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Impact of Nitrates and Phosphates on Bacterial Communities in Model Hydroecosystems

[1]O.V. Morozova, [1]A.A. Ratushnyak, [1]O.Yu. Tarasov and [1,2]M.V. Trushin

[1]State Budgetary Establishment Research Institute for Problems of Ecology and Mineral Wealth, Use of Tatarstan Academy of Sciences, Daurskaya 28, 420089 Kazan, Russia
[2]Kazan (Volga Region) Federal University, Faculty of Biology and Soil Sciences, Kremlyovskaya 18, 420008 Kazan, Russia

Abstract: Changes in the amount and structure of bacterioplankton in conditions of pollution with nitrates and phosphates were investigated in model mesocosms with natural water and aquatic macrophytes (*Typha angustipholia*) in a seasonal dynamics. The obtained data were suggested on the role of bacterioplankton in the processes of elimination of nitrates and phosphates from the polluted model hydroecosystems. Dynamics of denitrifiers and nitrifiers as well as polyphosphate-accumulating bacteria were found in water and ground of model mesocosms. It was found that aquatic macrophyte favored to the process of bacterial autopurification of the polluted waters.

Key words: Nitrate · Phosphate · Polyphosphate-accumulating bacteria · Denitrifier · Nitrifier · Mesocosm · Hydroecosystem

INTRODUCTION

Bacterioplankton is one of the main groups of hydrobionts participating in elimination of nitrates and phosphates from the polluted aquatic ecosystems [1-5]. Bacteria, able to accumulate phosphorus in the form of volutin granules, actively participate in elimination of this compound from hydroecosystems [1, 3, 4]. It was detected that a synthesis and isolation of polyphosphates by bacteria from the ground directly influences the phosphorus exchange between water and ground [6-8].

It was previously reported that aquatic macrophytes play a significant role in the turnover of nitrates and phosphates [9-11]; however, their impact on bacterioplankton is poorly investigated. Early studies showed important interactions between macrophytes and bacterioplankton [12, 13]. However, these interactions were not studied in details.

The aim of the present work was to investigate the structure of bacterioplankton and its ability to change concentrations of nitrates and phosphates in model mesocosms.

MATERIALS AND METHODS

To simulate mesocosms, tuns of 0.7 and 0.2 m in diameter and height, respectively, with 5 cm oozy bottom were used. The tuns contained natural water (30 L) and plants of *Typha angustipholia*. Experimental variants (overgrown and open mesocosms) were added with $NaNO_3$ (600 mg/L) and Na_2HPO_4 (600 and 30 mg/L) that corresponds to 15 maximum allowable concentrations (MAC) for nitrogen and phosphorus for water regulations of fish-economics values. Samples of water and ground were taken before the addition of biogens and 2 weeks later. Then, samples were taken every month during all vegetation period (June-September, 2010).

Bacterioplankton number and the amount of polyphosphate-accumulating bacteria were assessed using a direct calculation on membrane filters with MBI-4 microscope. For this purpose, water samples were placed to sterile flasks with 2% formalin. Ground samples were taken from depths of 0.5-1 cm. Then, 1 g of the sample was placed to sterile tube with 4 mL of distilled water and 2% formalin. To separate the adsorbed bacteria, standard

Corresponding author: M.V. Trushin. State Budgetary Establishment Research Institute for Problems of Ecology and Mineral Wealth Use of Tatarstan Academy of Sciences, Daurskaya 28, 420089 Kazan, Russia. E-mail: mtrushin@mai.ru.

Table 1: Classification of water quality according to RosHydroMet index

Class of water quality	Level of water pollution	Total amount of bacteria, 10^6 cell/mL
1	Very pure	< 0.5
2	Pure	0.5-1.0
3	Slightly polluted	1.1-3.0
4	Polluted	3.1-5.0
5	Dirty	5.1-10.0
6	Very dirty	> 10.0

procedures were used [14-16]. To intensify the separation, ultrasound device UZV-1,3TTC was used (35 kHz, «Sapphire», Russia) for 6 min (2 min with 30 s interruption). Then, samples were washed with 5 mL of distilled water and the necessary volume was sampled for the desired dilutions in distilled water. All samples were filtered through Vladipor filters (Russia) (pore size of 0.2 microns). Then, the filters were stained with toluidine blue to obtain metachromatic color of volutin granules [17]. Over 500 cells in 10 visual fields were assessed at each filter to check the number of bacterioplankton and bacteria. Considering the number of bacterioplankton, waters in model mesocosms were assessed according to nomenclator of ROSHYDROMET (Table 1). The remaining groups of microorganisms were detected via cultivation on selective growth media. To reveal denitrifiers, Hilti growth medium consisting of two solutions (solution 1 - KNO_3 2 g, asparagine 1 g, H_2O 250 mL, solution 2 – citric sodium 5 g, KH_2PO_4 2 g, $MgSO_4$ 2 g, $CaCl_2$ 2 g, $FeCl_3$ traces, H_2O 500 mL) was used [18]. 2 mL of bromthymol blue (1.6%) was then added to the medium. Nitrates deoxidation and aerogenesis were used as markers for the development of denitrifiers. To reveal nitrifiers (ammonium-oxidizing bacteria), Vinogradskii medium was used (per 1 L of distilled water): $(NH_4)_2SO_4$ 2 g, K_2HPO_4 1 g, $MgSO_4$ 0.5 g, NaCl 2 g, $FeSO_4$ 0.4 g and $CaCO_3$ 5 g [18]. The presence of nitrites was assessed using Griss reagent (10% Griss reagent in 12% solution of glacial acetic acid). To assess the number of denitrifiers and nitrifiers, McCredi table was used [18]. To evaluate an amount of polyphosphate-accumulating bacteria, growth medium with the following content was used: (per 1 L of distilled water): D-glucose 10 g, $(NH_4)_2SO_4$ 0.5 g, $MgSO_4$ 0.3 g, NaCl 0.3 g, KCl 0.3 g, $FeSO_4$ 0.036 g, $MnSO_4$ 0.03 g, $CaCO_3$ 5 g, lecithin 5 g and agar-agar 15-18 g [19]. Litholysis of chalk-stone around bacterial colonies was used to detect bacteria mineralizing organic compounds with formation of orthophosphoric acid. Paired t-tests were used for statistical analysis; $p<0.05$ was considered to indicate significance; data were presented as mean ± SD.

RESULTS

Introduction of nitrates and phosphates resulted in the active growth of bacterioplankton in the beginning of the vegetation period in comparison with control biotopes and biotopes polluted only with nitrates (Fig. 1). By the end of observation period, in overgrown biotopes with load on one and two biogens, water conditions might be characterized as lightly polluted ($2.1\text{-}3\times10^6$ cells/mL), while in open biotopes as polluted (4.2×10^6 cells/mL) (with load on nitrates) or dirty (5.8×10^6 cells/mL) (with load on nitrates and phosphates) (Table 1).

We detected increasing of the amount of ground bacterioplankton in all biotopes just after starting the experiment (Fig. 2). The amount of bacteria reached $3.5\text{-}6.5\times10^9$ cell/g of wet ground that suggests on eutrophication of model hydroecosystems. Total amount of bacteria in the ground was reduced till $1.2\text{-}4{,}7\times10^8$ cell/g of wet ground in the second part of the vegetation period. In the open biotope with load on nitrates and phosphates, the amount of bacteria increased to 2.2×10^{10} cell/g of wet ground and reduced to 7.4×10^8 cell/g of wet ground by the end of observation period (Fig. 2).

The amount of polyphosphate-accumulating bacteria in biotopes with load on nitrates and phosphates reached $1.05\text{-}1.8\times10^6$ cell/mL and reduced 0.56×10^6 cell/mL by the end of the vegetation period (Fig. 3).The percentage of the bacteria was also increased (Fig. 4). The percentage reached 12% and 22% (for open and overgrown biotopes, respectively) during a second part of the vegetation period. The activation of the growth of polyphosphate-accumulating bacteria was also in the ground (Fig. 5). While total amount of bacterioplankton was reduced, the percentage of polyphosphate-accumulating bacteria reached 14% and 31% in the open and overgrown biotopes, respectively (Fig. 6).

In all biotopes, denitrifiers and nitrifiers were found (Table 2). There was a positive correlation link between the growth of polyphosphate-accumulating bacteria and

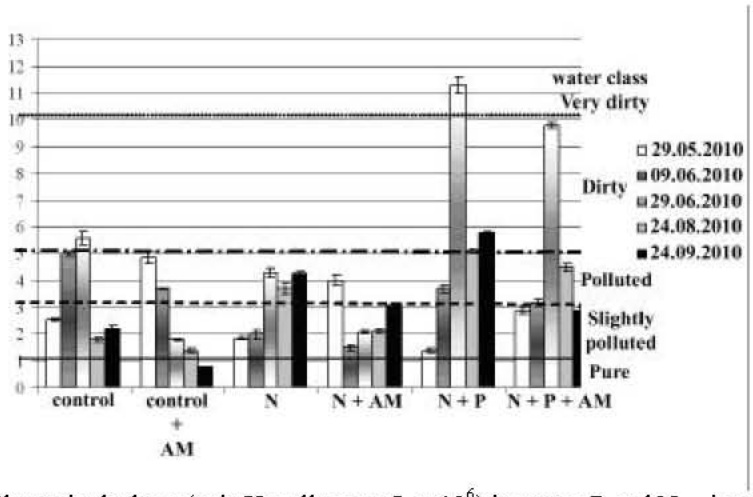

Fig. 1: Total amount of bacterioplankton (axis Y: cells per mL x 10^6) in water. P and N – inorganic phosphate and nitrogen, respectively; AM – aquatic macrophytes. Class of water quality is indicated according to nomenclator of ROSHYDROMET.

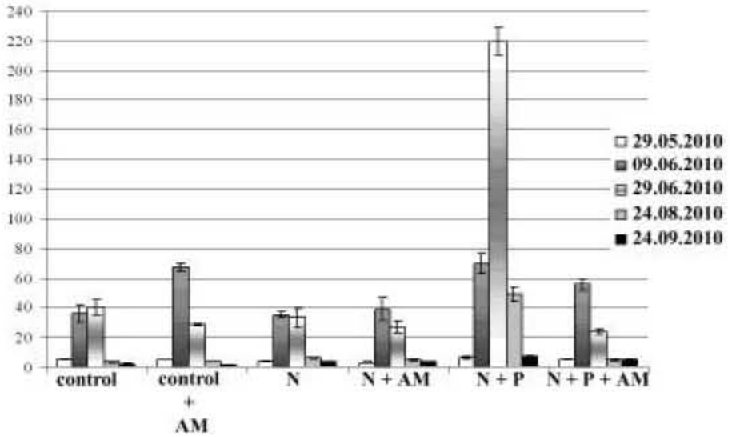

Fig. 2: Total amount of bacterioplankton (axis Y: cells per g x 10^8) of the ground. P and N – inorganic phosphate and nitrogen, respectively; AM – aquatic macrophytes.

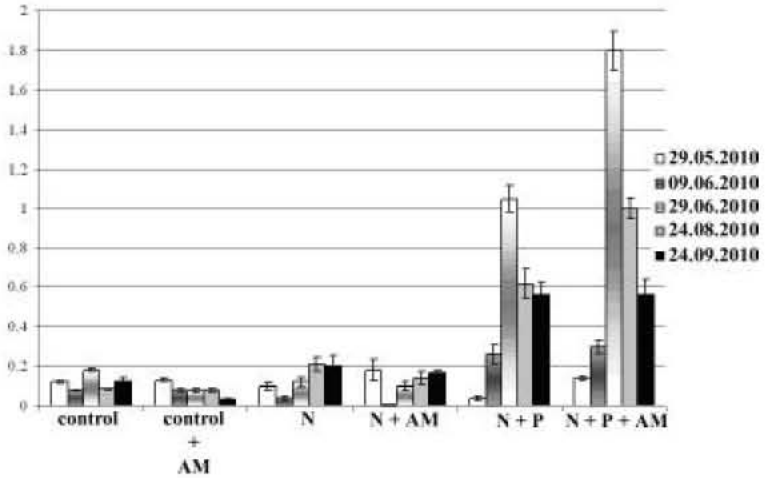

Fig. 3: Total amount of polyphosphate-accumulating bacteria (axis Y: cells per mL x 10^6). P and N – inorganic phosphate and nitrogen, respectively; AM – aquatic macrophytes.

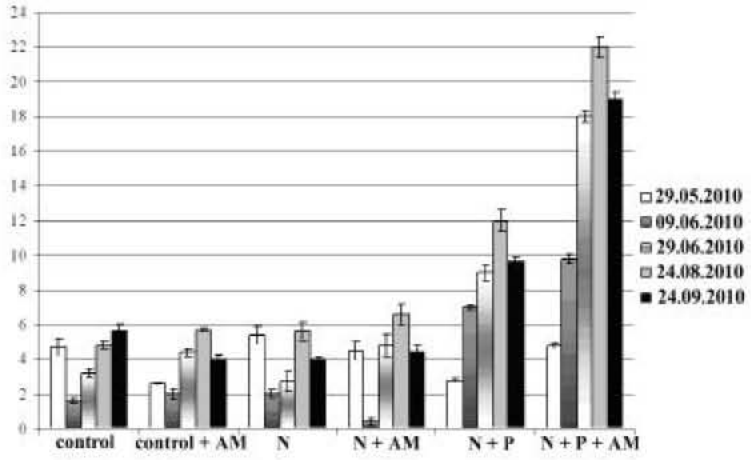

Fig. 4: The percentage of polyphosphate-accumulating bacteria. P and N – inorganic phosphate and nitrogen, respectively; AM – aquatic macrophytes.

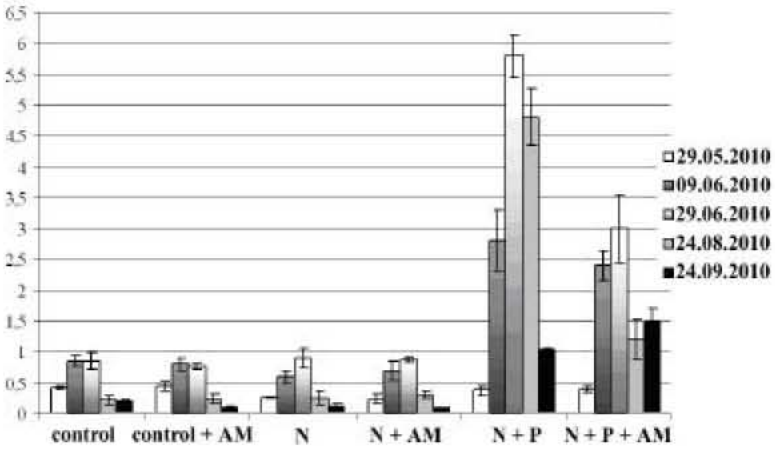

Fig. 5: Total amount of polyphosphate-accumulating bacteria (axis Y: cells per g x 10^8) of the ground. P and N – inorganic phosphate and nitrogen, respectively; AM – aquatic macrophytes.

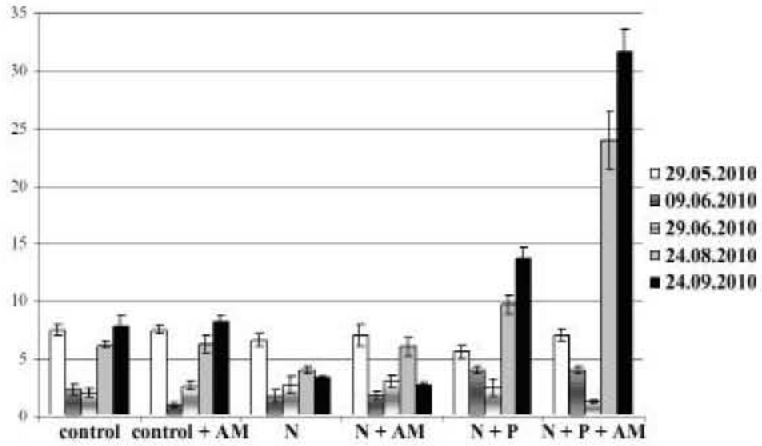

Fig. 6: The percentage of polyphosphate-accumulating bacteria of the ground. P and N – inorganic phosphate and nitrogen, respectively; AM – aquatic macrophytes.

Table 2: The amount of denitrifiers, nitrifiers and phosphate-accumulating bacteria (1000 cells / mL). P and N – inorganic phosphate and nitrogen; AM – aquatic macrophytes

	Variant of experiment					
Type of bacteria	Control	Control + AM	N	N + AM	N + P	N + P+ AM
May 29, 2010 Denitrifiers	0,6	6	6	0,6	6	6
Nitrifiers	6	6	0,6	60	0,0006	60
June 9, 2010 Denitrifiers	0,6	6	600	60	6	60
Nitrifiers	6	60	0,6	60	0,06	6
Phosphate-accumulating bacteria	0.28±0.05	0.76±0.09	0.02±0.01	0	0.04±0.01	0.36±0.07
June 29, 2010 Denitrifiers	0,6	60	6	6	0,6	6
Nitrifiers	600	60	600	600	60	600
Phosphate-accumulating bacteria	0.157±0.01	0.49±0.06	1.3±0.07	1.6±0.15	1.3±0.12	10±1.25
August 24, 2010 Denitrifiers	0,6	0,6	6	60	6	60
Nitrifiers	60	60	60	60	60	600
Phosphate-accumulating bacteria	8±0.87	1.1±0.11	0.2±004	1±0.11	7±1.16	9±1.12
September 24, 2010 Denitrifiers	6	6	0,5	2,5	2,5	2,5
Nitrifiers	60	0,6	6	60	0,6	6
Phosphate-accumulating bacteria	0.3±0.08	1±0.12	0.5±0.14	0.1±0.01	28±2.08	2.3±0.32

Table 3: The content of phosphates (mg/L) in experimental mesocosms in seasonal dynamics. P and N – inorganic phosphate and nitrogen; NW – natural water, AM – aquatic macrophytes

	Experimental variant					
	N + P		N		Control	
Date of observation	NW	AM	NW	AM	NW	AM
1.06.	<0.05	<0.05	<0.05	<0.05	<0.05	<1.0
3.06.	13.3	17.1	<0.05	0.07	<0.05	0.07
9.06.	0.52	0.86	<0.05	<0.05	<0.05	<0.05
21.06.	0.86	0.14	<0.05	<0.05	<0.05	0.05
29.06.	0.51	0.05	<0.05	<0.05	0.09	0.1
23.08.	0.06	0.89	<0.05	0.06	0.05	<0.05
22.09.	<0.05	<0.05	<0.05	<0.05	0.06	<0.05

nitrifiers in biotopes with load on two biogens (coefficient of correlation – 0.8). Bacteria taking phosphorus from organic compounds were also increased that suggest on enhanced amount of organic molecules in the biotopes (Table 2).

DISCUSSION

The obtained data suggest on the active role of bacterioplankton in processes of elimination of nitrates and phosphates in the polluted model ecosystems. At high concentrations of phosphates, polyphosphate-accumulating bacteria were very active. The addition of nitrates resulted in activation of denitrifiers. It is interesting to note here that some bacteria may show denitrification activity even in conditions of aerobiosis [20-22]. As was stated above, there was a positive correlation between the growth of nitrifiers and polyphosphate-accumulating bacteria. Both processes (accumulation of phosphates and denitrification) need the presence of oxygen [23-25]. The detected correlation suggested on favorable conditions in the biotopes for these simultaneous activities. Moreover, some nitrifiers may accumulate polyphosphates [26-28].

Similarly, in the ground conditions of eutrophication and owing to the presence of a large amount of organic compounds, heterotrophic bacteria could utilize organic substrates. During autopurification (when the amounts of organic compounds and biogenic elements tended to reduce) the function of bacteria with volutin granules was evident in retention of phosphorus inside the planktonic community within the ground ecosystem. It was detected in some research works that in eutrophic hydroecosystems polyphosphates are accumulated

mainly by phytoplankton, therefore bacteria do not play any significant role in elimination of phosphorus from water and ground [29, 30]. It is also known that there is a negative correlation between the density of macrophytes and phytoplankton biomass [9, 12, 31]. It is reasonable to suggest that bacterioplankton of the overgrown hydroecosystems plays a main role in the accumulation of polyphosphates (especially in autopurification) due to the lack of phytoplankton biomass necessary for phosphorus accumulation.

Thus, there is limited data on key mechanisms for phosphate and nitrate dynamics in hydroecosystems. The investigation of bacterial structure may be important for clarification of the autopurification processes.

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