Vol. 79: 127–135, 2017
https://doi.org/10.3354/ame01827

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online May 9

Distribution and activity of benthic bacteria in four lakes in the Bory Tucholskie National Park (Poland)

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ABSTRACT: Benthic bacteria play a major role in the decomposition and transformation of organic matter in water bodies. The imbalance between organic matter input and decomposition leads to its accumulation in the benthos and thus to the conversion of aquatic into terrestrial systems. This is a global problem, exacerbated by anthropic activities. The factors that determine the metabolic activity of bacteriobenthos have been extensively discussed. The complexity and importance of this information inspired us to study the occurrence and activity of benthic bacterial populations in different environmental conditions. We investigated 4 lakes of different trophic status (eutrophic, mesotrophic, oligotrophic and dystrophic) in the Bory Tucholskie National Park, Poland. The results indicated that the abundance of benthic bacteria was not determined by the trophic status of these water bodies; the lowest number was recorded in the eutrophic lake, and was considerably higher in meso- and oligotrophic lakes. Organic carbon oxidation was most intense in the eutrophic lake, despite containing the lowest number of benthic bacteria. Our results show no significant correlation between the total number of benthic bacteria and their respiratory activity, expressed as the rate of organic carbon oxidation. Taxonomic analysis demonstrated that species of the *Firmicutes* and *Proteobacteria* phyla were predominant among benthic strains. In conclusion, our research shows that the abundance and activity of benthic bacterial communities are lake-specific and can be determined by many environmental factors.

KEY WORDS: Benthic bacteria · Taxonomic diversity · Respiratory activity · Lakes

INTRODUCTION

Bottom sediments, defined as material deposited on the bottom of surface waters, are an extremely important element of all aquatic ecosystems (Kalwasińska & Donderski 2005a). Bacteriobenthos seems to play a major ecological role in biogeochemical cycles in these ecosystems (Dzyuban 2003). The concentration and activity of benthic bacteria are several times higher than those of bacterioplankton, which indicates their high ecological and metabolic potential. Moreover, benthic bacteria play a key role in the degradation and transformation of organic matter, and as a result, in the self-purification of water bodies (Kosolapov et al. 2005). In addition, *Bacteria* and *Archaea* constitute the base of the food chain (Radl et al. 2005). The theory of the microbial loop developed by Azam et al. (1983) suggests the existence of specific trophic relationships between dissolved organic matter (DOM) and micro- and macroorganisms. The main part of this trophic pathway consists of the incorporation of DOM into the biomass of bacterial cells, which are then consumed by zooplankton, mainly protozoa. Protozoa, in turn, become a source of food for aquatic vertebrates, thereby transferring the DOM to higher trophic levels. The efficiency of these processes is determined by the size of the bacterial population. Reducing bacterial abundance by nutrient availability is referred to as bottom-up regulation in the trophic system, as opposed to top-down

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regulation, which means reducing bacterial growth by the activity of zooplankton that feed on bacteria. Sanders et al. (1992) observed that the efficiency of the microbial loop depends on the trophic status of the lake. In oligotrophic lakes the availability of nutrients is a key factor limiting bacterial biomass. In contrast, top-down mechanisms are more important in eutrophic lakes. For these reasons, many recent studies have investigated the sediment microbial community (Dzyuban 2002, Siuda & Chróst 2002, Krevs et al. 2006, Suslova et al. 2009, Kiersztyn et al. 2012). Microbial analysis of sediments is difficult due to, among other factors, the heterogeneous, complex structure of the sampling material, and the small size of prokaryotic cells and their ability to adhere to particles of organic matter, which impedes correct assessment of their abundance (Kosolapov et al. 2005). However, despite extensive analyses, researchers still disagree about the factors that determine the abundance and activity of benthic bacteria. It is generally believed that bacterial abundance is determined by the trophic status of the lake. In lacustrine ecology, a lake's trophic status seems to influence the density and diversity of microbial communities. On the one hand, higher numbers of bacteria have been recorded in hypertrophic and eutrophic water bodies, and lower numbers in oligotrophic lakes (Chróst & Siuda 2006). On the other hand, Wobus et al. (2003) noted that the bottom sediments of lakes with different trophic statuses were characterized by a large diversity of bacterial species and different activity of their exoenzymes. As a result, the trophic status of lakes has little effect on the abundance of bacteria in sediments. The above examples indicate the need for new studies to expand the existing knowledge of microorganisms in aquatic ecosystems. Increased awareness of the significance of benthic bacteria encourages researchers to analyze factors that regulate their activity in the environment (Proctor & Souza 2001).

The aim of this study was to determine the variation in abundance, activity and diversity of benthic bacteria in lakes of different trophic status. We hypothesized that higher organic matter content in a lake could favor a greater abundance and activity of benthic bacteria as well as modify their diversity.

MATERIALS AND METHODS

Sampling strategy

The present study was conducted in 4 lakes in the Bory Tucholskie National Park, Poland. It is one of the youngest and smallest national parks in Poland with a total area of 4798.23 ha (Tobolski 2006). Within the park there are 21 lakes with a total water volume of 41.6 million m^3 (Zdanowski 2004). The total area of lakes constitutes 11% of the park, which makes it the third largest total lake area among 23 Polish national parks (Choiński et al. 2012). For our study we selected 4 lakes of different trophic status: eutrophic lake Mielnica (Site I), mesotrophic lake Olbrachta (Site II), oligotrophic lake Głuche (Site III) and dystrophic lake Rybie Oko (Site IV). The last 3 are legally protected. The trophic status of each lake was established by park authorities, based on (1) the content of chlorophyll *a* (chl *a*), (2) nitrogen and total phosphorus in the surface water layer, and (3) water transparency measured with a Secchi disc. The morphometric parameters and location of research stations are presented in Table 1.

Sediment samples were collected from the deepest part of the studied lakes using a sediment corer (custom made tube of plexiglass) to sample from the surface layer of bottom sediments down to 10 cm depth. Triplicate sediment samples were collected at each research site for analysis. All samples were transported to the laboratory in sterile glass containers at ±6°C, and examined immediately. Sampling was conducted following a seasonal cycle from summer 2013 to spring 2015. *In situ* physico-chemical parameters of water from above the bottom sediments at all research sites were measured directly in a sediment corer tube (Table 2). Temperature, pH and oxygen concentration were measured using WTW ProfiLine probes. Electrolytic conductivity was measured using WTW ProfiLine Cond 3210 kit meter.

Table 1. Selected parameters of the 4 research sites in the Bory Tucholskie National Park, Poland. Lakes Olbrachta, Głuche and Rybie Oko are legally protected

Lake	Site	Trophic status	Area (ha)	Depth (m)	Location
Mielnica	I	Eutrophic	11.3	1.0	53° 48.396′ N 17° 31.171′ E
Olbrachta	II	Mesotrophic	2.46	3.5	53° 48.289′ N 17° 31.717′ E
Głuche	III	Oligotrophic	3.3	8.0	53° 49.265′ N 17° 32.635′ E
Rybie Oko	IV	Dystrophic	0.2	3.5	53° 48.828′ N 17° 32.315′ E

Table 2. Physico-chemical parameters of water collected from above the bottom sediments at all 4 research sites. Results are presented as means ± SD (n = 4; 1 season^{-1}); range is given in parentheses

Site	Temp. (°C)	pH	Electrolytic conductivity (µS cm^{-1})	Oxygen conc. (mg dm^{-3})
I	9.6 ± 6.77 (1.8–18.1)	7.5 ± 0.3 (7.1–7.8)	451.3 ± 121.24 (327.5–591.5)	5.7 ± 1.35 (3.8–6.8)
II	10.1 ± 6.66 (3.3–19.0)	6.7 ± 0.77 (6.1–7.8)	193.3 ± 40.71 (149.1–246.0)	3.5 ± 1.45 (2.1–5.5)
III	10.0 ± 5.95 (2.2–16.3)	6.2 ± 0.25 (5.9–6.4)	37.9 ± 13.68 (28.6–57.8)	3.2 ± 0.66 (2.4–3.8)
IV	8.3 ± 4.71 (3.0–17.5)	5.8 ± 0.2 (5.6–6.0)	49.2 ± 10.91 (39.0–64.3)	3.5 ± 1.79 (1.7–5.7)

Abundance of benthic prokaryotes

The total abundance of prokaryotes in the sediment samples was determined using the LIVE/DEAD® *Bac*Light™ bacterial viability kit. Samples were 1:100 diluted in sterile 0.2 µm filtered water. Subsequently, 1000 µl of each sample was put into Eppendorf tubes, to which SYTO®9 fluorochrome was added together with propidium iodide (PI) (150 µl of each; 1:1 ratio). The tubes were shaken to achieve even distribution of the dye and left for 20 min in the dark at room temperature. Then, the samples were filtered through 0.2 µm porosity black membrane filters (Merck Millipore) followed by repeated washing with sterile water. After drying in the dark, the filters were fixed to slides with immersion oil and covered with coverslips. The slides were viewed immediately using an Eclipse E600 epifluorescence Nikon microscope with a B-2A filter set (EX 450-490 nm, DM 505 nm, 520 nm BA). The 20 fields of view prepared for each slide were later archived using Lucia G software. The total abundance of bacteria was calculated based on:

$$x = SN/sR \quad (1)$$

where x is the number of prokaryote cells g^{-1} of fresh sediment, N is the number of cells in a particular field of view, S is the effective area of filtration, s is the analyzed area of the field of view, and R is the sample dilution.

The results were calculated per gram of dry weight (DW) (obtained by drying the sample at 105°C until it reached stable weight) and expressed as cells g^{-1} DW.

The same technique was applied to determine the number of active and damaged benthic prokaryote cells. Based on the results, we determined the percentage of cells with active membrane (MEM+), which stained green after using a fluorochrome SYTO®9 and cells with damaged membrane (MEM–), which stained orange after using PI dye.

Rate of organic oxidation by the benthic prokaryotes

The rate of microbial organic oxidation was calculated on the basis of the respiratory activity of benthic bacteria, measured using the OxiTop (WTW) system. For this purpose, 10 g of sediment from each research station was diluted in 90 ml of sterile water (0.2 µm filtered water). Previously, the water was filtered through cellulose filters (pore size 0.2 µm) to remove prokaryotes and phyto- and zooplankton. Subsequently, the sediment samples were placed in sterile, dark OxiTop bottles with a capacity of 500 cm^3. Five drops of NTH 600 nitrification inhibitor were put into each bottle. A rubber hood insert containing 2 tablets of sodium hydroxide (ca. 0.4 g) for carbon dioxide absorption was then placed in each bottleneck. Finally, the bottles were sealed with OxiTop-C measuring heads. After setting the appropriate parameters, the samples were incubated under constant shaking. Samples were incubated for 24 h at 20°C. The results are expressed as mg of oxygen used per dm^3 of sediment. The amount of organic carbon oxidized by benthic prokaryotes was determined on the basis of the respiratory activity of these prokaryotes. We assumed, according Goossens et al. (1984), that the consumption of 1 mg O_2 enables bacteria to oxidize 0.29 mg organic carbon. The results are expressed as mg C_{org} dm^{-3} d^{-1}.

Identification of aerobic bacterial strains

The taxonomic analysis of culturable aerobic benthic bacterial strains was prepared using BIOLOG technology to obtain metabolic fingerprints. For this purpose, sediment samples were aseptically diluted in 0.85% sodium chloride and streaked out on agar as recommended by the manufacturer. After 18 to 24 h of incubation, 20 single colonies were picked up randomly from each site, used as strains (80 in total) and transferred into IF-A inoculation liquid (density of 90 to 98% turbidance) with sterile swabs. This bacterial suspension was inoculated in the wells of GEN III MicroPlate™ (BIOLOG). The plates were then incubated for 24 h at 26°C. The readings were performed using MICROSTATION™ ID Microlog 3 software.

Statistical treatments

Statistica 12 software was used for statistical analysis of the results according the methods described by Sokal & Rohlf (2012). Due to the fact that the analyzed data did not show normal distribution, which is required for ANOVA, differences between the sets of data were determined using a non-parametric Friedman test. Furthermore, a post-hoc test for Friedman was used to determine the differences within the sets of data. Pearson's correlation coefficient was determined in order to analyze the relationship between the obtained values. The level of significance was fixed at $p = 0.05$.

Table 3. Statistical differences in the total abundance of benthic bacteria between the analyzed datasets based on post-hoc for Friedman test. ns: not significant; *p < 0.05; **p < 0.01; ***p < 0.001

	I	II	III	IV	Spring	Summer	Autumn	Winter
I								
II	***							
III	***	ns						
IV	***	**	ns					
Spring								
Summer					ns			
Autumn					***		***	
Winter					*		**	*

RESULTS

The total abundance of prokaryotes was affected by both trophic status of the lake and season. The results were also affected by physico-chemical parameters of the water. The highest average number of benthic prokaryotes (10.87×10^9 cells g^{-1} DW) was recorded at Site II, in the mesotrophic lake. The lowest number (2.77×10^9 cells g^{-1} DW) was recorded at Site I, in the eutrophic lake. Benthic prokaryotes were more numerous in summer (9.56×10^9 cells g^{-1} DW) than in winter (6.25×10^9 cells g^{-1} DW) (Fig. 1). Statistical analysis showed significant differences among sampling sites and seasons. Significant differences in the abundance of bacteriobenthos were noted between the eutrophic lake and other lakes, and between the mesotrophic and dystrophic lakes. The differences between samplings in spring and summer were not statistically significant (Table 3). Analysis of the relationship between the physico-chemical parameters of water collected from above the bottom sediments and the presence of benthic prokaryotes indicated a strong negative, statistically insignificant correlation ($r = -0.95$) between the number of these prokaryotes and oxygen concentration. Water temperature was the only parameter positively correlated with the number of benthic prokaryotes, but it was statistically insignificant ($r = 0.22$).

As apparent from Fig. 2, biologically active prokaryotes constituted from 90.3% of bacteriobenthos in the mesotrophic lake to 94.3% in the oligotrophic lake. Differences in the activity of prokaryote membranes were recorded among seasons. As can be seen, the highest number of cells with active cytoplasmic membrane (98.2%) was recorded in spring, the lowest (84.0%), in autumn.

The rate of organic carbon oxidation in the sediments of the investigated lakes was highest in the eutrophic lake (24.95 mg C_{org} dm^{-3} d^{-1} on average). It was slowest in the dystrophic lake, which contains

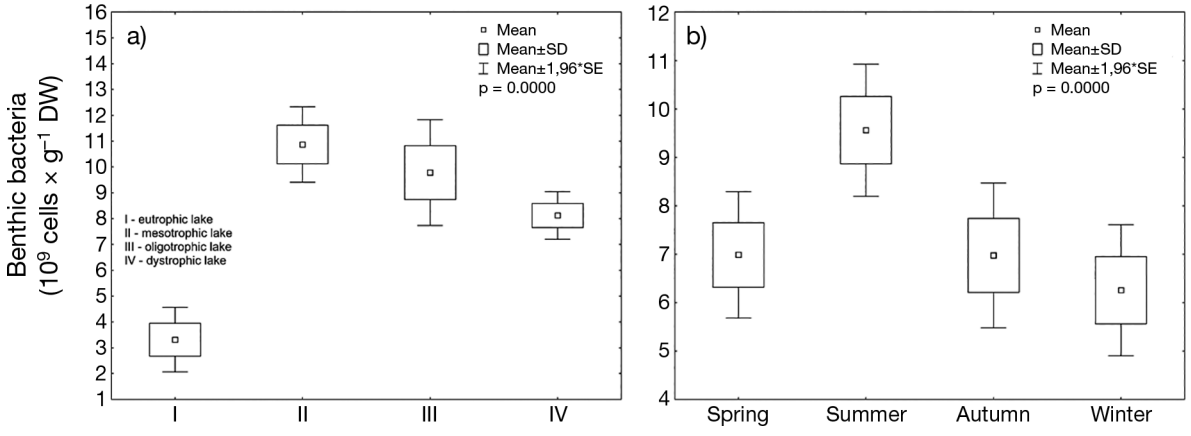

Fig. 1. Total abundance of benthic bacteria at the 4 research sites in (a) all seasons and (b) in the research seasons at all sites; n = 3 for each box. DW: dry weight

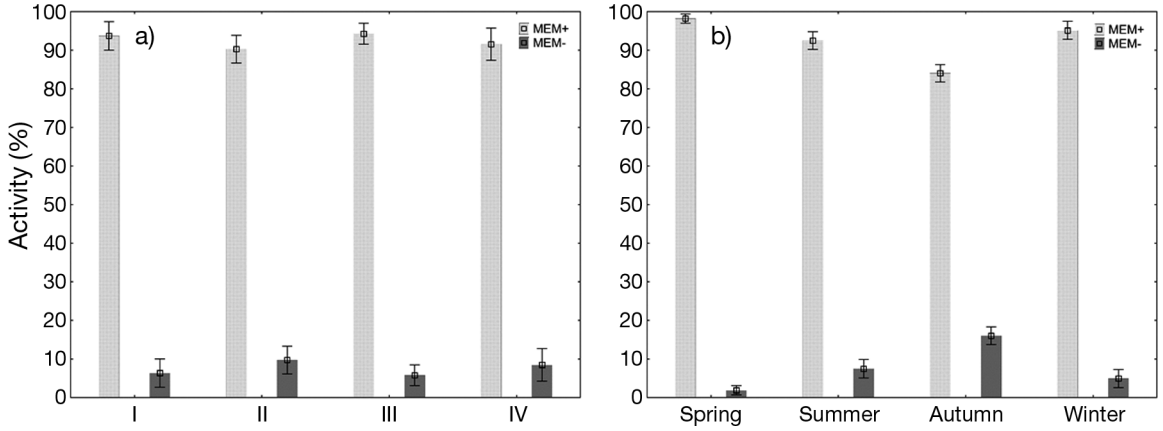

Fig. 2. Activity of the cytoplasmic membrane (MEM) of benthic bacteria at the research sites (a) in all seasons and (b) in the research seasons at all sites; n = 3 for each box

Table 4. Rate of organic carbon oxidation by benthic bacteria (mg C_{org} dm^{-3} d^{-1}). Results are presented as means ± SD (n = 3); range is given in parentheses; na: not applicable

	I	II	III	IV
Spring	28.48 ± 2.29 (25.3–31.12)	8.51 ± 1.36 (7.12–9.83)	14.28 ± 0.58 (13.85–14.94)	7.79 ± 1.9 (6.45–9.96)
Summer	24.89 ± 1.09 (23.77–25.94)	14.66 ± 0.63 (14.01–15.26)	18.82 ± 0.34 (18.48–19.16)	13.09 ± 0.17 (12.89–13.21)
Autumn	21.48 ± 0.36 (21.09–21.79)	18.79 ± 0.47 (18.43–19.32	22.29 ± 0.29 (21.99–22.56)	14.38 ± 0.45 (13.87–14.72)
Winter	na	na	na	na

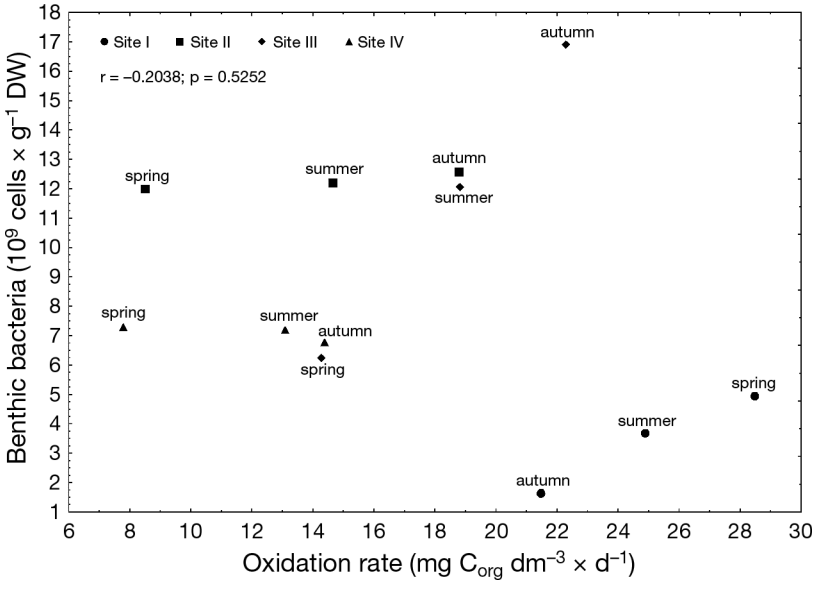

Fig. 3. Correlation between organic carbon (C_{org}) oxidation rate and total abundance of bacteria at the 4 research sites

high amounts of humic substances (Table 4). The reverse proportion was observed in the eutrophic lake, where organic carbon oxidation was the most intense although the total abundance of prokaryotes was lowest. *In situ* measurements of the abundance and respiratory activity of benthic prokaryotes showed no significant correlation between the analyzed parameters. The results of the analysis of the correlation between the described parameters is shown in Fig. 3.

As can be seen in Table 5, the cultured benthic strains (n = 80) comprised 37 species belonging to 22 genera, 18 families, 6 orders and 3 classes. The phylum *Firmicutes* was represented by 15 species, *Actinobacteria* by 9 species, and *Proteobacteria* by 13 species. In the bottom sediments of the studied lakes the following species were the most numerous among the cultured strains: *Serratia fonticola, Serratia liquefaciens* and *Aeromonas allosaccharophila*. *Serratia* species and other strains of the family *Enterobacteriaceae* were isolated mainly from the bottom sediments of the dystrophic lake. Trophic status showed an influence on the taxonomic diversity of cultured bacteriobenthos. The greatest species richness was recorded at Site I (eutrophic lake) and the lowest at Site III (oligotrophic lake).

Table 5. Taxonomic affiliations of cultured bacterial strains and their occurrence in the benthic bacterial populations of the 4 studied lakes

Phylum / Class / Order	Family	Genus/Species	%	I	II	III	IV
Firmicutes							
Bacilli							
Bacillales	*Staphylococcaceae*	*Macrococcus brunensis*	1.6				
		Macrococcus equipercicus	3.3				
		Staphylococcus fleurettii	1.6				
	Paenibacillaceae	*Paenibacillus motobuensis*	1.6				
		Paenibacillus popilliae	1.6				
		Paenibacillus anaericanus	1.6				
		Paenibacillus illinoisensis	1.6				
		Paenibacillus xylanexedens	1.6				
	Incertae sedis	*Exiguobacterium acetylicum*	1.6				
	Balillaceae	*Bacillus carboniphilus*	1.6				
		Bacillus pumilus	3.3				
		Bacillus amyloliquefaciens	1.6				
		Bacillus oleronius	1.6				
Lactobacillales	*Leuconostocaceae*	*Weisella viridescens*	1.6				
	Aerococcaceae	*Eremococcus coleocola*	1.6				
Actinobacteria							
Actinobacteria							
Actinomycetales	*Nocardiaceae*	*Rhodococcus erythropolis*	1.6				
	Actinomycetaceae	*Trueperella bernardiae*	3.3				
	Cellulomonadaceae	*Cellulomonas hominis*	1.6				
	Microbacteriaceae	*Clavibacter michiganensis*	1.6				
	Corynebacteriaceae	*Corynebacterium pilosum*	1.6				
	Brevibacteriaceae	*Brevibacterium linens*	3.3				
	Intrasporangiaceae	*Janibacter hoylei*	1.6				
		Kytococcus sedentarius	1.6				
	Tsukamurellaceae	*Tsukamurella pulmonis*	1.6				
Proteobacteria							
Gammaproteobacteria							
Pseudomonadales	*Pseudomonadaceae*	*Pseudomonas marginalis*	1.6				
		Pseudomonas caricapapayae	1.6				
		Pseudomonas putida	4.9				
	Moraxellaceae	*Acinetobacter baumanii*	3.3				
		Acinetobacter beijerinckii	1.6				
		Acinetobacter johnsonii	1.6				
Enterobacteriales	*Enterobacteriaceae*	*Serratia fonticola*	11.5				
		Serratia liquefaciens	6.6				
		Serratia proteamaculans	4.9				
		Yersinia intermedia	1.6				
		Yokenella regensburgei	1.6				
Aeromonadales	*Aeromonadaceae*	*Aeromonas allosaccharophila*	6.6				
		Aeromonas veronii	1.6				
			Order	5	3	5	4
			Family	9	7	8	5
			Genus	9	7	8	7
			Species	13	10	8	10

DISCUSSION

One of the basic microbial parameters for studying prokaryotes in the natural environment is their abundance, which depends on a combination of biological, physical and chemical factors. In our study, the highest total abundance of prokaryotes was recorded in summer, and the lowest in winter. Our results are in agreement with the results obtained in other studies of benthic as well as planktonic and neustonic prokaryotes (Hadas & Berman 1998, Donderski & Kalwasińska 2003, Kalwasińska & Donderski 2005a,b, Sabae &

Rabeh 2007, Swiontek-Brzezinska et al. 2008). A large number of prokaryotes in summer may result from high temperature, which, in addition to factors such as nutrients and light availability, promotes the growth of phytoplankton. The latter represents a major source of organic matter for the prokaryotes. Our results indicate that for the 4 studied lakes, trophic status apparently did not influence the abundance of bacteriobenthos. The lowest number of benthic prokaryote cells was recorded in the eutrophic lake. Oleynik & Kabakova (1998), studying bacteriobenthos in Danube lakes, showed that its high abundance was determined by the content of organic matter. Our research indicates a significant negative correlation between the number of prokaryotes and oxygen concentration. These results suggest that prokaryotes that are adapted to low oxygen levels can play an important ecological role in the studied sediments. In many natural environments the concentration of oxygen is reduced to very low or even undetectable levels (Stolper et al. 2010). Bacteria with the ability to grow at low oxygen concentrations are phylogenetically diverse and common in nature (Morris & Schmidt 2013). This adaptation expands the diversity of microbial life, extending the number of ecological niches unavailable for aerobic prokaryotes (Han et al. 2011).

The results also suggest that the availability of organic compounds does not determine the growth of benthic prokaryotes. Their abundance seems to depend on the physical and chemical parameters of the environment in which they live. Dzyuban (2003), too, noted that the structural and functional differences between bacteriobenthos from different areas may result from specific physico-chemical properties of a particular aquatic environment.

One method of showing biological activity of microorganisms through biodegradation of available substrates involves measurement of used oxygen using the respirometric technique (Hund & Schenk 1994, Ozimek & Kope 2012). Our results indicate that the rate of organic carbon oxidation in the sediments of the studied lakes was highest in the eutrophic lake, and lowest in the dystrophic lake with a high content of humic compounds. Thus, the low number of benthic prokaryotes in the eutrophic lake did not impair biodegradation processes. High efficiency of these processes could result from the shallow depth of the lake and photosynthesis occurring near the bottom. At the remaining research sites there was no correlation between the activity of prokaryotes and their abundance. Donderski (1983), studying the respiration activity of benthic and planktonic prokaryotes, did not find any relationship between the oxidation rate of various organic compounds and the total abundance of these microorganisms.

Another important indicator of prokaryote activity is the condition of the cytoplasmic membrane (Breeuwer & Abee 2000, Mikš & Warmińska-Radyko 2008). The detection of living cells in bacterial and archaeal populations is based on a popular method using the LIVE/DEAD® *Bac*Light™ bacterial viability kit (Leuko et al. 2004). Regardless of the lakes' trophic status, the number of cells with active membranes in the sediments exceeded 90%, which does not correspond to the majority of results available in the literature. Luna et al. (2002), who analyzed the activity of cytoplasmic membranes of bacteria in the bottom sediments of the Adriatic Sea, recorded 70 to 74% dead cells. Freese et al. (2006) also noted a high percentage of cells with damaged membranes in the eutrophic waters of the Warnow River (Germany). According to these authors, viable cells accounted for only 24% of the prokaryote population. Haglund et al. (2003) recorded a slightly higher number of viable cells (57 to 63%) in the bottom sediments of mesotrophic Lake Erken (Sweden). In our research, the high number of viable cells was probably caused by the specific nature of the studied microbial communities, which were not exposed to external stress factors such as anthropogenic pressure.

The taxonomic structure of the aquatic microbial community can be diversified. According to Zwart et al. (2002) *Proteobacteria* (*Alphaproteobacteria*, *Gammaproteobacteria* and *Betaproteobacteria*), *Actinobacteria*, *Bacterioidetes*, *Cyanobacteria* and *Verrucomicrobia* are predominant among freshwater bacteria. The 16S rRNA gene analyses demonstrate that *Gammaproteobacteria* also dominated in the sediments and surface layers of saltwater environments (Stolle et al. 2011, Jamieson et al. 2013). Our results, based on the identification of cultivable aerobic strains of bacteria, suggest that *Gammaproteobacteria* species were the most numerous in the studied sediments. Our study relied on the BIOLOG automated microbial identification system, which is used with increasing frequency for the characterization of microbial communities, and is based on their ability to oxidize different sources of carbon (Smalla et al. 1998). The evaluation of the BIOLOG system has shown that it guarantees the identification of approximately 93% of cultured strains isolated from water samples (Klingler et al. 1992). Our results confirm the observations of Leon et al. (2012), who examined the taxonomic structure of benthic prokaryotes in oligotrophic lakes in Chile using molecular methods. *Gammaproteobacteria* were represented by aquatic

bacteria of the families *Pseudomonadaceae* and *Enterobacteriaceae*. *Enterobacteriaceae*, which are regular components of the microbiomes of many organisms, are widespread in the environment. They can be isolated from water, sediments and soil (Byappanahalli & Fujioka 1998, An et al. 2002, Hong et al. 2010). The presence of *Enterobacteriaceae* in the studied sediments may be associated with the soils surrounding the lakes and the presence of birds in the park. The greatest species diversity was recorded within the phylum *Firmicutes*. Data concerning the occurrence of this taxon in benthic communities vary greatly. Bacteria of the phylum *Firmicutes* have been identified among the bottom sediments of many aquatic ecosystems such as lakes, seas and even wetlands (Wang et al. 2012, Zhu et al. 2013, Wunderlin et al. 2014). Rarely do they constitute a dominant taxon, although studies by Köchling et al. (2011) indicate that in the bottom sediments of the Gulf of Cadiz (Spain) *Firmicutes* comprised as many as 24 taxa, represented mainly by the classes *Bacilli* and *Clostridia*. A high number of Gram-positive bacteria in the sediments of the studied lakes, mainly of the genera *Bacillus* and *Paenibacillus*, may be associated with the inflow of these soil bacteria from the basin covered with forests. Differences in species composition between the lakes were probably caused by the different trophic status of these water bodies. Similar conclusions were reached by Dai et al. (2016), studying the diversity of benthic and planktonic bacteria in eutrophic and mesotrophic lakes.

CONCLUSIONS

The results of our research led to the following conclusions: (1) trophic status did not determine the abundance of benthic prokaryotes in the 4 studied lakes; (2) the respiratory activity of benthic prokaryotes does not depend on their abundance, but only (to some extent) on the trophic status of the lakes; and (3) taxonomic analysis of the cultured strains isolated from the benthic prokaryote population indicates that it comprised mainly bacteria from the phyla *Firmicutes* (class *Bacilli*) and *Proteobacteria* (class *Gammaproteobacteria*), which indicates that the numbers of Gram-negative and Gram-positive bacteria in the bottom sediments of the studied lakes were similar.

Acknowledgements. The research was financed by the National Science Center, based on DEC-2012/07/N/NZ9/02082 decision. Special thanks to Joanna Studzińska for her help in planning and conducting laboratory analyses.

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Editorial responsibility: Rutger de Wit, Montpellier, France

Submitted: October 7, 2016; Accepted: March 21, 2017
Proofs received from author(s): May 5, 2017