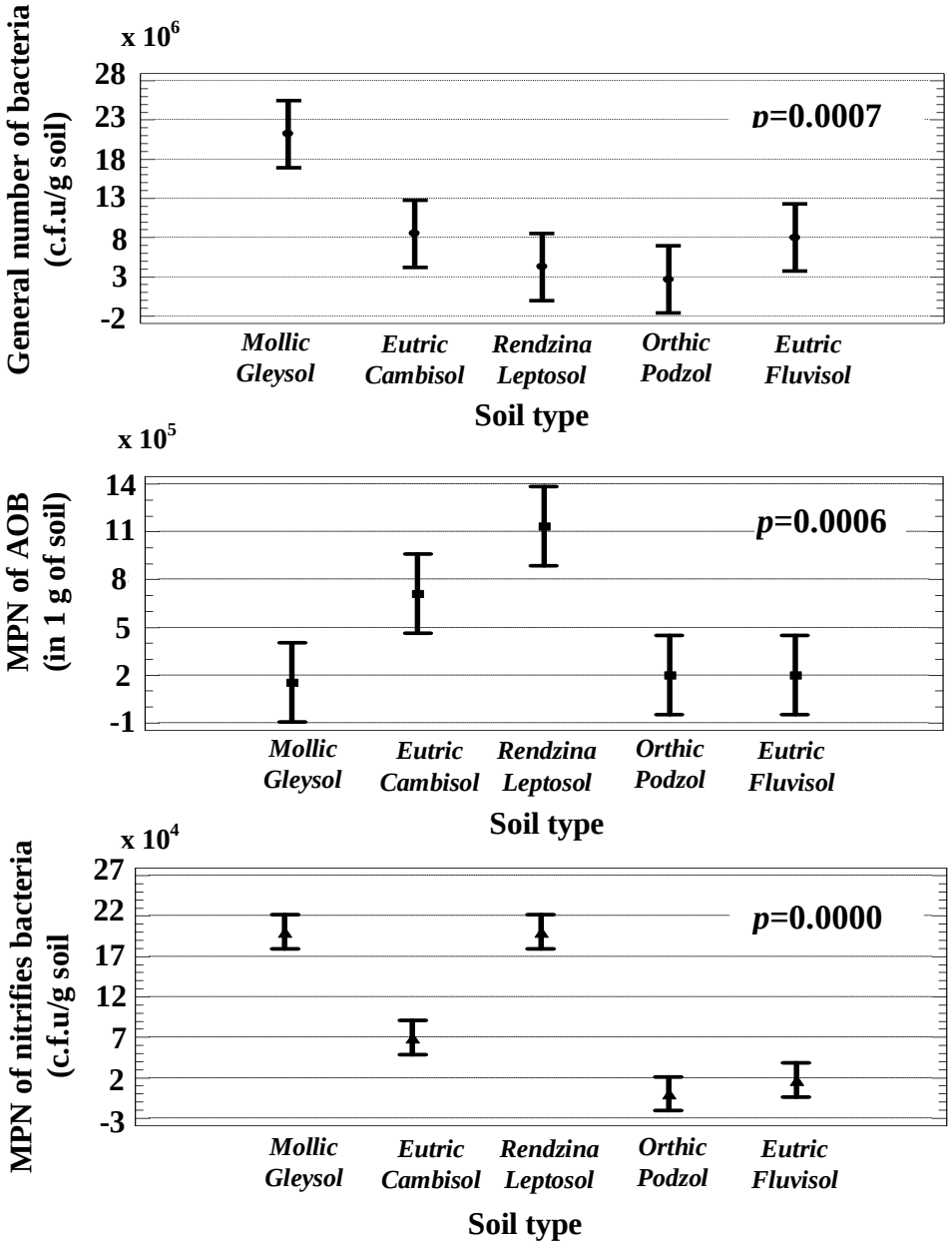

Figure 3. The total number of bacteria, most probable number of ammonia oxidizing bacteria and most probable number of nitrifying bacteria, representing individual soil types. Mean values with 95% LSD intervals (n = 15) are presented.

belonging to *Delftia* and *Clostridium* genus (96 to 99% identity). Moreover, representatives of *Nitrosospira* and *Nitrosomonas* (96 to 97% identity) were found in *Mollic Glyesol*, *Eutric Cambisol* and *Rendzina Leptosol* samples.

DISCUSSION

Soil water content was described by pF curve as a function of soil water tension, which provided an information on the ability to retain water by soil pores at a particular water tension, showing how tightly water was held between soil aggregates. A value of pF which is equal to 0 corresponds to the full aquatic capacity, such that all soil pores are filled with water. pF of 2.2 is typical field water capacity, whereas a pF value as high as 4.2 is considered as wilting point for the plant (Stępniewska and Wolińska, 2006). Our results are in agreement with other studies showing increase of microorganisms number with soil moisture (Rigobelo and Nahas, 2004; Schimel et al., 2007; Finlay and Esteban, 2009). Seasonally-variable

Table 3. Percentage similarity based on the partial 16S rRNA gene sequences to the closest relatives in the NCBI nucleotide sequence database.

Soil sample	Closest relative in NCBI database	Accession number	Identity (%)	Phylogenetic division
	Pseudomonas species	EU111721.1	96	*Betaproteobacteria*
	P. fluorescens	EU373377.1	96	*Betaproteobacteria*
	P. jessenii	FM209480.1	96	*Betaproteobacteria*
	P. toalaassi	FM202487.1	97	*Betaproteobacteria*
	P. putida	EU601175.1	96	*Betaproteobacteria*
	P. clemancea	AM419155.2	96	*Betaproteobacteria*
Mollic Gleysol	*P. teessidea*	AM19154.2	96	*Betaproteobacteria*
	P. gingerii	EU14476.1	96	*Betaproteobacteria*
	P. lindanilytica	EF633256.1	97	*Betaproteobacteria*
	P. chlororaphis	EF620458.1	96	*Betaproteobacteria*
	P. moorei	FM955889.1	96	*Betaproteobacteria*
	Nitrosospira sp. 39-19	AF042170	97	*Betaproteobacteria*
	Nitrosomonas europea	AF058692	96	*Betaproteobacteria*
	P. plecoglossicida	FJ577676.1	99	*Betaproteobacteria*
	P. species	EU747694.1	99	*Betaproteobacteria*
Eutric Cambisol	*P. putida*	EU258552.1	99	*Betaproteobacteria*
	P. fluorescens	FJ588702.1	99	*Betaproteobacteria*
	Nitrosospira multiformis 24C	AF042171	96	*Betaproteobacteria*
	Delftia species	EF440612.1	99	*Betaproteobacteria*
	D. tsuruhatensis	EF421404.1	99	*Betaproteobacteria*
	D. acidovorans	CP000884.1	99	*Betaproteobacteria*
Rendzina Leptosol	*Comamonas acidovorans*	AF078774	98	*Betaproteobacteria*
	C. testosteroni	M11224	97	*Betaproteobacteria*
	Nitrosospira tenuis NV-12	U76552	96	*Betaproteobacteria*
	Nitrosomonas europea	AF073793	96	*Betaproteobacteria*
	Clostridium jejuense	AY494606.1	96	*Firmicutes*
	C. xylanovorans	AF116920.1	96	*Firmicutes*
	C. aminovalericum	M23929.1	97	*Firmicutes*
Orthic Podzol	*C. citroniae*	DQ279737.1	96	*Firmicutes*
	C. phytofermentas	CP000885.1	96	*Firmicutes*
	Ruminococcus sp. M-1	AB125231.1	96	*Firmicutes*
	R. gauvreaui	EF529620.1	96	*Firmicutes*
	R. schinkii	X94965.1	96	*Firmicutes*
	Pseudomonas monteilli	EU512943.1	99	*Betaproteobacteria*
	P. taiwanensis	EU857417.1	99	*Betaproteobacteria*
Eutric Fluvisol	*P. mosselii*	EU921228.1	99	*Betaproteobacteria*
	P. putida	EF620456.1	99	*Betaproteobacteria*
	P. plecoglossicida	FJ493170.1	99	*Betaproteobacteria*

environmental factors, like soil moisture and oxygenation may strongly influence the soil microbial community activity (Rigobelo and Nahas, 2004; Gleeson et al., 2008; Iovieno and Bååth, 2008). It is well known that some microbial populations have more effective stress tolerance mechanisms than others. Gleeson et al. (2008) suggested that AOB, as Gram-negative bacteria, are more sensitive to rewetting stress (less water) than other soil microorganisms, what was confirmed by our results, as we also found higher abundance of AOB as a consequence of soil rewetting. Iovieno and Bååth (2008) indicated that the increase in bacterial growth after

rewetting was largely due to the dormant bacteria becoming active after rewetting. The activation may be a probabilistic event, similar to colony formation (Gleeson et al., 2008; Iovieno and Bååth, 2008). On the other hand, Kaprelyants et al. (1993) defined the dormancy as a reversible state of low metabolic activity,in which cells can persist for extended periods without division; this correspond to a state in which cells are not 'alive' in the sense of being able to form a colony when plated on a suitable solid medium, but one in which they are not 'dead' that is, when conditions are more favourable they can revert to a state of 'aliveness' as so defined.

Presented results confirmed high biological capability of the soils, despite their long term storage (19 years) in the Soil Bank (4°C), as 10-days pre-incubation (at room temperature) with non-limited water availability seemed to be enough for activation of biological life. The microorganisms abundance noted in the current study remained on the comparable level with results published by other researchers (Dąbek-Szreniawska et al., 1996; Taok et al., 2007). Moreover, Szostak et al. (2005) indicated that lower number of nitrifying bacteria than AOB abundance is comfortable, taking into account environmental aspects, as $N\text{-}NO_3$ from nitrification process is less stable that $N\text{-}NH_4$, and consequently an excess of nitrates in the soil leads to $N\text{-}NO_3$ accumulation in the plants, what is dangerous for animals and people health (Szostak et al., 2005).

As a results of sequencing procedure, the representatives of bacterial divisions belonging to *Betaproteobacteria* and *Firmicutes* were found. It is however, not a surprising effect as in literature database e.g. Schloss and Handelsmann (2006) reported that more than 92% of the 16S rRNA gene sequences is assigned to *Proteobacteria* (48.6%) and *Firmicutes* (0.8%). What is more, *Firmicutes* and *Actinobacteria* generally represent a high proportion of the permafrost microbial community, accounting for up to 100% of Canadian high Arctic isolates (Hebsgaard and Willerslev, 2009). Similar findings were demonstrated by Dunbar et al. (2002) and Janssen (2006), who indicated that 16S rRNA genes from soil bacteria are associated with at least 32 phylum-level groups. The dominant phyla in the libraries are: *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi*, *Planctomycetes*, *Gemmatiomonadetes* and *Firmicutes*. Apart from these nine major phyla, members of a number of other phylum-level lineages, such as *Chlamydiae*, *Chlorobi*, *Cyanobacteria*, *Deinococcus-Thermus*, *Fibrobacteres*, *Nitrospira,* as well as non-cultivable representatives of: BRC1, NKB19, OP10, OP11, OS-K, SC3,SC4, termite group I, TM6, TM7, WS2 and WS3 (Janssen, 2006), can be found in the global data set. In this context, our data are compatible with that presented above.

However, we should always realize that our understanding of the links between microbial diversity and soil environment are still poor due to the fact that we cannot determine easily the microbial communities, even if we can detect uncultivable microorganisms by molecular techniques.

Conclusions

As a result of laboratory experiment, it was demonstrated that soil rewetting with non-limited water availability resulted into activation of soil biological life (dormant bacteria become active), despite the long period of soil storage.

Moreover, with changeability in microorganisms abundance posed by different values of water potential corresponding with the spectrum of available water, usefulness for microorganisms was found. General number of soil bacteria (p = 0.0098) as well as MPN of AOB (p = 0.0025) indicated significant negative correlations with pF; however, the abundance of nitrifying bacteria were not essentially correlated ($p>0.05$) with pF, even though they displayed gradual drop as a result of soil rewetting in the range of pF 0 - pF 3.2.

Comparison of biodiversity in the long term storage soils revealed that genera of *Betaproteobacteria* (*Pseudomonas*, *Nitrosospira*, *Nitrosomonas*, *Delftia*) and *Firmicutes* (*Clostridium*, *Ruminoccous*) are common bacterial groups, which quickly become active form of dormancy state after rewetting dry soils.

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