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Effect of Organic Phosphorus and Nitrogen Enrichment of Mesotrophic Lake Water on Dynamics and Diversity of Planktonic Microbial Communities – DNA and Protein Case Studies (Mesocosm Experiments)

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Abstract

Effects of mesotrophic lake water enrichment with organic phosphorus and nitrogen substrates (DNA and model protein, bovine serum albumin – BSA) on dynamics and diversity of natural microbial communities (bacteria, heterotrophic nanoflagellates, ciliates) were studied in mesocosm experiments. Simultaneous enrichment with DNA and BSA strongly increased the abundance and biomass of all studied groups of microorganisms and induced changes in their morphological and taxonomic structure. The increased participation of large heterotrophic nanoflagellates cells (larger than 10 μm) in their total numbers and shifts in taxonomic and trophic structure of the ciliates, from algivorous to small bacterivorous, species were observed. Grazing caused changes in bacterial size distribution in all enriched mesocosms. Large (10–50 μm) filamentous bacteria significantly contributed to the total bacterial numbers and biomass. Pronounced increase in populations of β- and γ-*Proteobacteria* was found in lake water enriched with organic P and N sources, whereas α-*Proteobacteria* did not change markedly in the studied mesocosms. DNA additions stimulated the rates of bacterial secondary production. BSA shortened the rates of bacterial biomass turnover in lake water. Relatively high and constant (~ 30%) percentage contribution of active bacteria (MEM+) in two mesocosms enriched with DNA and DNA+BSA suggested the important role of nucleic acids as a source of phosphorus for bacterial growth, activity and production. Numerous and statistically significant correlations between bacteria and protists indicated the direct and selective predator-prey relationship.

K e y w o r d s: organic P and N sources, microbial diversity, lake water

Introduction

Numerous investigations have suggested that origin, chemical composition and availability of organic matter, together with others environmental factors, such as water temperature, pH and nutrients, influence the development, activity and composition of microbial loop and diversity of bacterial communities (Münster and Chróst, 1990; Cottrell and Kirchman, 2000; Kritzberg *et al.*, 2006). Dissolved organic matter (DOM) constitutes more than 90% of the total organic matter in natural waters and is the major source of nutrients and energy for heterotrophic microbial activities (Münster and Chróst, 1990). DOM is a mixture composed of a variety of organic compounds such as amino acids, peptides, carbohydrates, proteins, nucleic acids and refractory compounds of various structures (Münster, 1984; Münster and Chróst, 1990). The concentrations of easily utilizable DOM in freshwater environments are very low and limit the growth and production of heterotrophic bacteria. Heterotrophic microorganisms play a key role in the decomposition and transformation of organic matter within the microbial loop (Chróst *et al*., 1989; Chróst and Siuda, 2006). Mesocosm studies have shown that heterotrophic bacteria are the heterogeneous population composed of different bacterial groups, that are highly dynamic and can differ strongly in their response to resource availability (different organic substrates and inorganic nutrients) and to food web

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structure (Cottrell and Kirchman, 2000; Fisher *et al*., 2000; Lebaron *et al*., 2001; Joint *et al*., 2002). Thus, the trophic interactions between dissolved organic matter, bacteria and others microbial loop organisms are crucial for the carbon cycle, functioning and an ecological stability of aquatic ecosystems (Chróst and Siuda, 2006).

Proteins are one of the most common nitrogen rich constituents in DOM fraction, and they are a source of the most important easily utilizable nitrogen, carbon and energy for aquatic microheterotrophs (Williams, 1986). Especially free amino acids derived from proteins are significant part of organic nitrogen in waters, which are preferentially utilized by bacteria (Hollibaugh and Azam, 1983).

Numerous investigations report that dissolved DNA containing plentiful carbon, nitrogen and phosphorus, may serve as nutrient source for bacterial growth after hydrolysis by both cell-associated and dissolved nucleases of bacteria (Paul *et al*., 1987; Jørgensen and Jacobsen, 1996; Siuda and Chróst, 2001; Chróst and Siuda, 2006). In freshwaters, low contribution (less than 0.015%) of carbon derived from dDNA to the total DOC pool diminishes the role of dDNA in carbon cycle, whereas it may be important source of N and P for microorganisms (Siuda *et al*., 1998). Results obtained by Chróst (2002, 2004) indicated that free, extracellular nucleic acids (dsDNA, ssDNA, RNA) after microbial enzymatic hydrolysis and dephosphorylation (mainly by 5'nucleotidase) constituted an important source of inorganic phosphorus for planktonic microorganisms in Polish Mazurian lakes. Depending on the trophic status of a lake, phosphorus bound in nucleic acids contributed from 51 to 92% (DNA – from 11 to 30%) to the pool of dissolved organic phosphorus in lake water.

The major aim of the study was to investigate the effect of mesotrophic lake water enrichment with DNA and BSA (Bovine Serum Albumin) on numbers, biomass and composition of microbial communities, *i.e.* bacteria, heterotrophic nanoflagellates (HNF) and ciliates, as well as to identify the trophic interactions between them. During our mesocosm experiment we tested the hypothesis that the organic matter-rich water habitats and the rates of microbial and biochemical processes are the main mechanisms generating diversity of microbial communities within the microbial loop. The rates of microbial regeneration of mineral nutrients such as C, N and P from the organic matter pools affected species diversity of the phytoplankton communities and their photosynthetic activity in the organic matter supply of microheterotrophic activities within microbial loop. We assumed that DNA may play a significant role as a crucial source of phosphorus, whereas albumin as source of nitrogen and carbon for microorganisms in freshwater environments.

Experimental

Materials and Methods

Sampling. The mesocosm experiment was conducted during summer stratification period in July. Natural lake water (1200 l) was taken from the mesotrophic Lake Kuc (Mazurian Lake District, northeastern Poland). Lake Kuc is a typical dimictic lake with marked summer and winter stratification. Basic morphological and physico-chemical parameters of the lake were described by Chróst and Siuda (2006). Lake water was taken from the pelagial zone at the deepest site of the lake, from the upper trophogenic water layer corresponding to maximum visibility of the Secchi disk. Water samples from each sampling depth (at 0.5 m intervals) were mixed together and treated as a representative sample for the studied lake.

Mesocosm experiments. In order to determine the influence of organic matter supplementation on changes in microbial (bacterial and protistan) communities, four experimental mesocosms filled with 300 l of lake water were used. First mesocosm without any manipulations served as a control. The second mesocosm was supplemented with deoxyribonucleic acid (DNA, final conc. 50 ± 2 μg l^{-1}), the third mesocosm received a Bovine Serum Albumin (BSA, final conc. 5±0.5 mg l^{-1}). A mixture of DNA and BSA was added to the fourth mesocosm in the above concentrations. The concentrations of chlorophyll$_a$ (2.7±0.3 μg l^{-1}), DOC (13.3±0.3 mg l^{-1}) and DNA (0.5±0.1 μg l^{-1}) in lake water were measured at the sampling time. At the beginning of the experiments (day 0) organic substrates were added and their concentrations were controlled every day of the experiment. The losses of DNA and BSA concentrations caused by *e.g.* bacterioplankton utilization were successively filled up in order to maintain initial enriched concentrations. After DNA and BSA additions to experimental mesocosms 24-h period was kept in order to stabilize chemical conditions of mesocosms water. Water samples (10 l) were taken from each mesocosms to analyze chemical and biological parameters on 1, 3, 6, 8, and 10 day of the experiment.

Physical and chemical analyses. Temperature, pH, conductivity and oxygen concentration were measured in lake water and in each mesocosm with an YSI 6600-meter (Yellow Spring Instruments, USA). Chlorophyll$_a$, extracted with 98% acetone, was measured using a TD-700 fluorymeter according to Arrar and Collins (1997). The concentration of BSA was measured spectrofluorymetrically (Shimadzu RF 1500) using Albumin Fluorescence Assay Kit (Fluka, Germany). The concentration of DNA by means of PicoGreen® (Invitrogen, USA) was determined fluorometrically (Sambrook *et al.*, 1989) according to Turner

Biosystem protocol (http://www.turnerbiosystems.com/doc/appnotes/998_2630.php). Dissolved organic carbon (DOC) concentrations were determined in water samples, filtered through 0.2-μm pore-size polycarbonate membrane filters (Millipore), according to NPOC assay using a Shimadzu TOC 5050 carbon analyzer.

Bacterial numbers, biomass and size distributions. Triplicate water samples were preserved with 37% formaldehyde (final concentration 2%). Subsamples of 1 ml were stained with DAPI (4'6-diamidino-2-phenylindole, final concentration 1 μg ml^{-1}), filtered through a 0.2 μm pore-size black polycarbonate membrane filters (Millipore) and enumerated by epifluorescence microscopy (Porter and Feig, 1980). Bacterial biomass (BB) was calculated by converting DAPI-stained bacterial cell volume to carbon units using the biomass conversion factor of 250 fg C μm^{-3} (Psenner, 1993). Variations in DAPI-stained bacterial cell length (Pernthaler *et al*., 1996) were used to subdivide bacterial numbers and biomass into three size classes: small (0.2–1.0 μm), medium (1.0–2.0 μm) and large (>2.0 μm) according to Lebaron *et al*. (2001).

Percentage contribution of active bacteria with intact membrane (MEM+). In order to determine the numbers of active bacteria with intact membrane (MEM+) LIVE/DEAD *Bac*Light Bacterial Viability Kits were used (Schumann *et al*., 2003). Triplicate samples of water were preserved with 25% glutaraldehyde (final concentration 4%). For 1 ml-subsamples mixture of two *Bac*Light Kits stains: SYTO 9 and propidium iodide was added (1:1 ratio, both dyes final concentration 0.15%), then incubated for 15 min at the room temperature in the dark, filtered through a 0.2 μm pore-size black polycarbonate membrane filters (Millipore) and enumerated by epifluorescence microscopy. The percentage contribution of MEM+ bacteria was calculated as a ratio of MEM+ to the sum of MEM+ and MEM – bacterial cells.

Bacterial cells enumeration and measuring. Between 500 and 1000 DAPI and LIVE/DEAD-stained bacterial cells in at least 10–20 digital images of each filter were counted and measured by automated image analysis system according to Psenner (1993). It consisted of an epifluorescence microscopy (Nikon ECLIPSE E 400 with filter set) equipped with highly sensitive digital camera (Nikon DXM 1200F, 12 MP), and linked to a personal computer with a software LUCIA General v. 4.82 (Laboratory Imaging, Prague, Czech Republic). Images of stained cells were recorded with camera and processed by the image analysis software.

Fluorescent *in situ* hybridization (FISH). Triplicate water samples for community analysis (5 to 10 ml) were fixed with freshly buffered prepared paraformaldehyde (PFA, pH 7.4, final concentration 2%). Subsamples were filtered through a 0.2 μm pore-size white polycarbonate membrane filters (Millipore), rinsed twice with 5 ml of sterile water, dried at the room temperature, and stored at the temperature of –20°C. Whole-cell *in situ* hybridization of sections from polycarbonate filters were performed with the oligonucleotide probes: EUB338 (Amann *et al*., 1990), NON338 (Wallner *et al*., 1993), ALF968 (Neef, 1997), BET42a, GAM42a (Manz *et al*., 1992) and CF319a (Manz *et al*., 1996) as described previously by Pernthaler *et al.* (2001). Oligonucleotides labeled with the cyanine dye CY3 were synthesized by Interactiva (Ulm, Germany). After FISH, the filters were air dried and mounted on glass slides in a previously described mix amended with DAPI (final concentration 1 μg ml^{-1}) (Pernthaler *et al*., 2002). Bacterial cells on the filter sections were observed with an epifluorescence microscopy (BX 51, Olympus) equipped with filter sets for DAPI (Ex 330–380 nm, DM – 400 nm, BA – 420 nm), and for CY3 (Ex 450–490 nm, DM – 505 nm, BA – 520 nm). The fractions of FISH-stained bacteria in at least 1000 DAPI-stained bacterial cells per sample were quantified.

Bacterial production (BP) and biomass turnover rate (BTR). Bacterial secondary production (BP) was determined with the [^{3}H]-methyl-thymidine ([^{3}H]TdR) incorporation method (Chróst and Rai, 1994). The total BP rates and BP rates in two size fractions of bacterial cells: <1.0 μm (free-living) and >1.0 μm (large free-living and attached) were determined. In order to measure free-living BP rates water samples were filtered through a 1.0 μm pore-size polycarbonate membrane filters (Millipore). Triplicate samples of water (5 ml) and 37% formaldehyde-stopped blanks were incubated with 0.1 ml [^{3}H]TdR (spec. activity 60 Ci $nmol^{-1}$, final concentration [^{3}H]TdR in assays 16.68 nmol l^{-1}, MP BIOMEDICALS) in the dark at *in situ* temperature for 60 min, and finally fixed with 37% formaldehyde (final concentration 4%). After 30 min of cold (0°C) 60% trichloroacetic acid (TCA, final concentration 20%) at 0–1°C precipitation, the TCA-precipitates were collected on 0.2 μm pore-size cellulose nitrates membrane filters (Sartorius), and rinsed three times with 5 ml 5% cold TCA. Filters were placed in scintillation vials, dissolved with 5 ml of high-capacity scintillation cocktail (Rotiszint 2211), and assayed in scintillation counter (Wallac 1400 DSA) using the external standard channel ratio model. The amount of [^{3}H]TdR incorporated into bacterial DNA was converted to bacterial cell production using the conversion factor of 1.25×10^6 cells $pmol^{-1}$ TdR (Chróst and Rai, 1994). Bacterial cell production was transformed to bacterial organic carbon production using the conversion factor of 19.8 fg C $cell^{-1}$ (Lee and Fuhrman, 1987).

Bacterial biomass turnover rate (BTR) was calculated as a ratio of bacterial biomass to bacterial production according to Chróst and Faust (1999).

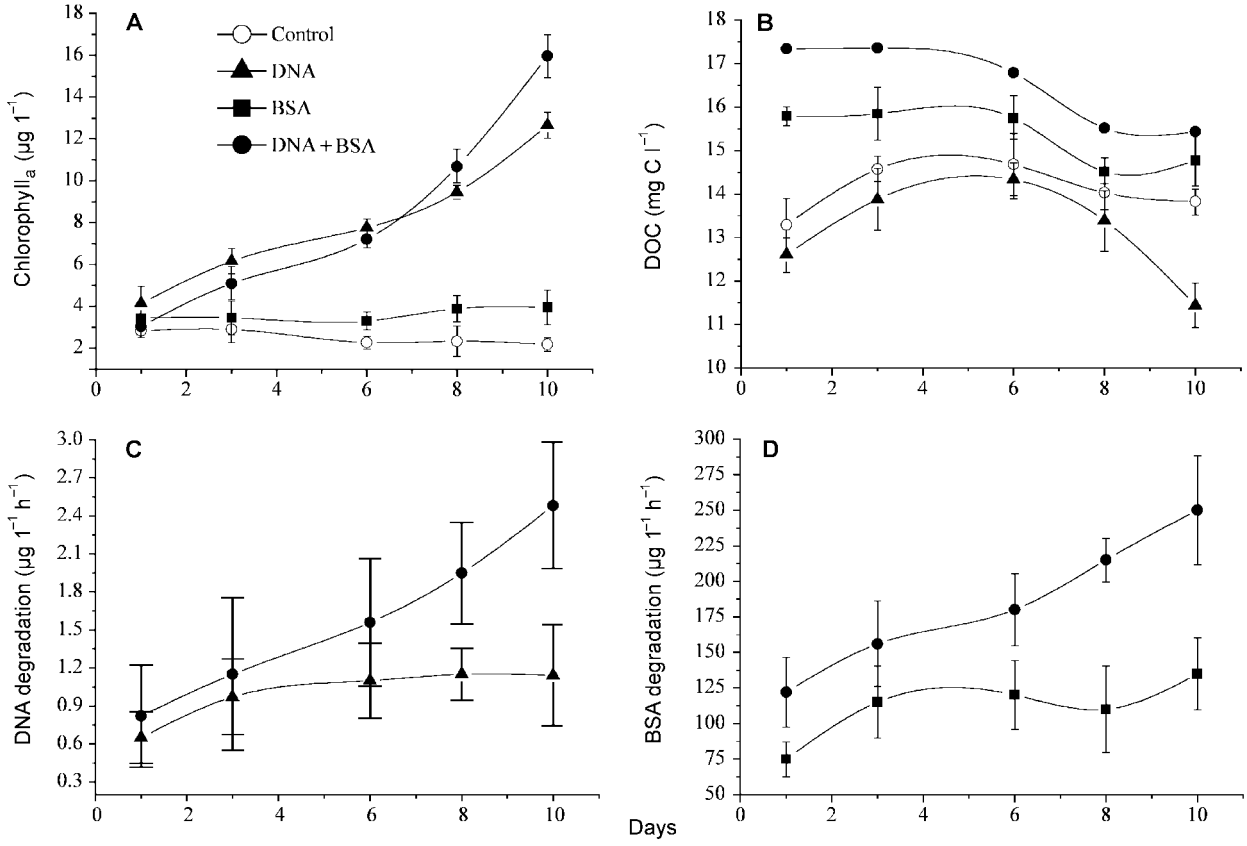

Fig. 1. Changes in concentrations of chlorophyll$_a$ (A), and dissolved organic carbon, DOC (B), and the rates of DNA (C) and BSA (D) degradations in four mesocosms: control, DNA, BSA, and DNA+BSA. Vertical bars show ±standard deviations values of the means.

Heterotrophic nanoflagellates (HNF) abundance, biomass and size distributions. Triplicate water samples were fixed with 37% formaldehyde (final concentration 2%). Subsamples of 5–20 ml, depending on the density of cells, were stained with DAPI (final concentration 1 µg ml^{-1}) (Porter and Feig, 1980), filtered through a 1.2 µm pore-size black polycarbonate membrane filters (Millipore), and enumerated by epifluorescence microscopy. Phototrophic and heterotrophic forms were differentiated by the presence or absence of chlorophyll autofluorescence. HNF biovolume was calculated from measurements of cells and approximations to simple geometrical forms. The carbon content was calculated by multiplying the biovolume with a conversion factor of 200 fg C µm^{-3} (Børsheim and Bratbak, 1987). Variations in DAPI-stained HNF cell length were used to subdivide their numbers and biomass into three size classes: small (<5 µm), medium (5–10 µm), and large (>10 µm).

Ciliate abundance, biomass and composition. Triplicate samples were fixed with Lugol's solution, then decanted to 5–20 ml and examined with light microscope (Nikon Optiphot 2). Species composition and measurements of ciliates were determined from living material using a phase contrast, immersion, and stains for the nuclei and food vacuoles, in samples drawn together with these for quantitative enumeration. Biovolume was calculated from measurements of cell dimensions and simple geometric shapes, and converted to carbon biomass using a conversion factor of 190 fg C µm^{-3} (Putt and Stoecker, 1989). Species identifications of ciliates were based mainly on Foissner *et al*. (1991–1995).

Statistical analyses. The data were statistically analyzed using computer software Origin v. 6.1 (Origin Lab, USA). Mean values, ranges, and standard deviations were used to compare results. Linear regression model was applied to study relationships among experimental data. Correlations were performed for all combined mesocosms. *T*-test was used to analyze differences among experimental mesocosms.

Results

Trophic parameters and organic compounds. The concentrations of chlorophyll$_a$ remained at a relatively constant level throughout the experiment in the mesocosms control (2.3±0.2–2.9±0.1 µg l^{-1}) and in BSA (3.3±0.04–3.9±0.2 µg l^{-1}), while increased gradually in mesocosms DNA (to 12.6±0.5 µg l^{-1}) and DNA+BSA (to 16.0±0.6 µg l^{-1}), Fig. 1A. The

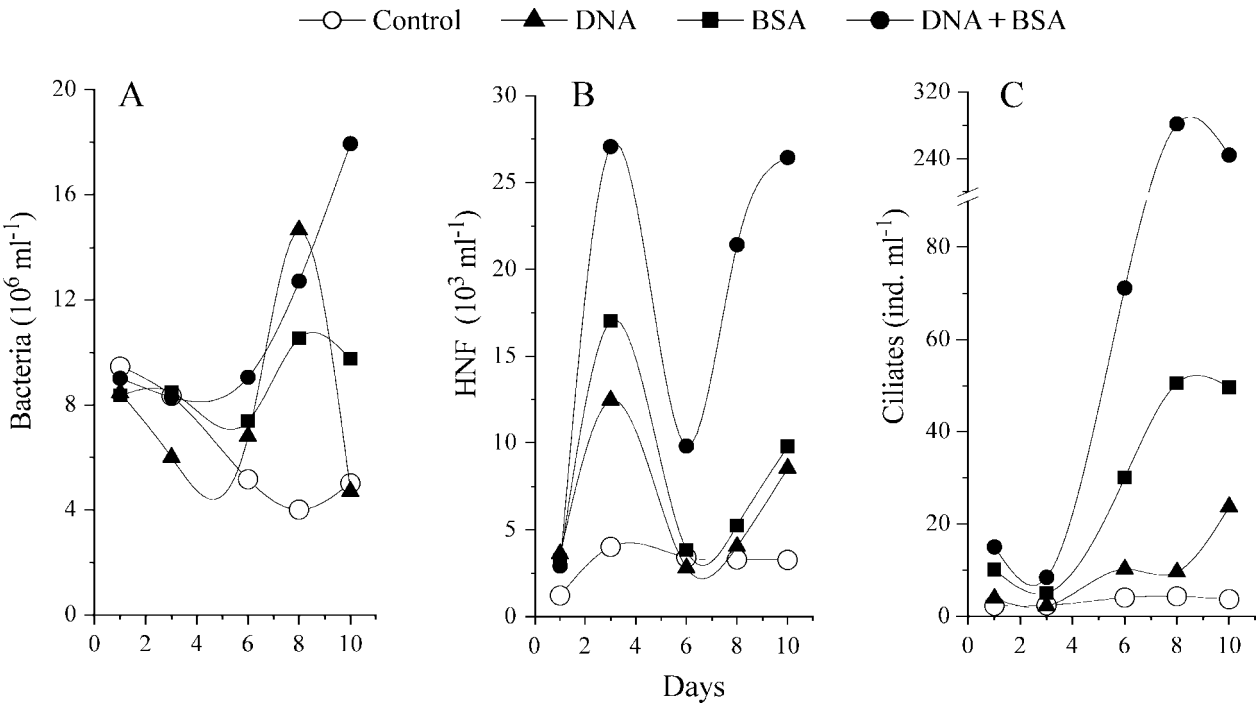

Fig. 2. Abundance of bacteria, heterotrophic nanoflagellates (HNF) and ciliates in four mesocosms: control, DNA, BSA, and DNA+BSA. For better visualization of the mean values, ±standard deviations values of the means are not shown (for ±standard deviations of mean values see Results).

differences among the control and all enriched mesocosms were statistically significant (*t*-test, $p<0.05$).

The concentrations of dissolved organic carbon (DOC) showed little variations in all mesocosms, Fig. 1B. They remained at almost the same level in the control and in the mesocosm DNA, and showed the highest concentrations in the middle of the experiment (14.7±0.2 mg l^{-1} and 14.3±0.03 mg l^{-1}, respectively). Slightly higher values were noted in the mesocosm BSA with maximum on day 8 (19.0±0.5 mg l^{-1}). In the mesocosm DNA+BSA, initially high DOC concentration decreased with time to 15.4±0.1 mg l^{-1}. Both mesocosms with BSA additions differed significantly from the control and DNA ones ($p<0.05$).

The rates of DNA degradation showed increasing trends in both mesocosms, however they increased more markedly in the mesocosm DNA+BSA (0.8±0.1–2.5±0.6 µg l^{-1} h^{-1}) than in the mesocosm DNA (0.6±0.1–1.1±0.4 µg l^{-1} h^{-1}), Fig. 1C. Similar trends were found in the rates of BSA degradation that increased distinctly in the mesocosm DNA+BSA (from 122.1±35.2 µg l^{-1} h^{-1} to 250±48.1 µg l^{-1} h^{-1}) while only slightly in the mesocosm BSA (from 75.0±23.1 to 135.1±18.0 µg l^{-1} h^{-1}), Fig. 1D. The differences between mesocosms were significant only in the rate of BSA degradation ($p<0.05$).

Bacterial and protistan numbers and biomass. At the start of the experiment bacterial numbers were almost the same in all mesocosms (about 9×10^6 ml^{-1}), Fig. 2A. During the subsequent days, the numbers of bacteria decreased gradually to $4.0\pm0.9\times10^6$ ml^{-1} in the control, remained at fairly constant level in the mesocosm BSA ($7.4–10.5\times10^6$ ml^{-1}), while increased considerably in both DNA-enriched mesocosms. Especially high increase to $17.9\pm2.3\times10^6$ ml^{-1} was observed in the mesocosm DNA+BSA whereas in the mesocosm DNA after the peak on day 8 ($14.7\pm2.1\times10^6$ ml^{-1}) bacterial numbers rapidly decreased to the minimal value of $4.7\pm0.7\times10^6$ ml^{-1} and coincided with the increase in protistan numbers. The differences in bacterial numbers were statistically significant between the control and BSA and also between the control and DNA+BSA (*t*-test, $p<0.05$).

HNF numbers followed similar trends in all mesocosms. The distinct peaks were observed on day 3 of the experiment with the highest value of $27.1\pm0.7\times10^3$ ml^{-1} in the mesocosm DNA+BSA. Then, the numbers remained at a constant level (about 3×10^3 ml^{-1}) in the control, while declined markedly on day 6 and increased thereafter in all enriched mesocosms (Fig. 2B). The HNF numbers was significantly higher in the mesocosm DNA+BSA than in the control and the mesocosm DNA ($p < 0.05$).

Ciliate numbers maintained on similar level (~4 ind. ml^{-1}) in the control while increased distinctly throughout the experiment in remaining mesocosms (Fig. 2C). The highest ciliate numbers of 281.6±34.6 ind. ml^{-1} was recorded in the mesocosm DNA+BSA on day 8, which was considerably higher than maximal amounts noted in mesocosms DNA (23.7±3.6 ind. ml^{-1}) and

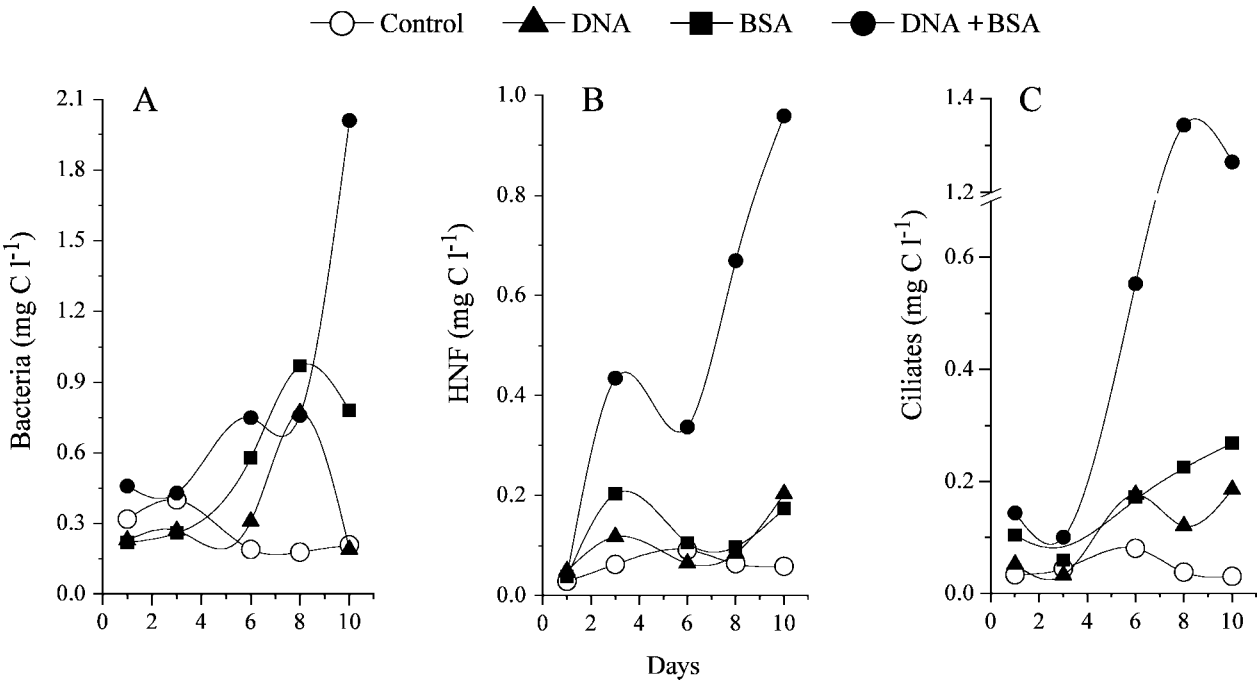

Fig. 3. Changes in biomass of bacteria, heterotrophic nanoflagellates (HNF) and ciliates in four mesocosms: control, DNA, BSA, and DNA+BSA. For better visualization of the mean values, ±standard deviations values of the means are not shown (for ±standard deviations of mean values see Results).

BSA (50.5±4.3 ind. ml^{-1}). However, the differences in the ciliate numbers were significant only between the control and the mesocosm BSA ($p<0.05$).

Variations in the biomass of all the studied groups of microorganisms were similar to changes in their numbers (Fig. 3). Only in the mesocosm BSA bacterial biomass increased continuously to 1.0 mg C l^{-1} on day 8. However, the most distinct increases in the biomass of all groups were observed in the mesocosm DNA+BSA in which high values of bacterial (2.1±0.1 mg C l^{-1}), HNF (195.8±59.2 µg C l^{-1}) and ciliate biomass (240.3±20.5 µg C l^{-1}) were 10, 17 and 41 times, respectively, higher than in the control. There were no significant differences in the bacterial biomass among mesocosms whereas significantly higher values of HNF biomass were found in the mesocosm DNA+BSA than in remaining mesocosms. The significant differences in ciliate biomass were found among the control and all enriched mesocosms (*t*-test, $p<0.05$).

In general, the effects of organic matter additions on bacterial biomass were more evident than on their numbers. In all mesocosms, increases in the bacterial numbers and biomass were usually observed after the peaks of protistan abundances.

Bacterial size distribution. Small-sized bacterial cells (0.2–1.0 µm) dominated in all mesocosms, constituting from 45% to 85% of the total bacterial numbers (Fig. 4). Their contribution remained almost constant in the mesocosm DNA, while it changed markedly in other mesocosms. The greatest variations in this class were observed in the mesocosm DNA+BSA, where the two distinct peaks were recorded. The contribution of the medium-sized class of bacterial cells increased gradually in the mesocosm BSA, while fluctuated distinctly in the mesocosm DNA+BSA and in the control. The contribution of large bacterial cells (>2.0 µm) mainly consisted of filamentous forms up to 20 µm in length (even up to 50 µm in mesocosms BSA and DNA+BSA), remained on almost similar levels during the whole experiment both in the control and in the mesocosm DNA. In the mesocosm BSA the highest contribution (18%) was recorded in the middle of the experiment and thereafter decreased to 5%. In the mesocosm DNA+BSA the contribution increased gradually until the end of the experiment, reaching 26% of the total numbers. Significant differences in the medium-sized class were found between the mesocosm BSA and DNA+BSA and also in the large class of bacterial cells between the mesocosm DNA and BSA (*t*-test, $p<0.05$).

Significant differences were observed in the contribution of particular size classes of bacterial cells to the total biomass (Fig. 5). Both in the control and in the mesocosm DNA, medium-sized class of bacterial cells (1.0–2.0 µm) dominated during the whole experiment with the highest values at the end of the study (75% and 65%, respectively). Thus, the differences between these mesocosms and others were statistically significant (*t*-test, $p<0.05$). In mesocosms BSA and DNA+BSA large-sized bacteria prevailed. Their contribution distinctly increased from 16 to 61% and from 39 to 63%, respectively. There were

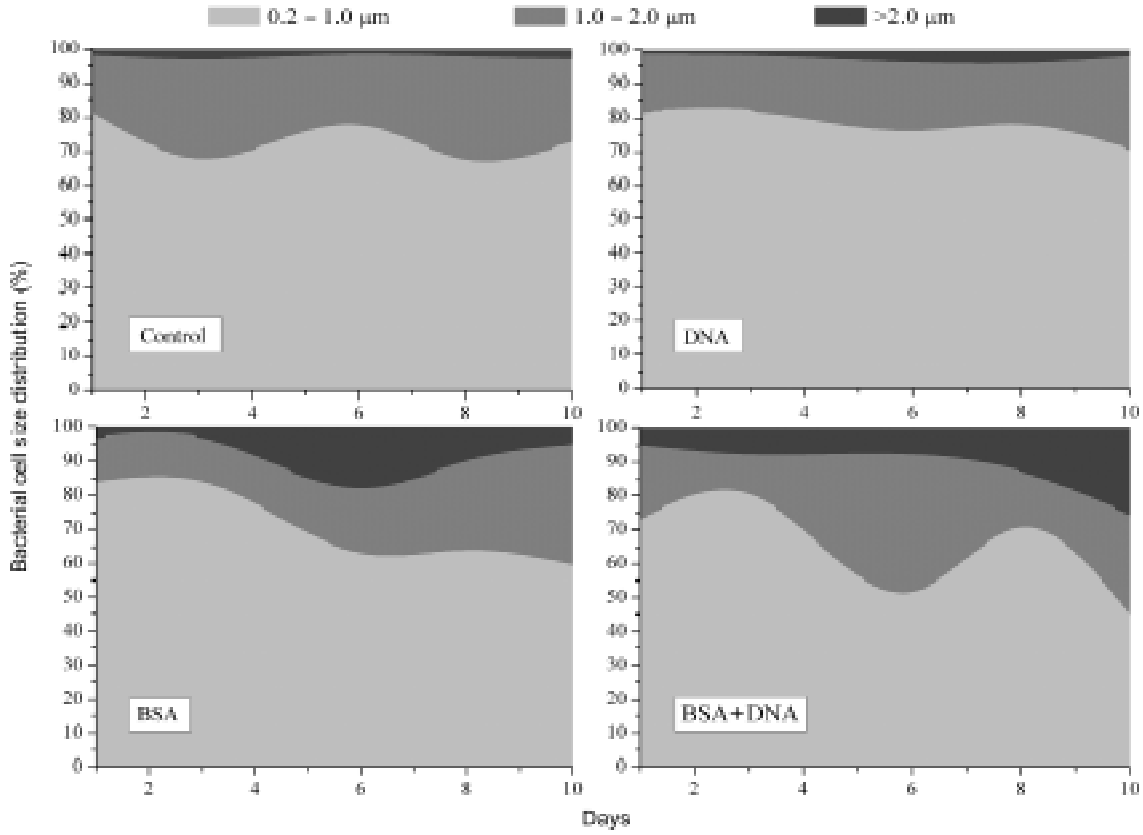

Fig. 4. Percentage contributions of three bacterial cells size classes: 0.2–1.0 μm, 1.0–2.0 μm and larger than >2.0 μm, to the total bacterial numbers in four mesocosms: control, DNA, BSA, and DNA+BSA.

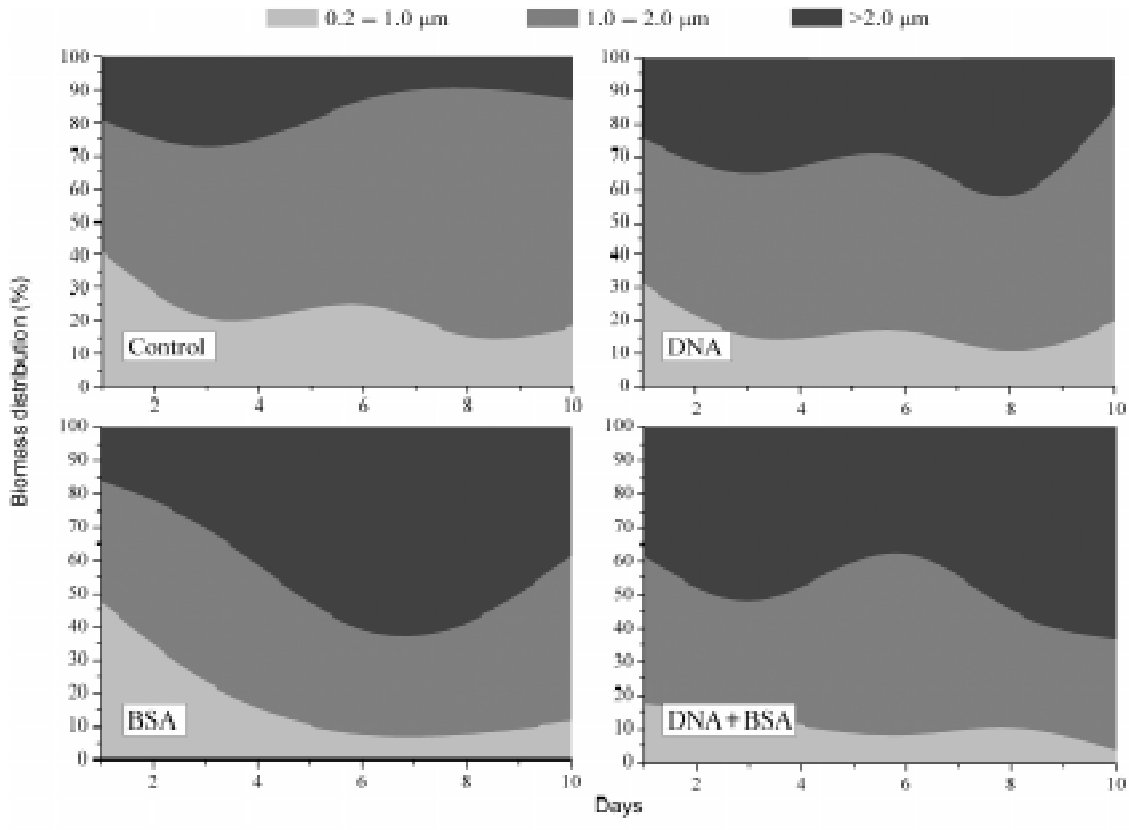

Fig. 5. Percentage contributions of three bacterial cells size classes: 0.2–1.0 μm, 1.0–2.0 μm and larger than >2.0 μm, to the total bacterial biomass in four mesocosms: control, DNA, BSA, and DNA+BSA.

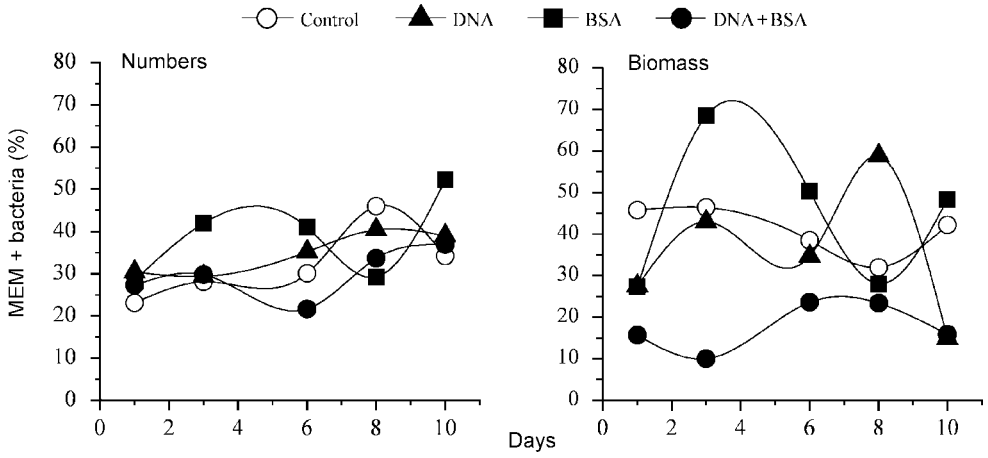

Fig. 6. Percentage contributions of active bacteria with intact membrane (MEM+) to the total bacterial numbers (A) and biomass (B) (presented as a sum of MEM+ and MEM – bacteria) in four mesocosms: control, DNA, BSA, DNA + BSA. For better visualization of the mean values, ±standard deviations values of the means are not shown.

highly significant differences in this class between the control and all enriched mesocosms (*t*-test, from $p<0.05$ to $p<0.001$) and between the mesocosm DNA and DNA+BSA ($p<0.01$). The contribution of small-sized bacteria was low in all mesocosms and decreased throughout the experiment. The significant differences were noted between the mesocosm DNA+BSA and the control and mesocosm DNA ($p<0.05$).

Protistan taxonomic composition. In all mesocosms, during the whole experiment, small (<5 μm), free-living forms dominated in total HNF density whereas medium-sized (5–10 μm) cells dominated in total HNF biomass. In the mesocosm DNA+BSA, large HNF cells (>10 μm) belonging mainly to the order of choanoflagellates appeared at the end of the experiment.

The taxonomic composition of ciliates was similar in the control and in the mesocosm DNA, in which small *Urotricha* sp., dominating at the beginning of the experiment, was replaced by omnivorous *Coleps* sp. in the second half of the studied period. Only on the final day of the experiment small bacterivorous *Scuticociliatida* sp. in the control and *Halteria grandinella* in the mesocosm DNA were the most abundant. In mesocosms BSA and DNA+BSA shifts from algivorous (*Rimostrombidium* sp.) to bacterivorous forms (*Cyrtolophosis mucicola*) were noted. With respect to biomass, during the first days of the experiment oligotrichs *Strombidium* sp. dominated in all mesocosms. During the following days omnivorous *Coleps* sp. constituted significant proportion of the ciliate biomass. Only in mesocosms +BSA and +DNA +BSA small bacterivorous scuticociliates and large bacterivorous vorticellids, respectively, dominated on the final day of the experiment.

Contribution of active bacteria with intact membrane (MEM+). Generally, the contribution of the active bacteria to the total numbers was similar in all mesocosms (Fig. 6A), thus the differences were not significant (*t*-test, $p>0.05$). In the control the contribution of MEM+ increased slightly, reaching maximum on day 8 (46%). In mesocosms DNA and DNA+BSA, MEM+ cells remained almost stable during the whole time of the experiment (30–40%). The most visible fluctuations were observed in the mesocosm +BSA, where the contribution of MEM+ increased at the beginning, remained stable during the following days and then increased distinctly up to 52% at the end of the experiment. The greater variations were noted in the contribution of MEM+ to the total bacterial biomass (Fig. 6B). In the control, these active bacteria did not change significantly throughout the experiment, constituting from 32 to 46% of the total biomass. In the mesocosm DNA two distinct peaks were recorded. After maximum (59%) on day 8, the percentage of MEM+ markedly declined to 15%. In the mesocosm BSA the contribution of MEM+ was very high and two distinct increases were noted: on day 3 (68%) and at the end of the experiment (48%). In contrast, very low contribution (10–24%) was observed in the mesocosm DNA+BSA and thus the differences between this mesocosm and others were statistically significant (*t*-test, from $p<0.05$ to $p<0.001$).

Fluorescent in situ hybridization (FISH). The fraction of microorganisms hybridizing with the *Bacteria* EUB338 probe was between 48 and 65% of total DAPI counts in the control (Fig. 7). In mesocosms DNA and BSA, from day 3 we observed a distinct increase of the proportion of the total bacteria detected with EUB probe (from 41–47 to 77%), whereas they remained on the same level in the mesocosm DNA+BSA (~74%). Statistically, significant differences were between the control and the mesocosm DNA+BSA and also between BSA and DNA+BSA (*t*-test, $p<0.05$). The γ-*Proteobacteria* had the largest response to the addition of BSA, with the population

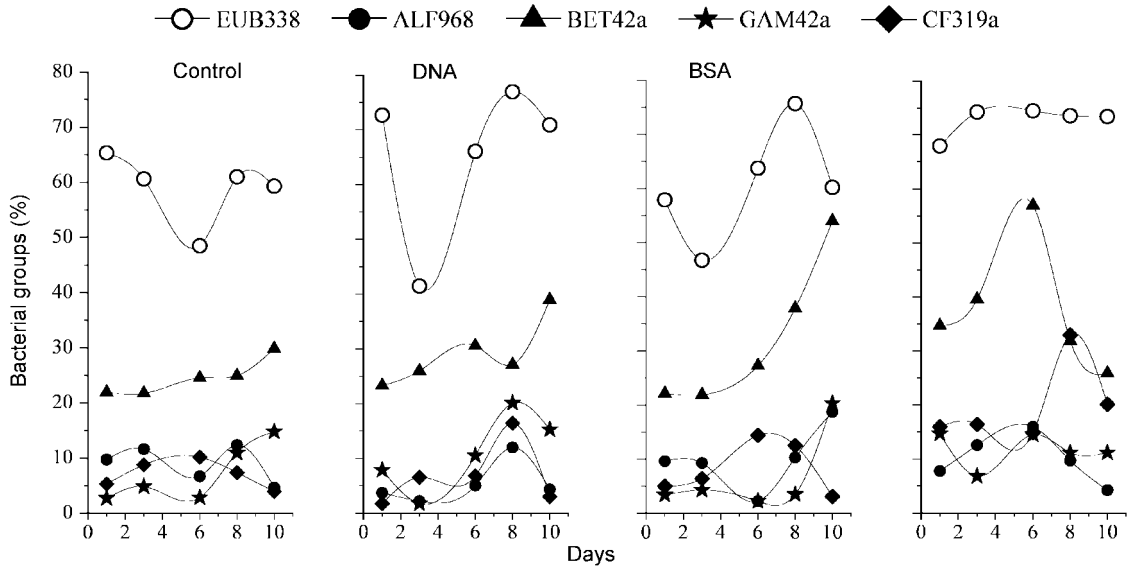

Fig. 7. Percentage contributions of bacterial taxonomic groups determined by fluorescent in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes: EUB338 for *Bacteria*, ALF968 for α-*Proteobacteria*, BET42a for β-*Proteobacteria,* GAM42a for γ-*Proteobacteria* and CF319a for *Cytophaga-Flavobacterium* cluster, related to the total number of DAPI-stained bacteria in four mesocosms: control, DNA, BSA, DNA+BSA. For better visualization of the mean values, ±standard deviations values of the means are not shown.

increasing from 3 to 20% as well as to DNA addition (from 8 to 20%). *Cytophaga-Flavobacterium* (CF) cluster also increased in all enriched mesocosms. In comparison to the control, where the detection rates of CF was low (4–10%), especially in the mesocosm DNA+BSA, the abundance of CF increased significantly to relatively high level (from 16 to 33%). Thus the differences between the control and all enriched mesocosms were statistically significant ($p<0.05$). The β-*Proteobacteria* was dominating population in all mesocosms throughout the experiment. The proportion of this lineage was rather constant (22–30%) in the control, whereas distinctly increased in mesocosms DNA and DNA+BSA. There were only statistically significant differences in the abundance of β-*Proteobacteria* between the control and DNA+BSA mesocosm ($p<0.05$). The changes in α-*Proteobacteria* abundances and their maxima (usually on day 8) were very similar in all mesocosms and their share never exceeded 20% throughout the experiment (Fig. 7).

Addition of organic compounds increased the sum of the detected α, β and γ-*Proteobacteria* and *Cytophaga-Flavobacterium* cluster populations from 40 to 55% in the control, 37–75% in the DNA, 40–96% in the BSA and from 73–103% in the DNA+BSA mesocosm. The coverage of the bacterial probe EUB-positive cells reflects the development of total counts and seems to be completed.

Bacterial biomass production and turnover rates. In all mesocosms the rates of bacterial production (BP) increased substantially during the first half of the experiment and then rapidly declined towards the end (Fig. 8). The highest rates of BP were noted in the mesocosms DNA (42.0±0.5 μg C l^{-1} h^{-1}) and DNA+BSA (53.4±3.2 μg C l^{-1} h^{-1}), which were about 4–5 times higher than in the control and in the mesocosm BSA.

During the whole time of the experiment large and attached bacteria (>1.0 μm in length) were responsible for the BP rates in all mesocosms, constituting from 52 to 98% of the total BP except the control, where at the beginning of the experiment, 66% of BP was connected with the free-living bacteria (<1.0 μm). In the mesocosm DNA+BSA, the contribution of the fraction <1.0 μm was the lowest (3–15%) among studied variants, while the contribution of large bacteria was very high during the whole experiment (85–97%), Fig. 9. The significant differences in the total BP were

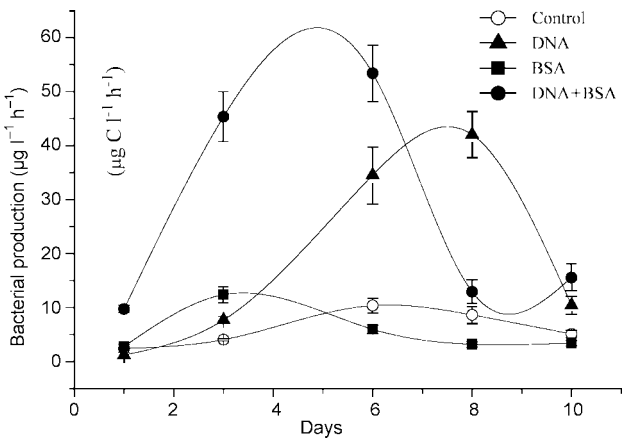

Fig. 8. The rates of total bacterial production in four mesocosms: control, DNA, BSA, DNA+BSA. Vertical bars show ±standard deviations values of the means.

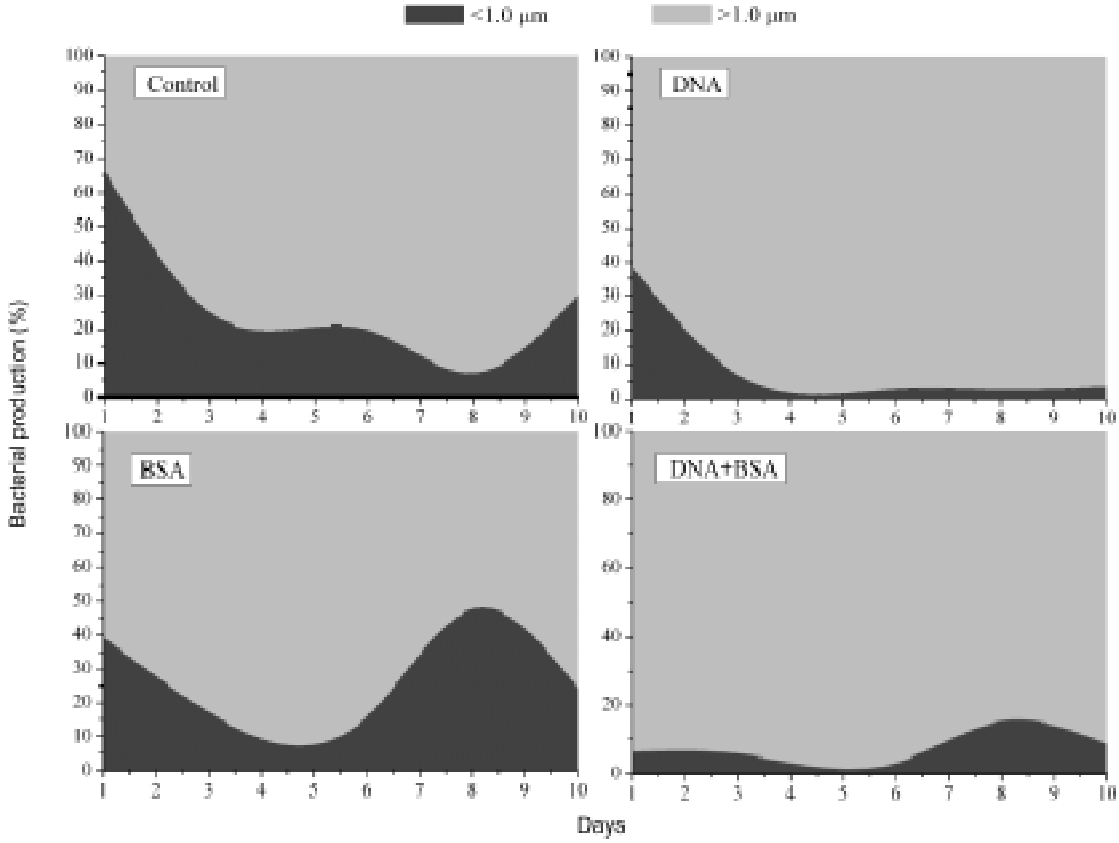

Fig. 9. Percentage contributions of two fractions of bacterial cells: free-living (<1.0 μm) and large free-living and attached (>1.0 μm) to the total bacterial production rates in four mesocosms: control, DNA, BSA, DNA+BSA.

found between the control and the mesocosm DNA+ BSA (*t*-test, $p<0.05$) and also between mesocosms BSA and DNA+BSA ($p<0.05$).

The longest mean turnover rate of bacterial biomass (BTR) was noted in the mesocosm BSA (14.5± 11.6 h), Fig. 10. In two mesocosms, enriched with DNA, mean BTR were identical (6.5 h) and comparable to the control. Thus, there were no significant differences (*t*-test, $p>0.05$) among mesocosms. High values of standard deviation indicate that BTR varied widely throughout the experiment. Generally, both in the control and in the mesocosm DNA, BTRs were the longest at the beginning of the study (13.3±1.2 h and 18.7±2.1 h, respectively) and showed decreasing trend. In contrast, in mesocosms BSA and DNA+BSA values of BTR were the shortest at the beginning of the experiment (2.1±2.4 h and 1.4±4.8 h, respectively) and showed increasing trend.

Statistical relations. Statistical analyses using data from all mesocosms showed numerous, in majority positive, and highly significant correlations between studied parameters (Table I and Table II). From among all presented results, the only negative correlations occurred between the contribution of MEM+ in the total bacterial biomass and HNF biomass and also between BTR and BP (Table II). Chlorophyll$_a$ was strongly correlated with all studied groups of microorganisms, whereas DOC was coupled only with *Cytophaga-Flavobacterium* and MEM+ numbers. The rates of DNA and BSA degradation were found to correlate both with trophic parameters and with bacteria and protists (Table I). As shown in Table II, bacterial numbers was more dependent on ciliates than on HNF, whereas bacterial biomass was stronger coupled with HNF. Correlations between particular bacterial and HNF size classes indicate the direct and selective

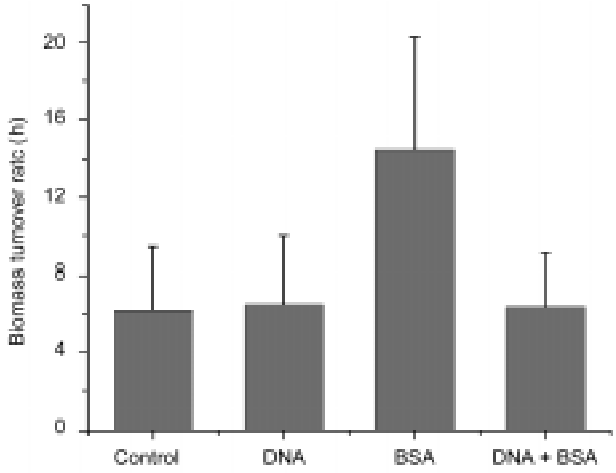

Fig. 10. Mean values of bacterial biomass turnover rates (BTR) in four mesocosms: control, DNA, BSA, and DNA+BSA. Vertical bars represent ±standard deviations of the mean values.

Table I
Matrix of Pearson's correlations for the microbial loop components and trophic parameters (Chl_a – chlorophyll$_a$, DOC – dissolved organic carbon) and rates of DNA (deoxyribonucleic acid) and BSA (bovine serum albumin) degradations

Parameter	Chl_a	DOC	DNA	BSA
Chl *a*	–	ns	0.80**	0.93***
DNA	0.80**	ns	–	0.96***
BSA	0.93***	ns	0.96***	–
Bacterial numbers	0.58**	ns	0.72*	0.81**
Bacterial biomass	0.63**	ns	0.87***	0.75*
MEM+ numbers	ns	0.45*	ns	ns
% of EUB338	0.46*	ns	ns	ns
% of CF319a	0.45*	0.45*	0.68*	0.72*
HNF numbers	0.56*	ns	0.70*	0.74*
HNF biomass	0.75***	ns	0.94***	0.95***
Ciliate numbers	0.68***	ns	0.90***	0.86**
Ciliate biomass	0.72***	ns	0.93***	0.90***

Correlations were performed for all combined mesocosms (n = 20 for trophic parameters; n = 10 for rates of DNA and BSA degradations, ns – not significant, $p < 0.05$*, $p < 0.01$**, $p < 0.001$***)

predator-prey relations between them. The most significant correlations were these between large bacteria (>2.0 μm) and large HNF (>10 μm). The numbers and biomass of HNF were strongly correlated with both numbers and biomass of ciliates, suggesting the close coupling between these protistan groups. The two of four studied bacterial taxonomic groups (β-*Proteobacteria* and *Cytophaga-Flavobacterium*) were correlated with HNF numbers.

Discussion

Literature data report that the concentration and composition of organic matter may regulate bacterial growth, production, activity as well as morphological and taxonomic structure (*e.g.* Chróst *et al.*, 1989, Pomeroy and Wiebe, 2001). The concentration of dissolved DNA, the most important source of phosphorus for planktonic microorganisms (Siuda and Chróst, 2001) of about 0.5 μg l^{-1} in the mesotrophic Lake Kuc from which water for experiment was taken, seems to be too low to support significantly bacterial growth in such low productive habitat. According to the data of Ferguson and Sunda (1984) and Hagström *et al.* (1984) the availability of substrates of low C:N ratio, such as proteins, peptides and amino acids often is not sufficient to supply all of the nitrogen required for bacterial growth.

In our study, both bacterial and protistan numbers showed large fluctuations depending on organic matter additions. Bacterial numbers was relatively stable in the mesocosm enriched with BSA, whereas increased distinctly in mesocosms enriched with DNA and with DNA+BSA. The high values of bacterial biomass in

Table II
Statistically significant linear correlations between microbial parameters in the studied mesocosms

Variables		p	r
x	y		
Bacterial numbers (BN)	HNF numbers	0.58	< 0.01
Bacterial numbers	Ciliate numbers	0.67	< 0.001
Bacterial biomass (BB)	HNF biomass	0.77	< 0.001
Bacterial biomass	Ciliate biomass	0.75	< 0.001
HNF numbers	Ciliate numbers	0.63	< 0.01
HNF biomass	Ciliate biomass	0.89	< 0.001
BN 1.0–2.0 μm	HNF numbers 5–10 μm	0.70	< 0.001
BN >2.0 μm	HNF numbers >10 μm	0.77	< 0.001
BB 1.0–2.0 μm	HNF biomass 5–10 μm	0.64	< 0.01
BB >2.0 μm	HNF biomass >10 μm	0.83	< 0.001
% MEM+ biomass	HNF biomass	–0.50	< 0.05
MEM+ numbers 0.2–1.0 μm	HNF numbers <5 μm	0.46	< 0.05
MEM+ biomass 0.2–1.0 μm	HNF biomass <5 μm	0.47	< 0.05
% of BET42a	HNF numbers	0.50	< 0.05
% of CF319a	HNF numbers	0.66	< 0.01
Biomass turnover rate	Bacterial biomass	0.47	< 0.05
Biomass turnover rate	Bacterial production	–0.53	< 0.05

Correlations were performed between all mesocosms, r – correlation coefficient, p – level of the significance

the mesocosm +BSA caused by large filamentous bacteria dominating during the most time of the experiment and constituting to about 60% of the total bacterial biomass resulted probably from the significant increase of HNF biomass, which through intensive grazing on bacteria may induced defence strategy among bacterial communities.

The addition of both DNA and BSA substrates caused the most evident and successive increases in bacterial and protistan numbers and biomass. At the end of the investigations bacterial and protistan communities reached 2–5 times higher level than in two remaining enriched mesocosms. Thus, our observations imply that studied microorganisms might be strongly regulated by the bottom-up mechanism. The highly significant correlations between the rates of DNA and BSA degradation and all microbial groups seem to confirm the above suggestion. Decidedly higher rate organic matter degradation in the mesocosm with the combined addition of both substrates than in mesocosms with the addition of DNA and BSA alone may suggest C, N and P limitation. The increase of P, N and C concentrations derived after enzymatic hydrolysis from added organic substrates stimulated an increase in bacterial numbers, which in turn resulted in the increase in numbers of nanoflagellates and finally in numbers of ciliates. It appears, also, that grazing by these two groups of protists was not sufficiently strong to effective decrease of bacterial numbers and biomass. Similar conclusions indicating nutrients impact on dynamic of microbial communities were described previously by Chróst *et al*. (2009), where the most distinct increases in bacterial numbers and production rates were noted under P and N loading. Moreover, nutrients released from decomposing organic material were available for primary producers, thus chlorophyll-a concentrations were positively related to the rate of organic matter degradation (Table I).

For precise estimation of the impact of DNA and BSA on bacterial numbers and biomass as well as on the contribution of active bacterial cells (MEM+) to the total bacterial numbers and biomass we performed short-term (48 h) dialysis bags experiment. In order to reduce the impact of the top-down control, we eliminated protists and other large organisms by water sample filtration through 1.0-µm pore-size filters. Water samples were put into dialysis bags (Spectra/Por – MWCO 50 000 Da, Roth) and incubated in all studied mesocosms. For detailed description of the dialysis bags experimental design, see Adamczewski *et al*. (2009). Results of this experiment indicated that bacterial communities changed strongly in response to different organic matter compounds addition (Table III). In comparison to the control, the most distinct increase both in bacterial numbers and biomass was noted in the mesocosm with BSA (60% in numbers, 124% in biomass). This short-term experiment provided valuable data with regards to the HNF distribution in lake water. Despite of the fact that most of HNF were eliminated from the sample through filtration, rapid increase of their new cells after 48 h incubation period was noted in the mesocosm DNA+BSA (from 0 to 1.2×10^3 cells ml^{-1}). Significant increases in HNF numbers and biomass in remaining enriched mesocosms were also observed. We suggest that these increases might derive from HNF cysts development. This confirms clearly that organic matter addition stimulated HNF excystment (Paranjape, 1980). Moreover, heterotrophic flagellates are able to uptake of dissolved organic matter, especially amino acids as a carbon and nutrient source (Bennett and Hobbie, 1972, Sherr and Sherr, 1984). Bennet *et al*. (1990) observed that during laboratory experiments, some of heterotrophic flagellates did not take up microspheres or algae and had no DAPI staining vacuoles suggesting uptake of dissolved organic carbon. The authors showed that this mode of nutrition require high supply of DOC. It seems that enrichment of lake water with BSA and DNA provided favourable conditions for osmotrophic nutrition. Thus, uptake of dissolved organic matter as well as grazing on bacterial cells directly caused high increase of the total HNF density in this mesocosm.

Results of the experiment with dialysis bags employment suggest that both bacterial as well as HNF communities were positively affected by DNA and BSA addition (the bottom-up control). However, the relatively low (below the expected level) increases in the bacterial numbers and biomass we noted in dialysis bag incubated in the mesocosm DNA+BSA. It means, that even if the rates of bacterial biomass production was very high in this mesocosm, large part of the

Table III
Changes in bacterial and HNF numbers and biomass and percentage contribution of MEM+ bacterial cells to the total bacterial numbers and biomass in dialysis bags after 48 h incubation period in the studied mesocosms (control, DNA, BSA, DNA+BSA)

Parameter	Mesocosm		
	DNA	BSA	DNA+BSA
	Changes %		
Bacterial biomass	32	124	36
MEM+ numbers	15	35	12
MEM+ biomass	1	15	18*
HNF numbers	104	72	203
HNF biomass	25	10*	96

Changes are expressed as a percentage of increase or decrease* of the studied microbial parameters noted in all enriched mesocosms in comparison to the to the control mesocosm after 48 h incubation period

newly produced cells were immediately ingested by HNF, which numbers distinctly increased during short (48 h) incubation period to 203% in comparison to the control (the top-down mechanism). Taking into account that changes in microbial communities are rapid and frequent, we consider that the results of short-term dialysis bags experiment are a good way to explain the complexity of the interactions between microbial communities studied during long-term investigations.

Close correlations between bacterial and protistan communities indicate strong predator-prey relationship. We found that bacterial numbers and biomass were positively correlated with both HNF and ciliate numbers and biomass (Table II). It means that not only HNF but also ciliates were important bacterivores. Also, very tight and positive correlations between HNF and ciliates densities and biomass may suggest the close coupling between these protistan groups.

Results of the studies on size distribution of bacterial communities indicate that variability of this parameter might be parallel affected by organic matter addition and protists pressure. In all studied mesocosms small, mainly spherical-shaped bacterial cells (<1.0 μm) dominated in the total bacterial numbers during the whole time of the experiment. It is in agreement with our earlier studies conducted in mesotrophic Lake Kuc by Chróst *et al*. (2000). The authors demonstrated that the most of the existing bacteria were very small and probably below the threshold size for larger organisms. In present study, the most visible changes in the contribution of small-, medium- and large-sized bacterial cells we observed in the mesocosm DNA+BSA. Similar, but less distinct shifts were recorded also in the mesocosm enriched with BSA. In these two mesocosms, continuous increases of medium (BSA mesocosm) and large (DNA+BSA mesocosm) bacterial cells contribution during the whole time of the study were noted. Generally, in the mesocosm BSA medium bacteria (36%), whereas in the mesocosm DNA+BSA large bacteria (26%) predominated in the total bacterial numbers. It is interesting, that lack of significant changes in bacterial size distribution was observed in the mesocosm DNA.

Similar to the distribution of bacterial size (Fig. 4), significant changes were also found in the distribution of bacterial biomass (Fig. 5), in which medium-sized cells dominated in all the studied mesocosms. However, significant increases of the contribution of large bacterial cells were noted in mesocosms BSA and DNA+BSA. At the end of the investigations in the mesocosm DNA+BSA the contribution of the large bacterial cells reached 64% of the total bacterial biomass. Similarly to the size distribution in bacterial numbers, the smallest fluctuations in bacterial biomass were recorded in the mesocosm DNA. Taking into account, that organic matter play crucial role in development of bacterioplankton communities, we suggest that increases of medium and large bacterial cells in numbers and biomass were caused by organic substrates addition. Our results indicate, that especially albumin stimulated an increase of bacterial biomass. The lack of changes in the size distribution of bacterial numbers and biomass after dissolved DNA addition may confirm this fact. It is evident that proteins, after enzymatic hydrolysis may be important source of nitrogen and carbon, which bacteria can assimilate during growth and biomass production. On the other hand, we conclude that changes in size distribution of bacteria were also directly caused by protists (both HNF and ciliates) grazing pressure. Especially close relationships between bacterial and HNF size distributions confirm strong predator-prey coupling. It is known that protistan grazing is size selective and that most of HNF prefer medium-sized bacterial cells (Gonzales *et al*., 1990, Pernthaler *et al.*, 1996). In literature, information about the community structure of HNF in lakes and their ecological requirements are limited (Auer and Arndt, 2001). However, body size of HNF seems to play an important role in their preference to different size classes of bacteria. In our study, small bacteria dominated the bacterial numbers, although the dominance of these bacteria does not necessarily imply that they were not grazed by HNF. During the whole time of the experiment very small (2–3 μm in size), free-living and unidentified HNF were the most abundant. We suppose that they might have a strong impact on small bacteria. Thus, at the beginning of the experiment the increase of bacteria was not apparent and maintained on constant level. It seems that these small HNF were probably the only group of HNF preferring the smallest bacteria. As shown by Jürgens *et al*. (1999) principal bacterivores are these mostly <5 μm in diameter. In contrast, medium-sized HNF represented mainly by choanoflagellates attached to particles formed a major part of the HNF biomass. It appears that these protists with very high ingestion rates may influence bacterial biomass (Carrias *et al.*, 1996, Šimek *et al*., 2004).

The fact that different HNF size classes are related to bacterial size classes in different way may partly explain correlations between them. During our studies we found close correlation between medium and large bacterial and HNF numbers and biomass. These correlations indicated that different HNF groups are related to appropriate size structure of bacteria; medium-sized (5–10 μm) flagellates prefer the medium bacteria (size of 1.0–2.0 μm), whereas large HNF cells (>10 μm) feed on large bacteria (>2.0 μm). The results seem to confirm above conclusions on the important role of HNF size structure in structuring bacterial communities. It also suggests that HNF, through selective grazing on bacteria, had an important

impact on the structure of whole bacterioplankton community (Hahn and Höfle, 2001).

During our studies, especially at the end of the experiment, large bacterial filaments (>10 μm in length) constituted large fraction of all bacterial cells mainly in the mesocosm enriched simultaneously with DNA and BSA. It seems that these "grazing-resistant" or "grazing-protected" bacteria were not available for small bacterivorous protists (Pernthaler *et al.*, 1996, Weinbauer and Höfle, 1998, Hahn and Höfle, 2001, Lebaron *et al*., 2001) but constituted a potentially important food source for organisms of the higher trophic levels such as ciliates, rotifers and crustaceans (Corno and Jürgens, 2006).

In the second half of the experiment we observed very high numbers of small (20–30 μm) bacterivorous ciliates like *Cyrtolophosis mucicola* attached to organic particles. This species feeds not only on bacteria, but also on the detritus, is r-selected and thus abundant only in the absence of competitors (Foissner *et al*., 1999). Ciliate biomass was mainly composed of omnivorous *Coleps* species, which were replaced by bacterivorous scuticociliates and *Vorticella* sp. in mesocosms BSA and DNA+BSA, respectively. They can efficiently consume larger prey size and also medium-sized bacterial aggregates or detritus particles (Arndt, 1993, Šimek *et al*., 2000). Vorticellids occur especially in bacteria-rich habitats and feeds very effectively on bacteria (Šimek *et al.*, 1995). Due to relatively high abundance of bacterivorous ciliates in our studies one may infer their decisively limiting effect on bacteria, especially on large forms.

The amount of active cells in relation to inactive and all bacterial cells occurring in aquatic ecosystem is one of the most important parameter describing bacterioplankton community. It is evident that physiological status of bacterial communities, which are responsible for the most of all microbial processes within the microbial loop, determines trophic and ecological conditions of aquatic environment. During the whole time of the experiment the percentage contribution of MEM+ bacterial cells to total numbers was similar and maintained almost on constant level (avg. ~ 30%) in all enriched mesocosms indicating no direct organic matter impact on active cells, Fig. 6. However, the experiment with dialysis bags employment showed marked increases in the contribution of MEM+ cells both to total bacterial numbers and biomass in the studied mesocosms (Table III). In comparison to the control, MEM+ cells contribution increased to 15% in the mesocosm DNA, 35% in the BSA and 12% in the DNA+BSA. It suggests that bacterial response is more complex than a simple increase in numbers and biomass. Bacteria may respond by a direct increase in response to organic matter but this effect is not visible because effective and continuous elimination through protistan feeding. Moreover, in the mesocosm experiment, the pattern of change in MEM+ that was very similar to changes in HNF biomass in the mesocosm BSA, whereas followed inverse patterns to the HNF biomass in two mesocosms enriched with DNA, may support above suggestion. So, all results indicate clearly, that both numbers and biomass of active bacterial cells were controlled simultaneously by two, the bottom-up (DNA and BSA addition) and the top-down (HNF grazing pressure), mechanisms during our studies. Negative correlation between contribution of MEM+ cells to the total bacterial biomass and HNF biomass suggests that especially grazing pressure by HNF may be one of the most important factors responsible for keeping bacterial numbers rather on constant level in examined mesocosms. Also close relations between both numbers and biomass of small active bacterial cells (<1.0 μm) and small HNF (<5.0 μm) (Table II) confirm selective, size-dependent HNF grazing on active bacteria. Grazing on the bacterial populations may continue until the concentration of bacteria decrease below threshold value, where grazing becomes energetically unfavourable (Chróst *et al*., 2000). Many studies suggest that protists may eliminate preferentially the active fraction (growing and dividing) of the bacterial community and that grazing rates on live bacteria may be almost two times or even four or more times higher than on dead bacteria (del Giorgio *et al*., 1996, Koton-Czarnecka and Chróst, 2003).

The initial assumption that DNA and BSA additions would elicit various responses of abundances of different bacterial groups was proved by the results of this study. The addition of organic matter especially labile and low molecular weight component may affect directly on bacterial growth and production as well as on taxonomic diversity. Data obtained by Cottrell and Kirchman (2000) from MICRO-FISH suggested that *Cytophaga*-like bacterial group dominated the consumption of high-molecular weight materials (protein and chitin), while the abundances of α- and β-*Proteobacteria* groups were more closely related to concentration of low-molecular weight compounds (amino acids). Furthermore, Covert and Moran (2001) separated estuarine DOM into high and light molecular weight and found clear differences in relative abundance of *Proteobacteria* (α, β, γ) and *Cytophaga-Flavobacterium*. On the other hand, Schutter and Dick (2001) suggested that carbon supply had strong effect on functional and structural characteristics of microbial communities.

In general, responses among the various bacterial groups (*Cytophaga*-like bacteria and *Proteobacteria*) were most distinctive in the mesocosm amended with DNA and BSA, Fig. 7. The *Eubacteria* (EUB338 probe) increased from 41 to 77% in the mesocosm

DNA, and from 47 to 76% in the mesocosm BSA. Water enrichment with BSA and DNA alone significantly increased γ-*Proteobacteria* in the mesocosms. It is evident that proteins, after enzymatic hydrolysis may be an important source of nitrogen and carbon, which bacteria can assimilate during growth and biomass production. In this case we can assume that bacterial growth was stimulated (by the quantitative supply of BSA) by available N and C components present in albumin and by inorganic phosphorous present in DNA. The γ-*Proteobacteria,* which represents a relatively small group in freshwater habitats in comparison to marine waters, increased after 3 days of incubation with DNA and after 8 days with addition of BSA (from 8 to 20% and from 3 to 20%, respectively). It is evident that they have the potential for rapid growth and to overgrow other bacteria under nutrient conditions (Pernthaler *et al*., 2001, Hornák *et al.*, 2005). Our data support the hypothesis that β-*Proteobacteria* possess ability of adaptation to patchy distribution of substrates, and that they grow more rapidly than other bacteria. It also seems self-evident, that similarly to changes in the total bacterial numbers and biomass, the strong impact on the contribution and the response of individual groups was caused by different treatments. Other investigations, in which glucose was added as a carbon source in enrichment experiments, reported similar results (Flaten *et al*., 2003, Olapade and Leff, 2005).

Addition of DNA and BSA also caused a large increase in the abundance of the *Cytophaga-Flavobacterium* cluster (average 24% or 2.4×10^6 ml^{-1}). This may be a consequence of consumption of proteins, which represent potentially large components of high-molecular-weight DOM in marine systems (Cottrell and Kirchman, 2000). In contrast, in the control mesocosm the *Cytophaga-Flavobacterium* cluster accounted for average 7% or 0.4×10^6 ml^{-1}. According to Weinbauer and Höfle (1998) changes in community composition are important because individual bacterial populations may display different characteristics, such as carbon substrate utilization, patterns or growth efficiencies, and thus may also influence community processes.

Results of our studies indicated that bacterial secondary production rates (BP) were rapidly stimulated by addition of nucleic acids, Fig. 8. Significant increases in BP rates noted in two DNA-enriched mesocosms (about 4–5 times higher than in the control and in the mesocosm +BSA) and simultaneously low level of BP rates in the mesocosm BSA (similar to the control) may confirm the importance of nucleic acids. Chróst (2002, 2004) reported that depending on the trophic status of a lake, phosphorus bound in free nucleic extracellular acids (dsDNA, ssDNA, RNA) may contribute from 51 to 92% to the pool of dissolved organic phosphorus in lake water. Nucleic acids are the most favourable substrates for development of aquatic bacteria populations (Siuda and Chróst, 2001). Some of organic compounds liberated enzymatically from nucleic acids hydrolysis *e.g.* nucleosides can be directly incorporated by heterotrophic microorganisms into nucleic acids (Chróst *et al*., 1988), being essential constituents during cell division. Therefore, significant increase in PB in the mesocosms DNA and DNA+BSA might be caused by two, mentioned above, roles of DNA in aquatic ecosystems. These observations confirm results of studies carried out by Fisher *et al*., (2000) they found the greatest values of bacterial production in response to the P-treatment.

Numerous results of experimental studies (Pernthaler *et al*., 1996, Lebaron *et al*., 2001, Joint *et al*., 2002) indicated that HNF may, through intensive bacterial cells consumption, stimulate their cell division and in consequence increase in bacterial abundance and activity. Experimental studies conducted by Lebaron *et al.* (2001) reported that after the peak of HNF abundance thymidine incorporation rates increased, whereas leucine incorporation rates roughly stabilized. These clear and strong changes in incorporation values of thymidine and leucine suggest that bacteria under grazers control reduce their protein synthesis activity but still have increasing DNA duplication rates. On the contrary, bacterivorous ciliates pressure, especially at the end of experiments, caused distinct decline in BP rates to low level. On the one hand strong ciliates pressure might eliminate large (usually attached) and active bacterial cells. On the other, they might activate defence strategy leading to decrease of bacterial activity and consequently distinct inhibition of bacterial production rates. This suggests very efficient and rapid removal of bacterial biomass originating from bacterial production by ciliates and/or zooplankton of the higher trophic levels. In summary, when BP was relatively low, bacterial biomass was increasing in all enriched mesocosms together with a bacterial production, but when BP was high bacterial biomass was fluctuating. Furthermore, the lack of large changes in bacterial biomass observed in the mesocosm with DNA even when bacterial production increased greatly after DNA addition, points to the importance of grazing in controlling bacterial density.

In all mesocosms we observed continuous increase of large and attached bacteria (fraction >1.0 μm) contribution to total BP, Fig. 9. In the mesocosms DNA and DNA+BSA, high (80–100%) and generally constant level of this fraction during the whole experimental period was noted. On the contrary, in the control and in the mesocosm BSA, distinct decrease in large and attached bacteria to the total BP contribution at the end of the studies was observed. It is noticeable, that decreases in large and attached bacteria observed

during investigations, corresponded to maximal values of ciliates numbers and biomass. It seems that bacterivorous ciliate taxa might cause decrease in large and attached bacteria contribution at the end of the studies. On the contrary, HNF affected the contribution of small and large bacteria to total BP at the beginning of the experiment. Increase of size-selective HNF abundance noted during first days of experiment resulted from strong pressure on small (<1.0 μm) bacteria, which dominated at this time. Not only changes in protistan grazing on bacteria explain fluctuation in free-living and large and attached bacteria contribution to the total BP. In our opinion DNA had impact on the development of large bacteria cells and increase in the rates of BP to the largest extent. In spite of the fact that we did not find the significant correlation between attached bacteria production and contribution of cells size class >1.0 μm, our results might suggest that the bacterial activity increased adequate to the cell size. We agree with Fisher *et al*. (2000) who suggest that addition of organic substrates causes the greatest aggregate bacterial growth response while addition of nutrients alone causes mainly the largest shifts in community composition. Suspended particulate organic detritus composed mainly of dead or dying algal and larger zooplankton cells, was successively colonized by active bacterial cells. It is well know that attached bacteria display much higher enzymatic and metabolic activity than free-living bacteria (Chróst *et al*., 2000, Chróst and Siuda, 2006). Consequently, attached and active bacteria, being under low HNF pressure, were significantly responsible for predominate contribution of bacterial production rates in studied mesocosms.

The rates of bacterial biomass turnover (BTR) may provide interesting conclusions about microbial populations in the studied mesocosms. The bacterial production rates, bacterial biomass and biomass turnover rates indicate close relationships between each other. In our experiment, BTR were negatively correlated with bacterial production whereas positively with bacterial biomass. It is interesting, that the lowest values of BTR we noted in mesocosms DNA+BSA and DNA (5.4 h) as well as in the control (6.2 h) while the highest in the mesocosm BSA (14.5 h), Fig. 10. The high BTR value in mesocosm BSA indicate that bacteria existing in the mesocosm BSA were less metabolically active than in other mesocosms and evidently caused distinct decrease in BP rates. On the contrary, in mesocosms with DNA addition, rates of BP were significantly higher and BTR were markedly shorter. Therefore, nucleic acids indirectly, throughout values of BB and BP influenced on BTR rates as well.

In conclusion, similarly as in studies with nutrients additions (Chróst *et al*., 2009) we examined all microbial parameters in natural water samples without elimination of larger organisms like rotifers and crustaceans. We consider that studies on all natural microbial communities allow comprehensively assess the role and importance of microbial communities within the microbial loop. It is well known that large organisms, such as *Daphnia* sp., may significantly influence both bacterioplankton and protists communities as well nutrients and organic matter concentration (Jürgens and Stolpe, 1995). During the studies we hypothesized that organic matter-rich water habitat and the rates of microbial and biochemical processes are the main mechanism generating diversity of microbial communities within the microbial loop. We conclude that the hypothesis on influence of organic matter on the component of the microbial loop can be supported by results of this study. The numbers and biomass of bacteria and protists seem to be determined by organic matter, especially by DNA+BSA being the major source of all limiting nutrients. On the other hand protistan grazing seems to influence morphological, size and taxonomic structure of bacterial community. Bacterivores responsible for the decrease of the bacterial numbers and biomass were HNF particularly at the beginning of the experiment and ciliates in the second half of the experiment. Thus, the impact of organic matter enrichment on bacterial structure was not as distinct as on the numbers and biomass. It is interesting to note that the additions of organic matter caused very fast and more evident growth of bacterial and protistan abundances than inorganic nutrients. Moreover, it seems that DNA as the important phosphorus source had the greater impact on bacterial processes than BSA.

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