Polish Journal of Microbiology
2009, Vol. 58, No 1, 77–90

ORIGINAL PAPER

Inorganic Phosphorus and Nitrogen Modify Composition and Diversity of Microbial Communities in Water of Mesotrophic Lake

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Received 25 July 2008, revised 25 October 2008, accepted 17 December 2008

Abstract

The effects of inorganic nutrients (N, P) enrichment of mesotrophic lake water on changes in bacterial and protistan (heterotrophic nanoflagellates and ciliates) communities compositions were studied in the mesocosm experiment. Phosphorus (PO_4^{3-}) and nitrogen (NH_4^+) alone and in combination were added to three types of experimental mesocosms. Mesocosms results suggested that simultaneous addition of P and N stimulated phytoplankton growth and production rates of bacterial biomass its turnover rate. Strong positive correlations between chlorophyll *a* and bacterial secondary production rates suggested that bacteria were mainly controlled by organic substrates released in course of phytoplankton photosynthesis. Both nutrients increased distinctly protistan biomass and resulted in the shift in ciliate community composition from algivorous to large omnivorous species. The response of bacterial numbers and biomass to nutrients addition was less evident. However, intensive grazing caused their dynamic changes. Fluorescence *in situ* hybridization (FISH) revealed only small changes in bacterial taxonomic composition. There was an apparent shift in dominance from *Cytophaga-Flavobacterium* to the *Alphaproteobacteria* group in the mesocosm with simultaneous addition of P and N, which positively related to increased abundance of bacterivorous protists. Experiment demonstrated that inorganic N and P nutrients directly influenced the bottom-down control of microbial communities, which had a crucial effect on morphological diversity of bacteria.

Key words: lake microbial community, phosphorus, nitrogen

Introduction

One of the major goals in current aquatic microbial ecology is understanding of a specific role of bacteria in processes of organic matter degradation and nutrients remineralization, as well as to recognize factors which regulate bacterial growth rates and microbial community composition. It is well known that bacteria are influenced by bottom-up (organic carbon, inorganic nutrients, mainly nitrogen and phosphorus) and top-down (grazing pressure and viral lysis) control mechanisms (Wright and Coffin, 1984; Pace and Funke, 1991; Weisse 1991; Pace and Cole, 1996). However, it is still not definitively accepted which of two control mechanisms is more important in aquatic habitats. As shown by Carlson *et al.* (1995) both control mechanisms are interrelated, because nutrient availability (bottom-up factor) is also dependent on regeneration of nutrients by grazers (top-down component).

Inorganic nutrients necessary for microbial growth and metabolism are usually in low concentrations during summer stratification period in the upper trophogenic water layer of deep lakes and limit assimilation and biodegradation of organic compounds (Coveney and Wetzel, 1992). Phosphorus availability is an important factor controlling mainly phytoplankton primary production and its species composition in lakes of temperate zone (Wetzel, 1975; Vadstein and Jensen, 1988). It is also assumed the possibility of the N limitation of algal growth in several lakes (Vrede *et al.*, 1999; Kivi *et al*., 1993). Organic matter produced by phytoplankton is the major carbon source for heterotrophic bacteria, thus consequently organic carbon availability is one of the most important limiting factor

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of bacterial growth. On the other hand it is well documented that bacteria are limited by inorganic P and N alone and/or in different combination with organic C, both in freshwater and marine environments (Pace and Funke, 1991; Pace, 1993; Thingstad and Rasoulzadegan, 1995; Chrzanowski and Grover, 2001). Bacteria play the key role in re-mineralization of nutrients combined in organic matter making N and P available for primary producers through biological oxidation and subsequent release of CO_2 and inorganic nutrients (Vadstein and Jensen, 1988). In many cases natural bacterial assemblages are net consumers of inorganic orthophosphate ($P_{inorg.}$) rather than re-mineralizing organisms and often compete with primary producers for this growth-limiting nutrient (Currie and Kalff, 1984). Bacterial net assimilation of $P_{inorg.}$ can be four times higher than that of phytoplankton (Vadstein *et al.*, 1993). Many mesocosms experiments demonstrated that nutrient limitation of phytoplankton and bacterial growth and production, as well as community composition, are depended on season, trophic conditions and the food web structure in aquatic systems (Morris and Lewis, 1992; Kivi *et al.*, 1993; Chrzanowski *et al.*, 1995; Vrede *et al.*, 1999; Pinhassi and Hagström, 2000; Chrzanowski and Grover, 2001; Joint *et al.*, 2002).

There is also evident that heterotrophic protists are not only a crucial regulator of the bacterial populations which affects their size structure, taxonomic composition, function and processes in both marine and freshwater habitats (Pernthaler *et al.*, 1996; Jürgens *et al.*, 1999; Lebaron *et al.*, 2001; Hahn and Höfle, 2001; Šimek *et al.*, 2001; Chróst and Siuda, 2006), but they play an important role in the regeneration of nutrients fixed in the bacterial biomass. The most important role of bacterivorous protists in nutrient re-mineralization occurs in cases where the organic carbon/inorganic nutrient ratios of bacterial substrates are high and when bacteria are consumers rather than producers of inorganic nutrients (Caron *et al.*, 1988).

In this study, we tested the hypothesis that the main mechanism generating diversity of heterotrophic microbial communities within the microbial loop is an availability of inorganic N and P nutrients in mesotrophic lake water. We assumed that bioavailability and the ratios of N/P nutrient concentrations influence mainly phytoplankton community structure and their photosynthetic efficiency in the process of organic matter production. Through the photosynthetic production rates and the amount of released organic matter, mineral nutrients have an effect on species diversity and metabolic activity of heterotrophic components of the microbial loop. We present the data on the effects of nutrients enrichment of lake water on the heterotrophic microbial communities (bacteria, HNF, ciliates), their morphology, taxonomic structure, abundance and biomass, as well as grazing impact of protists on bacteria.

Experimental

Materials and Methods

Set-up of mesocosm experiment. The mesocosm experiment was conducted for 16 days during summer stratification period (August). Lake water from mesotrophic Lake Kuc (Mazurian Lake District, North-Eastern Poland) was taken from the pelagial zone at the deepest site of lake (27 m) from the upper trophogenic water layer (0–6 m) corresponding to the maximum depth of Secchi disk. Lake Kuc is a typical dimictic lake with marked summer and winter thermal stratification. Basic morphological and physico-chemical parameters of lake were described by Chróst and Siuda (2006). Lake water taken from each sampling depth (at 0.5 m intervals) was mixed (vol/vol) together, and the integrated sample was treated as a representative for the studied lake. Large water sample (*ca.* 1,200 l) was split to four mesocosms containers (300 l) that were kept in the conditions close to those *in situ*.

Four mesocosms with different N and P nutrients concentrations and ratios were used. First mesocosm served as a control to follow changes in microbial community without any nutrient addition. Second mesocosm was supplemented with inorganic phosphorus ($P\text{-}PO_4^{3-}$, final conc. 10 μg P l^{-1}). Lake water in the third mesocosm was enriched with inorganic nitrogen ($N\text{-}NH_4^+$, final conc. 160 μg N l^{-1}). To the fourth mesocosm a mixture of two inorganic nutrients (10 μg P l^{-1} $P\text{-}PO_4^{3-}$ + 160 μg N l^{-1} $N\text{-}NH_4^+$) was added together according to the Redfield's ratio N:P = 16:1 (Redfield *et al.*, 1963). After N and P addition to experimental mesocosms 24-h period was kept to stabilize physico-chemical conditions of water. N and P concentrations in all nutrient-amended mesocosms were kept almost constant (P ± 8%, N ± 6%) throughout experiment. Nutrient concentrations were checked every 24-hours, and if necessary, corrected to the initially set-up concentrations.

Chlorophyll *a*, DOC and nutrients ($P\text{-}PO_4^{3-}$ and $N\text{-}NH_4^+$) concentrations in natural lake water were measured before nutrients additions to the mesocosms. Concentrations of these parameters in Lake Kuc amounted for: chlorophyll *a* – 3.0±0.1 μg l^{-1}, DOC– 9.9±0.1 mg C l^{-1}, $P\text{-}PO_4^{3-}$ – 2.3±0.5 μg l^{-1}, $N\text{-}(NO_3^- + NO_2^-)$ – 18 μg N l^{-1}, $N\text{-}NH_4^+$ – non-detectable.

Sampling. Water samples (10 l) were taken from each mesocosm to analyze chemical and biological parameters on 1, 3, 8, 10, 13, 16 day of incubation period.

Physico-chemical analyses. Temperature, pH, conductivity and oxygen concentration were continuously measured in water of each mesocosm with the YSI 6600 probe (Yellow Spring Instruments, USA). The concentrations of soluble reactive phosphorus

($P\text{-}PO_4^{3-}$) and ammonium-nitrogen ($N\text{-}NH_4^+$) were analyzed in the filtrate (Whatman GF/C pre-combusted glass fibre filters) with the standard limnological procedures of molybdenum blue and phenylhypochlorite method, respectively (Golterman, 1969; Solórzano, 1969). Chlorophyll *a* (Chl*a*), extracted with 98% acetone, was measured using a TD-700 fluorometer according to Arar and Collins (1997). Dissolved organic carbon (DOC) concentrations were determined in water samples filtered through 0.2-µm pore-size polycarbonate membrane filters (Millipore) using Shimadzu TOC 5050 carbon analyzer. All chemical analyses were performed in triplicates.

Bacterial abundance, biomass and size distribution. Triplicate water samples were preserved with 37% formaldehyde (final conc. 2%). Subsamples of 1 ml were stained with DAPI (4'6-diamidino-2-phenylindole, final conc. 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 into four size classes (0.2–0.5 µm, 0.5–1.0 µm, 1.0–2.0 µm, larger than 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 BacLight Bacterial Viability Kits were used (Schumann *et al.*, 2003). Triplicate subsamples of water were preserved with 25% glutaraldehyde (final conc. 4%). To 1 ml water subsamples a mixture of two BacLight Kits stains: SYTO 9 and propidium iodide was added (1:1 ratio, both dyes final conc. 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 on 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 the 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 conc. 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 was performed with the oligonucleotide probes: EUB338 (Amann *et al.*, 1990), NON338 (Wallner *et al.*, 1993), ALF968 (Neef, 1997), BET42a (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 mixture amended with DAPI (final conc. 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). Bacterial secondary production (BP) was determined with the [^{3}H]-methylthymidine ([^{3}H]TdR) incorporation method (Chróst and Rai, 1994). Triplicate samples of water (5 ml) and 37% formaldehyde-fixed blanks were incubated with 0.1 ml [^{3}H]TdR (spec. activity 60 Ci $nmol^{-1}$, final conc. [^{3}H]TdR in assays 16.68 nmol l^{-1}, ICN) in the dark at *in situ* temperature for 60 min, and finally fixed with 37% formaldehyde (final conc. 4%). After 30 min of precipitation with cold (0°C) 60% trichloroacetic acid (TCA, final conc. 20%) at 0–1°C, the TCA-precipitates were collected on 0.2 µm pore-size cellulose nitrate 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, Germany), 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).

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

Ciliate abundance, biomass and composition. Triplicate water 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 according to Foissner *et al.* (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 models were applied to study relationships among experimental data. Correlations were performed for all combined mesocosms (n=24). *T*-test was used to analyze differences in all studied parameters among experimental mesocosms.

Results

Mesocosms trophic parameters. Chlorophyll *a* concentrations were very low in the control and mesocosms separately enriched with P-PO_4^{3-} (+P) or N-NH_4+ (+N), and remained at almost constant levels (from 1.9±0.1 to 4.3±0.1 μg l^{-1}) during the experiment. Only in the mesocosm where both nutrients were added (+P+N) chlorophyll *a* concentrations increased exponentially from 3.0±0.1 to 38.5±2.9 μg l^{-1} (Fig. 1A). Significant differences were observed between the mesocosm +P+N and remaining ones, and between the control and the mesocosm +P (*t*-test, $p<0.05$).

Dissolved organic carbon (DOC) concentrations did not show any distinct variations during the experiment, ranging from 8.9±0.1 mg l^{-1} to 11.6±0.3 mg l^{-1} (Fig. 1B). The differences between mesocosms were not statistically significant (*t*-test, $p<0.05$).

The concentrations of P-PO_4^{3-} depended on the mesocosm variant. In mesocosms: control and +N-enriched, *i.e.* without P addition, P-PO_4^{3-} concentrations originated from natural lake water and were similar (3.7 ± 1.2 to 6.3 ± 1.2 μg P l^{-1}) with a slight increase after 4th day of the experiment. In mesocosms: +P and +P+N inorganic phosphorus concentrations varied between 9±1.4 and 12±1.0 μg P l^{-1} (Fig. 1C). Statistically, the differences in inorganic P concentrations between control and +N mesocosms and between +P and +P+N were significant (*t*-test, $p<0.05$).

Ammonium-nitrogen concentrations showed increasing trend in all mesocosms. However, both in the control and +P mesocosms, N-NH_4 concentrations were in very low concentrations and increased slightly from 3.3 ± 5.8 to 30.0 ± 10.0 μg N l^{-1} and from

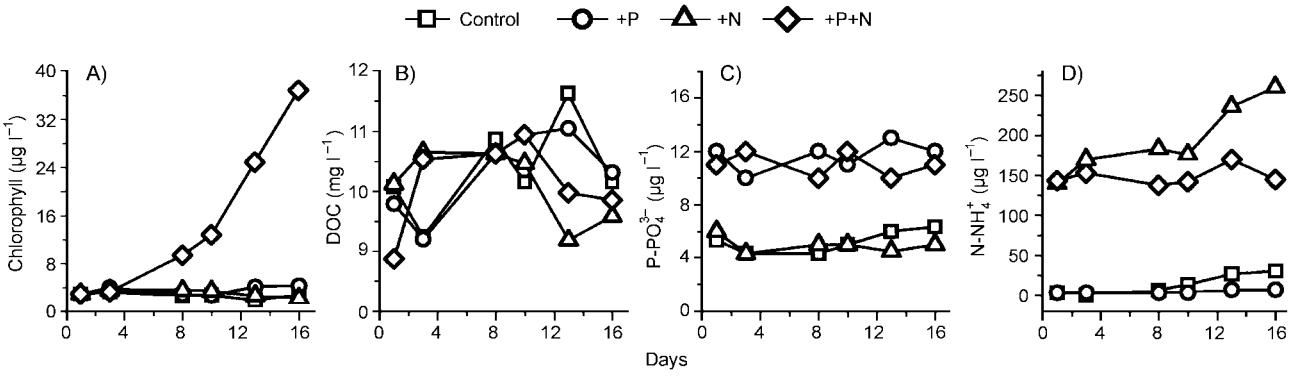

Fig. 1. Changes in concentrations of: chlorophyll *a* (A), DOC (B), P-PO_4^{3-} (C) and N-NH_4^+ (D) in four experimental mesocosms: control, P-enriched (+P), N-enriched (+N), and N- and P-enriched (+P+N).

For better visualization of the mean values, ± standard deviation (±SD) values are not shown (for ± SD values see Results).

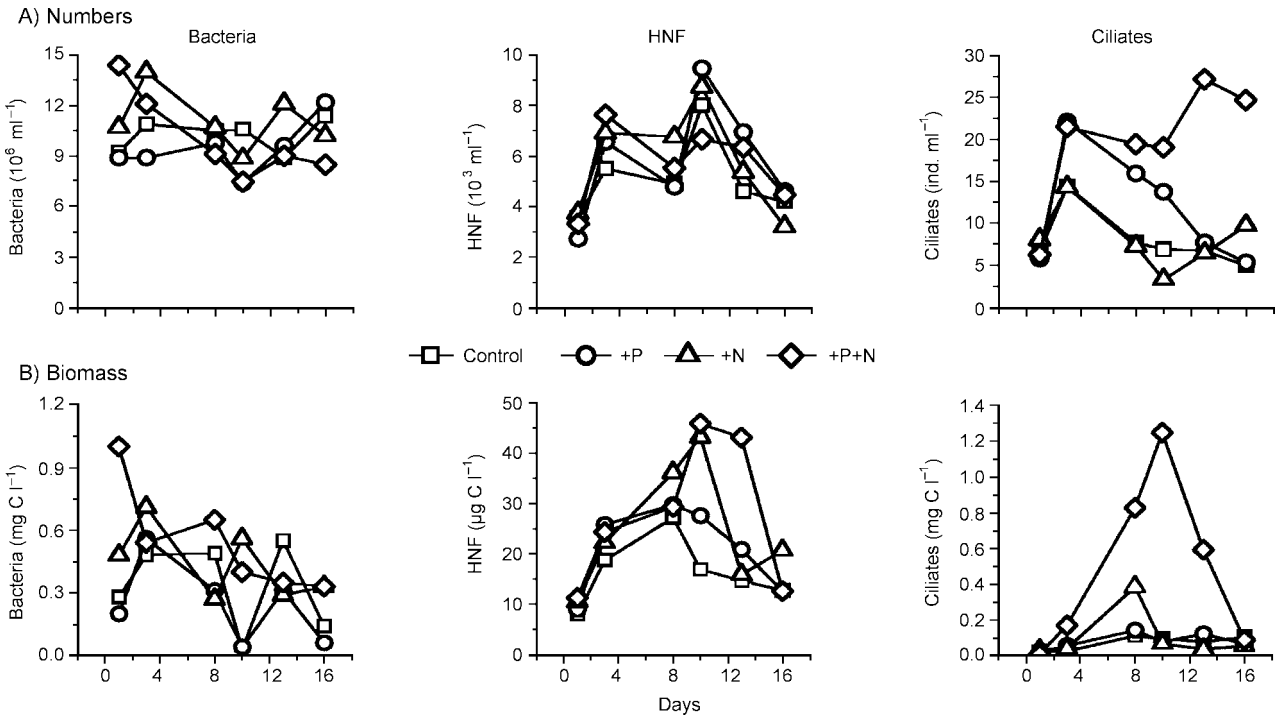

Fig. 2. Changes in numbers (A) and biomass (B) of: bacteria, heterotrophic nanoflagellates (HNF) and ciliates in four experimental mesocosms.

Mesocosms legends as in Fig. 1.
For better visualization of mean values, ±standard deviation (±SD) values are not shown (for ±SD values see Results).

3.3 ± 5.8 to 6.7 ± 11.5 µg N l^{-1}, respectively. Higher values were noted in both mesocosms with N addition, N-NH_4+ concentrations increased from 140.0 ± ± 10.0 to 260.0 ± 10.0 µg N l^{-1} in the mesocosm +N and from 143.3 ± 5.8 to 170.0 ± 10.0 µg N l^{-1} in the mesocosm +P+N (Fig. 1D). The statistical differences between mesocosms enriched with N and two remaining ones without N-additions were significant (*t*-test, $p<0.05$).

Bacterial and protistan numbers and biomass. Bacterial numbers changed distinctly in all nutrient enriched mesocosms, while remained relatively constant ($9.2\pm1.1-11.4\pm0.3\times10^6$ ml^{-1}) throughout the experiment in the control with a slight increase on the last sampling day (Fig. 2A). In the mesocosm +P, bacterial numbers remained steady to 8 day, they dropped to $7.4\pm0.5\times10^6$ ml^{-1} on day 10, and then gradually increased to $12.2\pm1.0\times10^6$ ml^{-1} at the end of the experiment. In the mesocosm +N, the bacterial numbers peaked twice; after the peak of $14.0\pm0.6\times10^6$ ml^{-1}, on day 3, bacterial numbers rapidly decreased and then their numbers increased again on day 13. In the mesocosm +P+N bacterial numbers decreased continuously up to day 10 (from 14.4 ± 0.6–$7.5\pm0.4\times10^6$ ml^{-1}) and then remained at constant level until the end of the experiment.

In control, +P and +N mesocosms, initially low HNF numbers ($2.7–3.7\times10^3$ ml^{-1}) increased on day 10, reaching maximal value of $9.5\pm2.3\times10^3$ ml^{-1} in the mesocosm +P, and then rapidly decreased (Fig. 2A). In the mesocosm +N+P, after the peak on day 3, the numbers of HNF gradually decreased until the end of the study. Small, 2–3 µm in size, cells dominated throughout the experiment, accounting from 42 to 82% of the total numbers.

In all mesocosms ciliate numbers increased distinctly from 6–8 ind. ml^{-1}, at the beginning of the study, to 14–22 ind. ml^{-1}, on day 3. Subsequently they decreased to low level in control and in the mesocosm +P while, after the slight decrease on day 10, started to the increase towards the end of the experiment in mesocosms +N and +P+N. (Fig. 2A). At the beginning of the study algivorous *Tintinnidium* sp. dominated in the control and the mesocosm +P, while bacterivorous peritrichs in mesocosms +N and +P+N. During the second half of the experiment ciliates of the genus *Coleps* sp. were most abundant in the mesocosm +P+N, while small-sized (less than 30 µm in size) bacterivorous oligotrichs (*Halteria grandinella*) dominated in remaining mesocosms.

The differences in bacterial and HNF numbers among mesocosms were statistically not significant (*t*-test, $p>0.05$). However, significant differences were found in the numbers of ciliates between the mesocosm +P+N and remaining three mesocosms ($p<0.01$).

More evident responses to addition of nutrients were observed in biomass of all the studied groups of microorganisms (Fig. 2B). High bacterial biomass

(1.0±0.02 mg C l^{-1}) at the beginning of the experiment in the mesocosm +P+N decreased dramatically at the end of the study. In remaining mesocosms bacterial biomass showed two distinct peaks, after which it rapidly decreased reaching minimal values of 0.04–0.1 mg C l^{-1} both in the control and in the mesocosm +P, and about 0.3 mg C l^{-1} in the mesocosm +N. These very low values corresponded to the high ciliate biomass.

HNF biomass increased markedly during the first half of the experiment and then decreased rapidly to low level (Fig. 2B). The highest values were noted in mesocosms +P+N (45.9±13.9 μg C l^{-1}) and +N (43.3±23.9 μg C l^{-1}), which were about three times as high as in the control mesocosm. Domination structure was very similar in all mesocosms in which 5–10 μm sized cells constituted 37–83% of the total biomass. The high values of HNF biomass both in mesocosms +N and +P+N were due to the presence of the large size HNF (>10 μm).

Ciliate biomass did not follow the same patterns as their numbers, because of their high individual weight. In all mesocosms maximal values were recorded on day 8 of the experiment (Fig. 2B). Especially high amounts of ciliate biomass was noted in mesocosms +P+N (1246.0±54.4 μg C l^{-1}) and +N (383.2±139.4 μg C l^{-1}) that were 13 and 4-times, respectively, higher than in the control, and resulted from mass occurrence of sessile very large (500–1200 μm-sized) heterotrichs *Stentor* sp. This omnivorous species dominated during almost the whole time of the experiment in all mesocosms accounting for 96% of the total ciliate biomass and affected the dynamics of variations of a biomass.

Differences in bacterial biomass were statistically significant between the mesocosm +P and both mesocosms +N and +P+N, whereas the studied mesocosms did not differ significantly in HNF biomass. The significant differences in ciliate biomass were recorded between the mesocosm +P+N and both the control and the mesocosm +P (*t*-test, $p<0.05$).

Bacterial size distribution. In all mesocosms small-sized bacterial cells (0.2–0.5 μm in length) dominated during the experiment constituting from 52 to 82% of the total bacterial numbers (Fig. 3). This size class of bacterial cells followed increasing trend in all mesocosms except the mesocosm +P+N, in which they ranged slightly from 53 to 68%. The highest increase was noted in the control (to 82%) while the lowest participation was found in the mesocosm +N (up to 74%). The most distinct differences among mesocosms were visible in the contribution of bacterial cell size fraction of 0.5–1.0 μm which percentage contribution distinctly increased from 11 to 29% in the mesocosm +P+N, while decreased to 9–12% in remaining mesocosms. The contribution of the 1.0–2.0 μm size fraction in the mesocosm +P+N was the highest at the beginning of the experiment (22%) and then decreased slowly to the end of the experiment reaching 8% of the total numbers. Maximal values of about 18% were recorded at the end of the experiment (13 day) in the control. The percentage contribution of the largest bacterial cells (>2.0 μm length) was relatively low (below 10%) and remained at constant level throughout the experiment in all mesocosm. No significant differences in the contribution of particular bacterial size classes were found among the mesocosms (*t*-test, $p>0.05$).

Contribution of active bacteria with intact membrane (MEM+). The percentage contribution of MEM+ bacteria to the total bacterial numbers was low in all mesocosms and did not exceed 12% (Fig. 4). Generally, the MEM+ contribution showed increasing trend. The most distinct increase in MEM+ contribution was found in the mesocosm +P, in which their participation ranged from 6 to 12% and thus this mesocosm differed significantly from remaining ones ($p<0.05$). Especially low contribution of MEM+ was

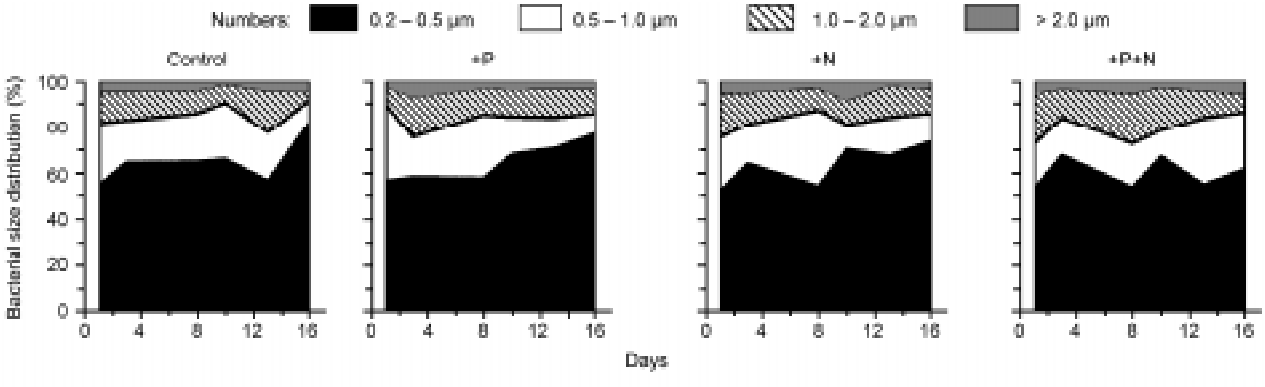

Fig. 3. Changes in percentage contributions of the studied bacterial cells-size classes: 0.2–0.5 μm, 0.5–1.0 μm, 1.0–2.0 μm and larger than 2.0 μm (>2.0 μm) to the total bacterial numbers in four experimental mesocosms.

Mesocosms legends as in Fig. 1.

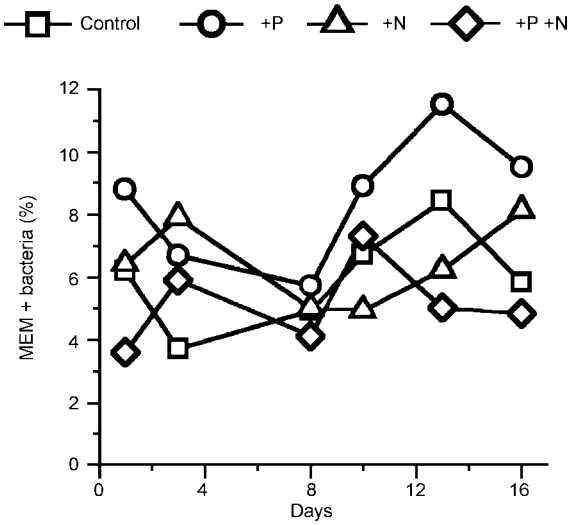

Fig. 4. Changes in percentage contributions of active bacteria with intact membrane (MEM+) to the total bacterial numbers (estimated as a sum of MEM+ and MEM– bacteria) in four mesocosms.
For better visualization of mean values, ± standard deviation values are not shown. Mesocosms legends as in Fig. 1.

found in the mesocosm +P+N, in which active bacteria fluctuated between 4 and 7% during the experimental period.

Fluorescent *in situ* hybridization (FISH). The percentage of DAPI stained cells that hybridized with probe EUB338 (*Bacteria*) ranged from 48 to 75% (Fig. 5). Gradual changes in the composition of the bacterial assemblages occurred both in the control and in the mesocosm with the addition of inorganic N and P nutrients. In all mesocosms we observed a distinct decrease of the proportion of *Bacteria*, to 48–49% in mesocosms +P and +N. From day 8 the proportion of cells hybridizing with this probe increased distinctly in mesocosms supplement with nutrients. The dynamics of particular bacterial groups was similar; however a much larger fluctuations were seen in the mesocosm +P+N. Generally, proportions of bacteria detected by BET42a probe increased from 13 to 36% in the control, from 24 to 37% in the mesocosm +P and from 17 to 32% in the mesocosm +N, whereas showed large variation in the mesocosm +P+N ranging between 19 and 27%. This subclass of Proteobacteria (BET42a) was the dominant group in all mesocosms except the mesocosm +P+N in which *Cytophaga-Flavobacterium* on day 8 and *Alphaproteobacteria* on day 13 prevailed. Among the studied groups, *Alphaproteobacteria* cluster (ALF968 probe) had the largest response to the addition of nutrients and showed two peaks. The first small peak was found on day 8, while the second large peak (26–29%) was observed on day 13. *Cytophaga-Flavobacterium* showed inconsiderable variations in mesocosms +P (8–14%) and +N (9–16%), increased markedly in the control (7–18%) while varied irregularly in the mesocosm +P+N (9–25%). The structure of the bacterial assemblages did not differ substantially among the studied mesocosms. Only minor differences in the presence of *Cytophaga-Flavobacterium* and *Alphaproteobacteria* populations were observed in the mesocosm +P+N (Fig. 5). Statistically, there were no significant differences in bacterial community composition among all studied mesocosms (*t*-test, $p>0.05$).

Bacterial production (BP) and biomass turnover rate (BTR). Bacterial secondary production rates (BP) both in the control and in the mesocosm +N remained at low and almost similar level during the whole time of the experiment and did not exceed 6 μg C l^{-1} h^{-1} (Fig. 6A). In these mesocosms two opposite trends were observed: increasing in the control and decreasing in the mesocosm +N. In mesocosms +P and +P+N, the rates of BP distinctly increased during the first day of the experiment (from 6.0±1.2 to 12.9±0.5 μg C l^{-1} h^{-1} and from 7.1±0.5 to 27.9±1.3 μg C l^{-1} h^{-1}, respectively), and then rapidly decreased to low values similar to those noted at the beginning of the study. Statistically significant differences (*t*-test, $p<0.05$)

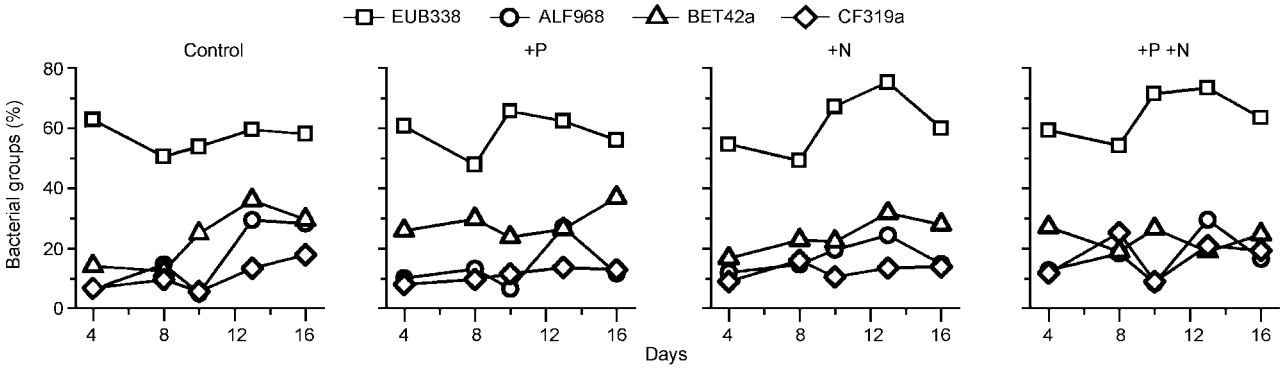

Fig. 5. Changes in percentage contributions of the studied bacterial taxonomic groups determined by fluorescent in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes: EUB338 for *Bacteria*, ALF968 for *Alphaproteobacteria*, BET42a for *Betaproteobacteria* and CF319a for *Cytophaga-Flavobacterium* cluster, related to the total number of DAPI-stained bacteria in four mesocosms: control, P-enriched (+P), N-enriched (+N), and N- and P-enriched (+P+N).
For better visualization of mean values, ±standard deviation values are not shown.

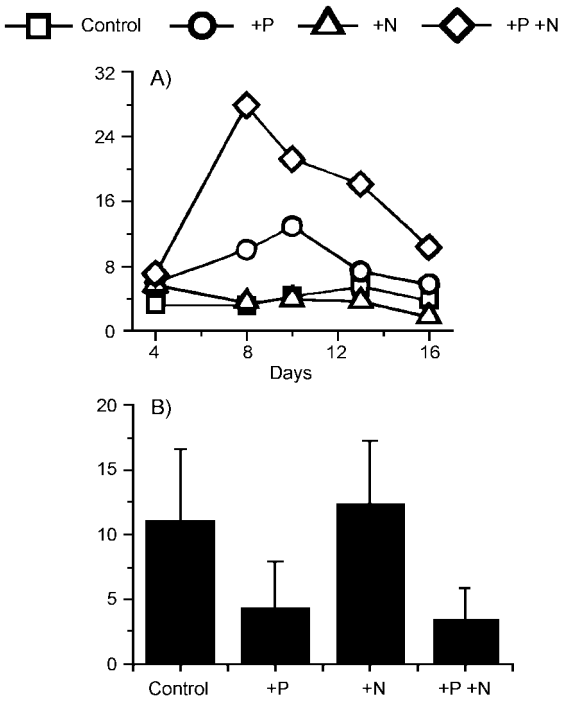

Fig. 6. Changes (A) in the rates of total bacterial production (BP) and (B) mean values of bacterial biomass turnover rates (BTR) in four mesocosms: control, P-enriched (+P), N-enriched (+N), and N- and P-enriched (+P+N).

For better visualization of mean BP values, ±standard deviation (±SD) values are not shown (for ±SD values see Results). Line bars in BTR represent ±SD.

were found among all mesocosms, except the differences between the control and the mesocosm +N.

The shortest mean turnover rates of bacterial biomass (BTR) were noted in mesocosms +P+N (3.4±2.4 h) and +P (4.4±3.5 h) (Fig. 6B). Considerably longer BTR rates were recorded in the control (11.1±5.5 h) and in the mesocosm +N (12.4±4.8 h) that differed significantly from mesocosms with P addition (*t*-test, $p<0.05$). During the experimental period the BTR values changed substantially from 7.7 to 19.4 h in the mesocosm +N with maximum at the end of the experiment. In remaining mesocosms BTR showed decreasing trends. However, in the control the BTR values were on higher level (14.6–3.7 h) than in mesocosms +P (9.3–1.1 h) and +P+N (7.7–1.9 h).

Discussion

A current subject of discussion is whether the abundance, activity and production of pelagic bacteria and also their morphological, functional and taxonomic diversity are preferentially controlled by nutrients or grazing. During our studies, we examined bottom-up effect on the heterotrophic microbial organisms in water from the mesotrophic Lake Kuc by experimental mesocosms enriched with inorganic nutrients (phosphorus and nitrogen alone and in combination). It should be emphasized that we did not employ any prefiltration method in order to eliminate from examined lake water larger organisms like rotifers and crustaceans. Thus interpretation of our results is not simple, however more realistic for the natural conditions in aquatic ecosystems, in which microbial loop, through zooplankton predation on microorganisms, is tightly connected with classical food chain. It is well known that cladocerans (especially *Daphnia* spp.) are a keystone group that, through their impact on HNF abundance, are able to determine the flux of carbon in freshwater planktonic food webs (Stockner and Shortreed, 1989; Jürgens and Stolpe, 1995). Zooplankton community structure may significantly influence microbial loop structure and, consequently, the fate of bacterial production as well as relationships between bacteria and HNF (Pace *et al.*, 1984; Gasol *et al.*, 1995). Moreover, elimination of large organisms can remove a substantial source of nutrients excreted by grazers, which through regeneration process supply nutrients for phytoplankton growth (Chrzanowski *et al.*, 1995). Ejsmont-Karabin *et al.* (2004) have shown that the role of small-bodied zooplankton, like protists, in process of phosphorus regeneration may be more important than the role of large daphnids. That is why, the positive correlation between HNF and phosphate was found (Table I). Due to short generation time and feeding on P-rich bacteria heterotrophic flagellates can excrete almost twice as much P as the zooplankton of the higher trophic levels such as rotifers and crustaceans (Vadstein *et al.*, 1993).

In this study a considerable variations were found in bacterial numbers and biomass throughout the experiment. However, the numbers of bacteria were almost in the same range both in all enriched mesocosms ($7–14\times10^6$ ml^{-1}) and in the control ($9–11\times10^6$ ml^{-1}).

Table I

Correlations between microbial and lake water trophic parameters. Correlations were performed for all combined mesocosms (n = 24, for bacterial production and biomass turnover rate n = 20)

Parameters		r*	p≤**
x	y		
Chlorophyll *a*	Bacterial production	0.45	0.05
Chlorophyll *a*	Ciliate numbers	0.66	0.001
$P\text{-}PO_4^{3-}$	Bacterial numbers	–0.57	0.001
$P\text{-}PO_4^{3-}$	% of MEM+	0.42	0.05
$P\text{-}PO_4^{3-}$	HNF numbers	0.46	0.05
% of MEM+	% of BET42a	0.51	0.05
HNF biomass	Ciliate biomass	0.66	0.001
Bacterial production	Bacterial numbers	–0.51	0.05
Bacterial production	Biomass turnover rate	–0.62	0.01

* correlation coefficient, ** probability

The only bacterial biomass showed about 2-times higher maximal value in the mesocosm enriched simultaneously with P and N (1.0 mg C l^{-1}) than in the control mesocosm. Thus, it seems that nutrient enrichment did not stimulate bacterial growth. On the other hand the negative relationship between bacterial numbers and mineral phosphorus may indicate a rapid uptake of phosphorus by bacteria. However, the positive correlation between MEM+ bacteria and P (Table I) may suggest that not all enumerated bacteria participated in PO_4^{3-} uptake. As mentioned above, grazers mainly excrete inorganic phosphorus, since the bacteria must have used inorganic phosphate as a main source of P (Vadstein *et al.*, 1993). The weak response of bacterial numbers and biomass in the present study may be explained by intensive grazing pressure, because bacteria were effectively grazed not only by HNF, but also by various groups of organisms like mixotrophic flagellates, ciliates, rotifers and crustaceans (Sanders *et al.*, 1989; Christoffersen *et al.*, 1990). Despite this, ciliates and other organisms are only occasionally important grazers of bacteria, while bacterial mortality due to grazing by heterotrophic flagellates always seems to be the most significant.

Generally, in our mesocosms experiment bacterial numbers and biomass increased when protistan numbers decreased, whereas their decreases coincided with the peaks of HNF and ciliate abundances. Results obtained by Chróst *et al.* (2000) have shown that grazing limits increases in bacterial abundance whereas concentration of substrates determines bacterial growth rates. During our experiment we noted relatively high numbers and biomass of HNF and ciliates, which responded mainly to the P+N enrichment. Very high cladocerans biomass observed at the end of the experiment in the mesocosm +P+N (3-times higher than in the control) probably had decisive effect on the reduction of HNF abundance (Ejsmont-Karabin – personal communication). In all mesocosms, small free-living nanoflagellates were found to dominate in numbers, whereas larger-sized choanoflagellates dominated in biomass. They are considered very efficient and selective bacterivores (Šimek *et al.*, 2004). Due to relatively high abundances of these forms, especially in the mesocosm with P+N addition one may infer their strong limiting impact on bacteria. It is generally assumed that when predator biomass more or less equals prey biomass, the former is limited by food and the latter by grazing (Wright, 1988). In our study, the mesocosm enriched concurrently with P and N was characterized by relatively high biomass of both HNF (about 50 μg C l^{-1}) and ciliates (up to 1.2 mg C l^{-1}) that were significantly higher than maximal values recorded in the control mesocosm. Thus, the ratio of bacterial to protistan (HNF and ciliates) biomass was almost equal (1.1 : 1) implying that grazing activity of bacterivorous organisms was probably very strong in this mesocosm. The average protistan biomass in remaining mesocosms was consistently lower (21–29%) than bacterial biomass (ratio 4–3 : 1). Our results also indicated that grazing pressure by protists appeared to be the major factor responsible for keeping bacterial numbers rather constant and that the top-down control was more important in regulating bacterial abundance than the bottom-up control by resources.

Not only fluctuations in bacterial numbers and biomass, but also important shifts in their community composition might be followed by development of nanoflagellates and ciliates. During the whole time of our experiment, we noted low contribution of active bacterial cells with intact membrane (MEM+) to total bacterial numbers (<12%). An extremely low participation of MEM+ active bacterial cells was found in the mesocosm with P+N supplementation (<7%). Taking into account results of heterotrophic nanoflagellate and ciliate abundances, which in this mesocosm were at a relatively high level, it seems that small contribution of active cells was a consequence of effective and continuous elimination through protistan selective feeding. Results of the field and laboratory experiments showed that protists eliminate mainly metabolically active cells within natural bacterial assemblages (Gasol *et al.*, 1995; del Giorgio *et al.*, 1996; Koton-Czarnecka and Chróst, 2003). The low contribution of active bacterial cells (MEM+) may confirm this hypothesis. However, alternative mechanisms have been also proposed. Some species of bacteria can adopt two distinct strategies to escape grazing pressure such as rapid growth and/or develop inedible, inactive filaments (Pernthaler *et al.*, 1996; Jürgens *et al.*, 1999; Lebaron *et al.*, 2001). Results of many studies indicated that small cells are not effectively consumed by protists and may constitute a refuge, where bacteria are able to escape predation. As shown by Šimek *et al.* (2005) after removing zooplankton from size-fractionated samples, the initially very small bacterial cells markedly enlarged their cell-size together with increasing proportions of bacteria with high nucleic acid content. Less active cells are more likely to survive than growing and productive cells, which do not develop grazing-resistance mechanisms. Consequently, the combination of preferential protistan feeding and regulation processes has a strong effect on composition and metabolic activity of the whole bacterial communities in aquatic environments (Lebaron *et al.,* 2001; Koton-Czarnecka and Chróst, 2003).

Different mechanisms may explain changes in both morphological and structural parameters of bacterial communities. Our results suggest that changes in morphology and dynamic of cell size distribution are a consequence of strong grazing pressure. The small bacterial cells (0.2–0.5 μm) dominated during

the whole time of incubation in all mesocosms. Our results are in good agreement with the studies conducted in mesotrophic Lake Kuc (from which water was taken for our investigation) by Chróst *et al.* (2000), who demonstrated that the most of the existing bacteria were very small due to weak grazing. The fraction of medium-sized cells (0.5–1.0 μm) increased in the mesocosm +P+N whereas decreased in remaining mesocosms. According to many authors these cells represent the majority of all grazed bacteria (Šimek and Chrzanowski, 1992; Pernthaler *et al.*, 1996; Koton-Czarnecka and Chróst, 2003). It means that biomass of the smallest bacterial cells should be only weakly related to the changes in protists predation, whereas slightly larger bacteria should reflect protistan bacterivory much more clearly. Furthermore, it has never been fully explained to what extent a selection due to grazing pressure affects the morphological and taxonomic structure and activity of natural communities. We did not observe distinct changes in large sized bacterial cells (mainly filaments and aggregates) throughout the experiments. Their contribution to the total bacterial numbers was below 10%. These bacterial forms are resistant to grazing by nanoflagellates (Hahn and Höfle, 2001; Šimek *et al.*, 2001) however, they are not resistant to the predation by the different ciliates (Corno and Jürgens, 2006), mainly small oligotrichs *Halteria grandinella* and prostomatids *Coleps* sp., which dominated numerically in our experiment. Omnivorous, very large-sized *Stentor* sp. formed a significant part of the total ciliate biomass during the whole time of the experiment, especially in the mesocosm amended concurrently with P+N. Microscopic observations showed that *Stentor* sp. was filled not only with bacteria, but also with *Scenedesmus* sp., *Cyclotella* sp. and green algae. Thus, we conclude that these both omnivorous ciliate taxa (*Coleps* sp. and *Stentor* sp.) feeds on bacteria, algae, flagellates, ciliates, and in the case of *Coleps* sp. on weak or dead invertebrates, might have the strong impact not only on bacterial abundance, dynamics and their size structure, but also on the dynamics other groups of organisms. It is supported by highly significant positive correlation between ciliate biomass and HNF biomass and between ciliate numbers and chlorophyll *a* (Table I). These ciliates are usually non-selective bacterial consumers and can efficiently consume larger prey sizes and also medium-sized bacterial aggregates or detritus particles (Arndt, 1993; Šimek *et al.*, 2000). Literature data show that many ciliates (especially these smaller than 50 μm) are efficient bacterivores and their effect on bacteria may exceed that of the heterotrophic flagellates. Ciliates may remove up to 39% of bacterial production supporting the idea that they create an important link between bacteria and higher trophic levels (Christoffersen *et al.*, 1990; Šimek *et al.*, 1999).

It is known evident that crustaceans, especially *Daphnia* sp. have strong impact on all planktonic components and may be more important as bacterial consumers than protists (Jürgens and Stolpe, 1995). Therefore, bacterial growth may be a function of the cascading trophic interactions. Consequently, we did not find relationship between bacteria and protists. It may suggest that bacteria were controlled by different bacterivorous species among studied groups of organisms. During our investigations, the high biomass value of small rotifers such as *Keratella cochlearis* feeding on bacteria and detritus-bound bacteria was observed especially in the mesocosm +P+N (Ejsmont-Karabin – personal communication). So, nutrients enrichment increased biomass both of protists and rotifers. However, these increases were not a direct effect of nutrient addition but resulted from bacterial growth. According to Fenchel (1986) bacterivorous organisms can keep bacterial density below the "carrying capacity" of the system. It seems, therefore, that protists as well as rotifers being present in high numbers, might stimulate the growth of bacteria, and simultaneously might maintain these cells on low levels, so the effect of nutrient addition on bacterial numbers and biomass was not directly visible.

Bacterial secondary production (BP) is a more sensitive measure of the bacterial activity and responds usually much more strongly to the nutrient addition than bacterial abundance (Pace, 1993; Vrede *et al.*, 1999). Lebaron *et al.* (2001) in the studies with nutrient enrichment applied thymidine incorporation to estimate bacterial cells replication and leucine incorporation to estimate the rate of protein synthesis, and found that bacterial thymidine and leucine incorporation give complementary information on the bacterial metabolism. The authors showed that nutrients and grazing play a key role in the activity of bacterial communities by regulating abundances and that both incorporation rates were inversely related to nano-protistan densities.

In our studies, ^{3}H-thymidine incorporation into DNA method was used to estimate bacterial secondary production (BP). Inorganic phosphorus alone and in combination with nitrogen caused strong increase in BP. On the contrary, inorganic nitrogen alone did not influence BP rate, which was similar to the control mesocosm. Our results are in agreement with experimental studies carried out in the eutrophic Lake Erken by Vrede *et al.* (1999) who showed that BP was P limited from May to August while N alone never stimulated BP. Similar conclusion was found by Morris and Lewis (1992) in a mesotrophic Lake Dillon indicating that P plays an important role in regulating bacterial growth. In this study bacterial production was negatively related to turnover rate of bacterial biomass (BTR) (Table I). Low bacterial activity and production

entailed long bacterial biomass turnover rate both in the control (11 h) and in the mesocosm +N (12 h). On the contrary, markedly shorter BTR in mesocosms +P and +P+N (4 and 3 h, respectively) resulted from very high rates of BP. The values of BTR might indicate that bacteria existing in the control and in the mesocosm with N addition were less metabolically active than in the mesocosms with P and P+N additions.

The direct and distinct increases in BP and short BTR after nutrients additions as well as the lack of large changes in bacterial biomass point to the importance of grazing in controlling bacterial density (Chróst *et al.*, 2000). Negative linear correlation between bacterial secondary production and bacterial numbers (Table I) seems to confirm this finding. Even, if bacterial production is very high, most of the newly produced cells are immediately consumed by bacterivorous HNF, ciliates and/or zooplankton of the higher trophic levels. As shown by Šimek *et al.* (2005) BP in the grazer-free treatments roughly reflected nutrient availability while in grazer-enhanced ones changes in BP were strongly affected by the grazer population dynamics and bacterivory and dropped due to grazing by the HNF assemblages.

As shown by Pace and Funke (1991) bacteria may respond directly to inorganic nutrient additions, indirectly to the effects of nutrients on phytoplankton, or to the combination of nutrient and phytoplankton changes. Although, simultaneous enrichment with P and N caused the rapid increase in BP suggesting nutrient limitation. A significant correlation between BP and chlorophyll (Table I) may indicate that bacterial production was controlled by organic substrates released by algae. Significant increase in the concentration of chlorophyll *a* (from 3.0 to 38.5 μg l^{-1}) by the addition of P and N together in comparison to remaining mesocosms, in which chlorophyll *a* reached the concentration only 4.3 μg l^{-1}, may confirm the assumed thesis of an influence of mineral nutrients on phytoplankton growth.

Bacterial community structure was analyzed using *in situ* hybridization (FISH). Mesocosm experiments with nutrient addition are of great interest for investigation, showing how some environmental factors induce temporal variations of specific populations among bacteria (Pinhassi *et al.*, 1999; Schäfer *et al.*, 2001). In our study, the sum of hybridized cells increased from 43 to 69% of total DAPI counts in the mesocosm that received phosphorous and nitrogen together. This increase was probably due to a grazing pressure and transformation of bacteria to an actively growing state with high numbers of ribosomes since in the control mesocosm we observed marked increase. In this study the *Betaproteobacteria* were clearly the most abundant group in all mesocosms throughout the experiment except the mesocosm +P+N. These result are consistent with others studies in limnetic habitats (Pearce *et al.*, 2005). This group of bacteria seemed to be less consumed by protistan predators, and predation led to large variations in the proportions of other groups of bacteria *e.g. Cytophaga-Flavobacterium* and *Alphaproteobacteria*. The observed significant positive correlation between active fraction of bacteria (MEM+ cells) and *Betaproteobacteria* abundance (Table I) might indicate that this group of bacteria was the most active among bacterial assemblages. There is increasing evidence that FISH method preferentially detects cells that may have a higher level of metabolic activity (Oda *et al.*, 2000). Cells hybridizing with the CF319a probe were present in very high densities especially in the mesocosm P+N (to 25%). Similarly to the results of Jürgens *et al.* (1999) and Labaron *et al.* (2001) we observed some very large and filamentous bacteria hybridized with this probe at the time of increased HNF grazing. Hahn *et al.* (1999) and Pernthaler *et al.* (2005) suggested that some members of *Cytophaga-Flavobacterium* group posses defense strategy against grazing which allows preserving the diversity of bacterial species in natural freshwater system. Lebaron *et al.* (2001) showed that species belonging to the genera *Cytophaga-Flavobacterium* may play a key ecological role along eutrophication gradients since they are ubiquitous species probably able to escape from the grazing pressure as it often occurs in highly productive areas (Chróst and Siuda, 2006). In our experimental mesocosm with addition of both N and P inorganic nutrients the high percentage of *Cytophaga-Flavobacterium* resulted probably from the preferential consumption by HNF. Selective predation by HNF on particular size fraction or actively growing populations of bacteria, or even certain taxa has already been demonstrated in many studies (Šimek and Chrzanowski, 1992; Jürgens *et al.*, 1999; Lindström, 2000; Hahn and Höfle, 2001; Jardillier *et al.*, 2004).

Interestingly, bacteria belonging to *Alphaproteobacteria* continued to increase throughout the experiment and dominated on one occasion in the mesocosm with both added inorganic nutrients. It suggests that *Alphaproteobacteria* may respond to added nutrients by fast growing cells, thus inducing rapid changes in the diversity of bacterial communities and to eventually outgrow the predation pressure (Pernthaler *et al.*, 1996). Bacterial populations consisted of cells with high ribosomal RNA content that become more abundant after peak of grazing belong to the *Alphproteobacteria*. These changes happened on a very short time-scale, which indicates that, the observed bacterial dynamics response to the environmental condition. Our results suggest that the nutrients do not limit the different bacterial groups except the mesocosm with P+N addition. However, resource availability

(the bottom-up control) affects bacterial community composition since individual bacterial populations differ in their response to nutrient fluctuations (Fisher *et al.*, 2000; Crump *et al.*, 2003). Lindström (2000) showed that the bacterial community composition is also depended on phytoplankton biomass in particular Cryptophyceae, on the other hand study by Richardot (2001) assumed that sloppy-feeding by Cladocera spp. could be involved in control of some bacterial groups. There is no doubt that various factors tend to show that the top-down control could preferentially favor the development of certain bacterial groups in laboratory studies (Lebaron *et al.*, 2001; Šimek *et al.*, 2005) while the availability of resources affects the growth rate and abundance of individual bacterial populations (Pace and Cole, 1996; Pinhassi and Hagström, 2000). It appears that the changes in the community composition and diversity were probably caused by the combined effects of nutrient additions, grazing and viral activity, since the proportion of *Alphaproteobacteria*, *Betaproteobacteria* and *Cytophaga-Flavobacterium* cluster changed only slightly in mesocosms with nutrients addition compared to the control mesocosm. Specifically, the added both nutrients might have increased the proportion of some groups of bacteria *e.g. Cytophaga-Flavobacterium* populations.

In conclusion, our results indicate that simultaneous P and N addition to lake water stimulated growth of protistan biomass. The impact of nutrients enrichment on the bacteria was not as marked as on protists, but the addition of P and N together resulted in significant increase in bacterial secondary production and reduced biomass turnover rate. On the contrary, the addition of nutrients had little effect on the bacterial taxonomic structure. It appears that large fluctuations in bacterial abundances were probably caused by the combined effect of nutrient addition and grazing. It seems, however, that the top-down control by predation had the strong effect on shaping the morphologic composition as well as the activity of bacterial communities by regulating their abundances. Therefore, the two control mechanisms acted probably simultaneously on bacteria but were not equally important (Weisse, 1991). These regularities confirmed results of the studies of Chróst *et al*. (2000) and Chróst and Siuda (2006) in the pelagial zone of Mazurian lakes of different trophic status. The authors underlined an importance of bottom-up and top-down mechanisms in ecological regulation of bacterial biomass and the rates of their secondary production.

Acknowledgements

This work was financially supported by projects: PBZ-KBN-087/P04/2003 and partially by N304 016 32/0959 from the Ministry of Higher Education and Science, Poland.

We are very grateful to Dr. Jolanta Ejsmont-Karabin for discussion about zooplankton effect on microbial activity, as well as, to Mrs. Halina Malinowska for nutrients analyses during our experiments, both from Hydrobiological Station of PAS in Mikolajki. Two anonymous reviewer's comments greatly improved presentation and discussion of our results.

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